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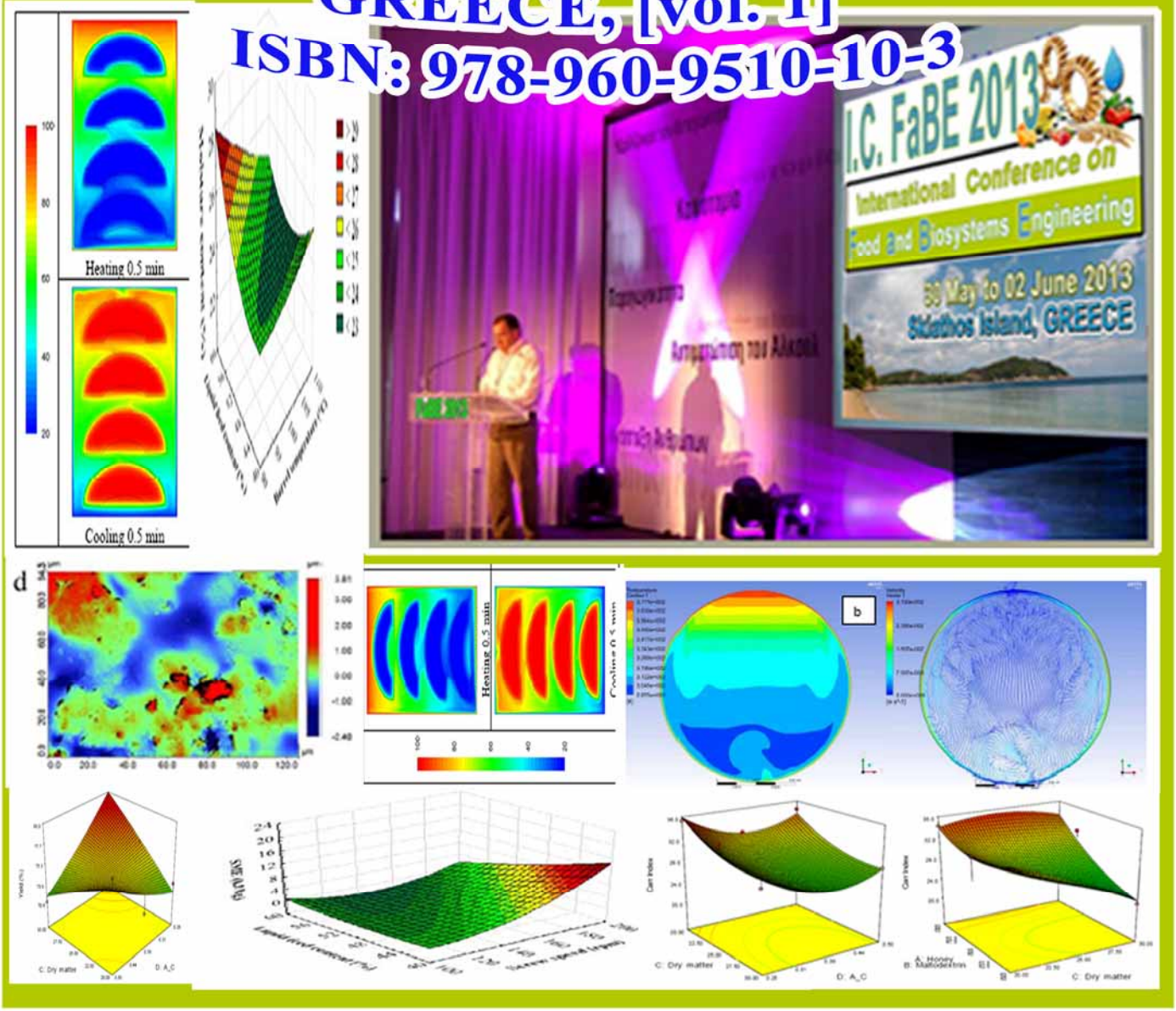

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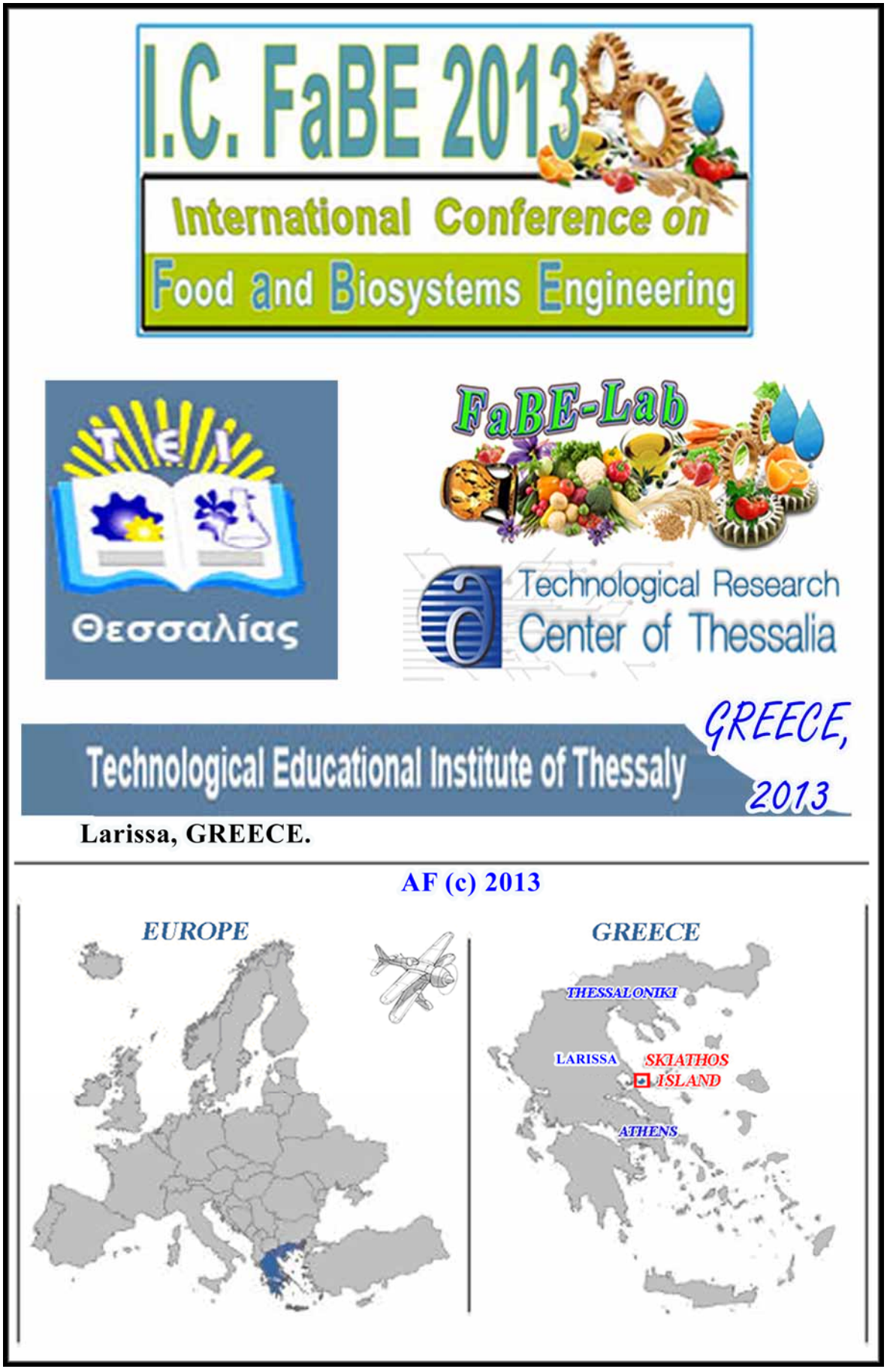
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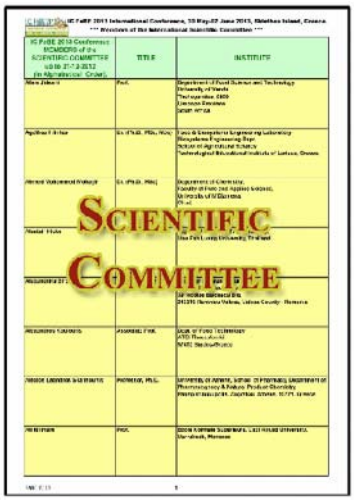

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Invited Speakers	Title of Presentation
Professor Dionysis D. Bochtis Department of Engineering, Faculty of Science and Technology, Aarhus University, Denmark.	**Management of bio-production systems in the era of automation and robotics.**
Professor Gustavo V. Barbosa-Cánovas Professor of Food Engineering at the Department of Biologicial Systems Engineering, Washington State University (Pullman, WA), USA. Director of the university's Center for Nonthermal Processing of Food (CNPF).	**Application of novel preservation technologies in food and biosystem engineering industries.**
Opening Speech Speaker	**Title of Presentation**
Professor Ferruh Erdogdu, Ph.D. **Professor of Food Process Engineering** **Department of Food Engineering** **University of Mersin** **Ciftlikkoy-Mersin** **33343 Turkey**	**Romancing with CFD in Food Engineering.**

PREFA CE

"**FaBE 2013 International Conference on Food and Biosystems Engineering**" is an international conference organized by the professors and researchers of the Food and Biosystems Engineering Laboratory, Department of Biosystems Engineering, of the Technological Educational Institute of Thessaly (TEI of Thessaly [former TEI of Larissa]), which is located in Larissa, Greece.

The International Conference **FaBE 2013 (Food and Biosystems Engineering 2013)** had a broad and scientifically interesting program on: 'Environmental Sustainability and innovation of Food and Biosystems Engineering in an Ever Changing World".

The gathering offered a platform where knowledge, innovative ideas, experiences and research achievements of people involved in the field of Food and Biosystems Engineering found a step of expression and dissemination.

In the middle of a financial crisis, the conference served as a reminder that innovative ideas for Food and Biosystems Engineering is not a luxury, that could be temporarily disregarded, but a basic prerequisite for a viable future of the population and the planet itself.

The International Conference FaBE 2013, took place in the Skiathos Palace in the exotic Greek Island of Skiathos (Central Greece) from Thursday 30 May till Sunday 02 June 2013.

Skiathos Island is a jewel among islands, set in the clear blue sea, within sight of the picturesque mountain Pelion. Skiathos is an enchanting corner of Greece, a masterpiece of nature, full of olive-trees, pines, fennel and thyme, an attracting pole for all kinds of visitors; mostly for those who can appreciate quality in pleasure, tradition and spirituality.

A number of marvellous beaches such as Lalaria with Tripia Petra, a rare beach site with an impressive white rock emerging from the sea, numerous old style monasteries and little white chapels fascinating even the most experienced traveller. The most famous spot of the island is Koukounaries, one of the best beaches in Europe. Overlooking the Koukounaries beach is a jet – setter hot spot, a wonderful holiday microcosm, the Skiathos Palace.

Participation has been very encouraging. Almost 180 papers have been selected for presentation, covering a wide range of topics, and reflecting the interdisciplinary nature of *Food and Biosystems Engineering* challenges. They have been classified in the following topics/sessions:

of ***FOOD ENGINEERING***: .

- ✓ Food Materials and Rheology.
- ✓ Advances in Food Process Engineering.
- ✓ Engineering of Novel Food Process.
- ✓ Food Product Engineering and Functional Foods.
- ✓ Food and Agricultural Waste Engineering.

- ✓ Advances in Food Packaging and Preservation.
- ✓ Modelling and Control of Food Process.
- ✓ C.F.D. applications in Food Engineering.
- ✓ Nano and microencapsulation in Food and Agriculture.
- ✓ Engineering and Mechanical Properties of Food.

of ***BIOSYSTEMS ENGINEERING***: .

- ✓ Extraction Technology for natural antioxidants and Phytochemical.
- ✓ Industrial Fermentations and Biotechnology.
- ✓ Advanced Greenhouse Technologies.
- ✓ Precision Agriculture and Variable Rate Irrigation.
- ✓ Water and Wastewater Management and Irrigation Engineering.
- ✓ Automation and Geoinformation novelty in Biosystem Engineering.
- ✓ Multi Criteria Decision Analysis, Remote Sensing, GIS and Fuzzy Logic Modelling.
- ✓ Hydrology, Hydrogeology, Geochemistry, Water Pollution and Water Resources Engineering.
- ✓ Wastewater and Sludge Reuse from Food, Agricultural and other Industries.
- ✓ Ecotoxicology, Transport and fate of pollutants and Environmental health.

The conference hosted also a special session:
"Innovation Session"

Finally, the editors would like to thank:
The authors of the papers, for contributing and sharing their own expertise.
The members of the organizing, the financial and the scientific committee, for their eager help.
The reviewers, for ensuring high scientific standards for the presentations.
The sponsors of the conference for their financial support.
All conference participants, for their active involvement in the exchange of knowledge, which is the essence of a conference.

The editors

Dr. Konstantinos PETROTOS and Dr. Agathos FILINTAS.

CONFERENCE PAPERS

SYNTHESIS OF STRUCTURED LIPIDS BY ACIDOLYSIS OF PUMPKIN SEEDS OIL

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ABSTRACT:

Structured lipids (SLs) are "taylor-made fats" with important medical and functional properties that can be produced by green enzymatic processes for food applications. They consist of novel triacylglycerols (TAGs) obtained by changing the native fatty acid (FA) profiles or by the incorporation of new desired FAs in the acylglycerol backbone.

This work aimed at the production of TAGs of MLM type, containing a medium-chain fatty acid (M) at positions sn-1,3 and a long-chain fatty acid (L) at sn-2 position, by acidolysis of oil extracted from seeds of *Cucurbita maxima* pumpkin with capric acid (C10:0), catalyzed by commercial lipase preparation from *Thermomyces lanuginose* (Lipozyme TL IM).

The reactions were performed in a batch bioreactor, in a solvent-free media or in n-hexane, at 45 °C, for 48 hours at a molar ratio of pumpkin seeds oil:capric acid of 1:2 and a fixed amount of immobilized lipase of 5 %, 10 %, 15% and 20 %. Reaction products were separated by thin layer chromatography (TLC) in silica gel plates. The TAG bands were scrapped from the TLC plate, methylated and assayed by gas chromatography.

The incorporations of C10:0 into pumpkin seeds oil increased with time and reached a plateau of 30 mol-% after 24 h reaction time. There were no significant differences in the FA incorporations obtained with different amount of immobilized lipase used, thus the subsequent experiments were carried out with 5 % of lipase load and a reaction time of 24 hours. In the presence of n-hexane, the FA incorporation obtained was nearly the same (28.8 ± 3.4 mol-%). Therefore, future studies of production of SLs by acidolysis of C10:0 with *Cucurbita maxima* pumpkin seeds oil will be conducted without solvent, since the use of solvent-free media is environmentally friendly and preferred in the food industry.

Keywords: acidolysis; capric acid; *Curcubita maxima*; Lipozyme TL IM; pumpkin seeds oil; structured lipids; triacylglycerols.

HONEY POWDER PRODUCTION BY SPRAY DRYING

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ABSTRACT:

In this study, honey was passed from liquid form to a powder form via spray drying. The effects of the ratio of honey to maltodextrin, dry matter content of mixture (feeding solution) and the amount of anti-caking agent (silicone dioxide) on flowability (Carr Index), product yield and Hunter b value (yellowness index) of powder were investigated. Combined D-Optimal design was used to arrange the experimental data. The honey (50-60% (db)) and maltodextrin ratio (40-50% (db)) was chosen as mixture variables whereas dry matter content of mixture (20-30%) and the amount of anti-caking agent in the end product (0.25-0.5% (db)) was chosen as independent process variables. The spray drying was carried out in a pilot-scale spray dryer and drying conditions as the inlet air temperature of 180 °C, outlet air temperature of 80 °C and atomization pressure of 588 kPa were kept constant in all experiments.

The flowability gets better with increasing dry matter content of feeding solution and honey content in the end product. The powder production yield and Hunter b value also increased with increasing dry matter content of solution. However, the amount of anti-caking agent in the powder was not found to be effective as dry matter content of solution on powder properties. As expected, the yellowness index, Hunter b value of powder reached maximum value for honey powder containing 60% honey and 40% maltodextrin. Besides, the best flowability value and production yield were obtained for honey powder containing 55% honey and 45% maltodextrin. As a conclusion, the optimum composition of feeding solution was determined as 30% dry matter, 0.25% anti-caking agent, 58% honey and 42% maltodextrin content in dry basis.

Keywords: Honey Powder, Spray drying, Maltodextrin, Flowability, Yellowness index

1. INTRODUCTION

Honey offered by nature to humankind is a remarkable food in terms of nutrition and health properties. The Codex Alimentarius Commission (1981) defined honey as "the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature". Honey naturally contains 80-85 % carbohydrates (53,5 % fructose, 43,6 % glucose, 2,5 % maltose, 0,3 % sucrose (Cui et al., 2008)), 15-17 % water, 0.3 % proteins, 0.2 % ash, and minor quantities of amino-acids and vitamins as well as other components in low levels of concentration. Since honey has high viscosity (1.36 Pa.s at 25°C and 21.5% moisture) (White, 1979) and sticky features, direct usage in the food industry is very difficult. The dried honey could overcome these viscous and sticky properties and has good commercial potential in dry mixes and seasoning. Honey powder has more advantages than liquid honey with respect to good uniformity with other food ingredients in a mixture, easy to handle and use, less space to keep the storage areas, low water activity and moisture content which lead to longer shelf life. Liquid honey mostly converts to powder form through spray dryer (White, 1978). Drum, microwave and freeze drying techniques are also used in honey powder production.

Spray drying is to date the most effective and economic way of producing of food powders having specific characteristics from liquid materials. In the spray-drying process, the liquid material is atomized into small droplets inside the drying chamber where the droplets come into contact with the hot air (up to 240 °C depending on the feed characteristics) (Chen, 1994; Pisecky, 1997). Since the liquid foods are dried at high temperatures during spray drying process, the physical and chemical properties of food powder are affected by these conditions. Rapid water removal during spray-drying results in an amorphous matrix that is susceptible to glass transition related changes, including stickiness, caking, and collapse (Aguilera et al., 1995; Chen, 2007; Foster et al., 2006; Phanindrakumar et al., 2005; Venir et al., 2007), as well as color changes (Acevedo et al., 2006). Pretreatment is needed during food powder production due to sugar, protein and lipid content of foods.

The glass transition temperature of honey which is one of critical parameter in spray drying is very low due to its sugar and organic acid contents. For this reason, the glass transition temperature of honey during spray drying should be increased using a carrier material such as maltodextrin, waxy starch (Takashi, 1984), diet fiber (Nobuhiko et al., 1992), dextrin and maltose (Hebbar et al., 2002). The present study was undertaken to optimize the feeding solution properties in terms of ratio of honey to maltodextrin (mixture components), and dry matter content of feeding solution and the amount of anti-caking agent in the end product (independent process variables) in order to obtain an optimum quality (flowability (Carr Index), product yield and Hunter b value (yellowness index)) honey powder with a maximum honey content.

2. MATERIAL and METHODS

2.1 Material

Liquid pine honey samples were provided by EGE NKM Gıda San. ve Tic. A.Ş., Turgutlu, Manisa, Turkey. The liquid honey sample was kept until drying at room temperature under dark conditions. Maltodextrin (DE 5-7) and silicone dioxide (SiO_2) as carrier material and anti-caking agent, respectively were supplied from MARK Gıda Ltd. Şti., İzmir, Turkey.

2.2 Feeding solution preparation

Maltodextrin and silicone dioxide were dissolved in distilled water at 25 °C with a magnetic stirrer and mixed with the liquid pine honey according to the compositions listed in Table 1. The mixture was mechanically aggregated with IKA T25 Ultra Turrax at 3500 rpm to obtain a fine mixture prior to spray drying.

2.3 Spray Drying

The drying process was conducted in a pilot scale spray drier (Mobile Minor Niro-Atomizer, Denmark). The feeding solutions having different compositions was pumped to the atomizer and atomized with a rotary atomizer into vertical, co-current drying chamber with dimensions of 0.87 m diameter and 1.2 m height. Experiments were carried out at the inlet drying air temperature of 180 °C, outlet drying air temperature of 80 °C and with an atomization pressure of 588 kPa. The drying conditions were kept constant in all experiments. The feed temperature and hot air flow rate was also fixed for all experiments at 25 °C and 1.54 m^3/min, respectively.

2.4 Analysis

Hunter b value

The Hunter b value, yellowness index of honey powders was measured with a colorimeter (Colorflex, CFLX 45-2 Model Colorimeter, HunterLab, Reston, VA).

Bulk density and Tapped density

The bulk density (ρ_b) of the honey powders was determined by measuring the weight of the powder and the corresponding volume. Approximately 20 g of powder sample was placed in a 100 ml graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder. For the tapped density (ρ_t), the cylinder was tapped steadily and continuously on the surface by hand until no further change in volume, where 100 tapping is determined to be enough, in three parallel measurements (Jinapong et al., 2008).

Flowability

Flowability of the honey powders was evaluated in terms of Carr index (CI) (Carr, 1965). CI was calculated from the bulk (ρ_b) and tapped (ρ_t) densities of the powder as shown below:

$$CI = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100 \quad (1)$$

According to Carr Index value the flowability of food powder was classified as very good (CI<15), good (CI in range of 15 to 20), poor (CI in range of 20 to 35), bad (CI in range of 35 to 45) and very bad (CI>45).

Production Yield

The process powder yield was defined as the ratio between the total collected powder and theoretical powder quantity from the sprayed solution. The spray drier production yield was calculated as shown in Eq. 2.

$$Yield = \frac{Powdered\ \ food\,(g)}{Feeding\ \ solution\ (g) * (Dry\ \ matter\,/100)} \quad (2)$$

2.5 Experimental design and statistical analysis

The effect of feeding solution properties in terms of the ratio of honey to maltodextrin, the amount of anti-caking agent and dry matter content of feeding solution on honey powder flowability (Carr Index), yellowness value (Hunter b value) and production yield was investigated during the production of honey powder by spray drying. D-Optimal mixture design was used to arrange the experimental data. The level of independent process (anti-caking agent and dry matter content) and mixture (honey and maltodextrin ratio) variables was given in Table 1. Optimum feeding solution properties, targeting the maximum or minimum or acceptable values of honey powder properties (flowability, yellowness and production yield) were selected by using Design Expert version 7.0 software (Statease Inc., MI, USA).

TABLE 1. Process and mixture variables for D-optimal mixture design

Independent variables	**Code**	**Variables type**	**Variables level (actual and coded)**				
Honey (%) (db)	A	Mixture	50 (0)	52.5 (0.25)	55 (0.5)	57,5 (0.75)	60 (1)
Maltodextrin (%) (db)	B	Mixture	40 (0)	42.5 (0.25)	45 (0.5)	47.5 (0.75)	50 (1)
Dry matter content (%) (wb)	C	Process	20 (-1)	22.5 (-0.5)	25 (0)	27.5 (0.5)	30 (1)
Anti-caking agent (%)(db)	D	Process	0.25 (-1)	0.3125 (-0.5)	0.375 (0)	0.4375 (0.5)	0.5 (1)

2.6 Optimization

The desirability function method was used for simultaneous optimization of the multiple responses. The desirability function method finds the desired goals for each variable and response. The honey ratio and dry matter content among independent variables were maximized while the maximum production yield and yellowness and minimum Carr Index value of honey powder were targeted. The numerical optimization finds a point that maximizes the desirability function.

3. RESULTS and DISCUSSION

In order to identify the feeding solution properties in terms of the ratio of honey to maltodextrin, dry matter content of feeding solution and the amount of anti-caking agent in the end product that affect the honey powder properties such as flowability, yellowness value and production yield were analyzed with D-optimal mixture design. Results of different runs of the spray drying experiments were shown in Table 2. The maximum Carr Index value (min flowability) (36.38) was recorded for the feeding solution (Sample No: 25) containing 20 %

dry matter and equal amounts of honey and maltodextrin whereas the minimum Carr Index value (max flowability) was observed for the feeding solution (Sample No: 24) containing 30 % dry matter and 60 % honey in dry matter. The effect of dry matter content of feeding solution and the ratio of honey to maltodextrin on honey powder Carr Index value was shown in Figure 1 as 3-D graph.

TABLE 2. Experimental D-optimal mixture design used in the study and experimental values of response variables

Run	A:Honey (%) (in db)	B:Maltodextrin (%) (in db)	C:Dry matter (%) (in wb)	D:Anti-caking agent (%) (in db)	ρ_b (kg/m^3)	ρ_t (kg/m^3)	Carr Index	Hunter b value	Yield (%)
1	55	45	30	0.5	375.5	524.2	28.36	12.58	70.65
2	50	50	20	0.5	396.9	600.9	33.95	10.79	70.41
3	60	40	20	0.5	476.4	680.2	29.91	15.05	61.22
4	50	50	30	0.5	379.9	522.6	27.30	11.32	78.57
5	60	40	30	0.25	471.8	588.0	19.71	14.41	68.40
6	50	50	20	0.25	361.9	513.6	29.47	10.91	71.07
7	50	50	30	0.25	343.2	539.0	36.28	11.53	76.12
8	55	45	20	0.5	381.6	581.4	34.34	12.13	78.37
9	60	40	30	0.5	445.9	577.8	22.77	15.95	69.73
10	60	40	20	0.25	458.6	650.0	29.42	14.30	72.86
11	55	45	20	0.25	385.6	595.3	35.25	12.34	72.76
12	55	45	30	0.25	377.6	535.5	29.36	12.74	78.78
13	55	45	25	0.25	351.6	531.2	33.82	12.50	75.47
14	50	50	25	0.25	352.0	547.6	35.72	10.90	76.90
15	60	40	25	0.25	466.0	607.5	23.30	15.59	64.49
16	55	45	20	0.375	368.2	538.0	31.57	11.96	71.63
17	50	50	20	0.375	355.7	541.1	34.20	9.74	65.66
18	60	40	30	0.375	491.0	629.3	21.98	15.56	67.59
19	52.5	47.5	25	0.437	340.4	468.9	27.40	11.69	74.45
20	60	40	25	0.437	432.9	602.3	28.12	15.67	67.14
21	52.5	47.5	30	0.375	345.5	503.4	31.38	11.62	77.28
22	52.5	47.5	22.5	0.312	377.7	551.6	31.46	10.94	74.29
23	57.5	42.5	27.5	0.312	441.1	597.6	26.20	13.16	74.95
24	60	40	30	0.5	531.3	617.0	13.88	16.17	65.48
25	50	50	20	0.5	346.6	544.7	36.38	10.92	69.80
26	50	50	30	0.5	356.1	492.0	27.64	11.23	77.45
27	50	50	30	0.25	358.9	553.8	35.19	10.98	75.71
28	60	40	20	0.5	429.7	601.7	28.59	14.19	66.53

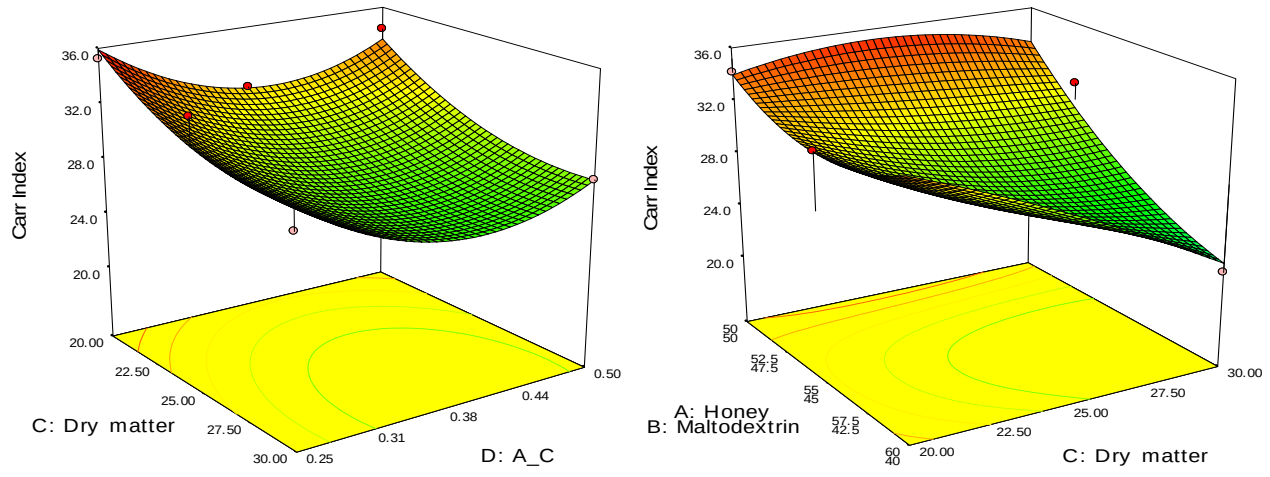
The Carr Index value of honey powder decreased with the increase in the ratio of honey and dry matter content of feeding solution. The ANOVA result showed that (Table 3) the mixture component (honey and maltodextrin) and interaction of honey, maltodextrin and dry matter content (AC and BCD) were significant parameters affecting the Carr index value (p<0.01).To obtain a honey powder having good flowability, higher dry matter concentrations of feeding solution should be used. Jinapong et al. (2008) explained this circumstance as the

lower the solids content of soymilk, which resulted in the lower feed rate and viscosity, the smaller liquid droplets formed during atomization resulting in much smaller dried particles than those obtained at high solids content. Finely sized powders show a worse flowability than coarser powders.

TABLE 3. ANOVA evaluation of linear, quadratic and interaction terms for each response variables

Source	df	Carr Index		Yield (%)		Hunter b value	
		Sum of squares	*p*-value	Sum of squares	*p*-value	Sum of squares	*p*-value
Model	17	732.5	0.0026*	574.5	0.0034*	96.49	< 0.0001*
Linear Mixture	1	346.5	< 0.0001*	201.8	0.0001*	87.71	< 0.0001*
AB	1	9.536	0.2638	12.53	0.1694	0.24	0.2546
AC	1	156.1	0.0007*	1.08	0.6734	0.95	0.0384**
AD	1	0.0109	0.9688	20.99	0.0842	1.09	0.0285**
BC	1	0.8034	0.7382	75.82	0.0045*	0.48	0.1222
BD	1	5.313	0.3976	0.33	0.8145	0.019	0.7448
ABC	1	0.2980	0.8384	2.27	0.5425	$4.20*10^{-2}$	0.6252
ABD	1	1.930	0.6059	$8.26*10^{-5}$	0.997	0.17	0.3427
ACD	1	0.3515	0.8248	23.69	0.069	0.6	0.0873
BCD	1	76.178	0.0074*	3.83	0.4317	$1.21*10^{-3}$	0.9338
AC^2	1	0.3395	0.8277	10.01	0.215	0.74	0.0615
AD^2	1	16.97	0.1453	3.55	0.4489	$7.44*10^{-3}$	0.8372
BC^2	1	1.984	0.6009	7.48	0.279	$8.80*10^{-3}$	0.8232
BD^2	1	2.071	0.5933	15.89	0.1263	0.56	0.097
ABCD	1	9.567	0.2631	72.96	0.0051*	$5.20*10^{-2}$	0.5898
ABC^2	1	4.676	0.4264	$6.20*10^{-3}$	0.9744	0.12	0.4188
ABD^2	1	24.33	0.0879	0.4	0.796	0.033	0.6672
Residual	10	68.04		57.11		1.67	
Lack of Fit	5	24.05	0.7382	33.1	0.3668	1.11	0.2338
Pure Error	5	43.99		24.02		0.56	
Cor Total	27	800.5		631.6		98.16	

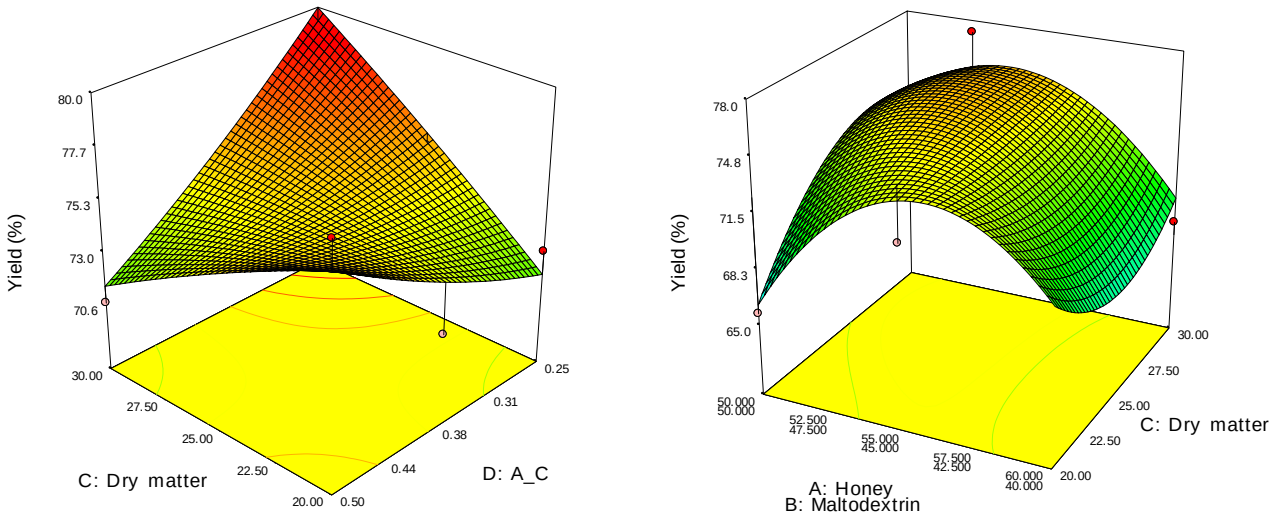
* significant at p<0.01; ** significant at p<0.05



(a) **(b)**

Figure 1. Calculated effect of feeding solution composition (a) dry matter and anti-caking agent content, (b) dry matter content and ratio of honey to maltodextrin on Carr Index value of honey powder

The honey powder production yield in spray dryer was significantly affected by the concentration of honey and maltodextrin in feeding solution (Table 3). The maximum yield (78.78%) was reached with 55% honey and 45% maltodextrin (db) of feeding solution containing 30% dry matter (Figure 2b). As shown in Figure 2b, the honey powder production yield was maximum at ~55% honey and ~45% maltodextrin concentration. The production yield decreased at the maximum or minimum ratio of honey in feeding solution (Figure 2b). The obtained honey powder has sticked on the wall of spray dryer when the ratio of honey in the mixture was greater than 55%. If the ratio of honey was smaller than 55% or the maltodextrin concentration of feeding solution was increased, the obtained honey powder was dustier. This would be the reason of lower production yield associated with the honey ratio in the mixture. The dry matter content of feeding solution had also an effect on production yield. The production yield increased with increasing dry matter content of feeding solution (Figure 2a).



(a) **(b)**

Figure 2. Calculated effect of feeding solution composition (a) dry matter and anti-caking agent content, (b) dry matter content and the ratio of honey to maltodextrin on production yield (%) of honey powder

Higher feed-solid concentration would decrease the sticky properties of honey powder and this would be the reason of higher production yield. Andrade and Flores (2004) also reported that the spray dryer production yield was increased with increasing of feed-solid concentration.

The yellowness (Hunter b value) of honey powder increased with increasing honey content in the feeding solution. The maximum Hunter b value of honey powder was obtained for a ratio of honey to maltodextrin 60:40 in feeding solution containing 30% dry matter. Honey powder produced from a feeding solution with higher dry matter possessed strong yellow color, indicating higher b value.

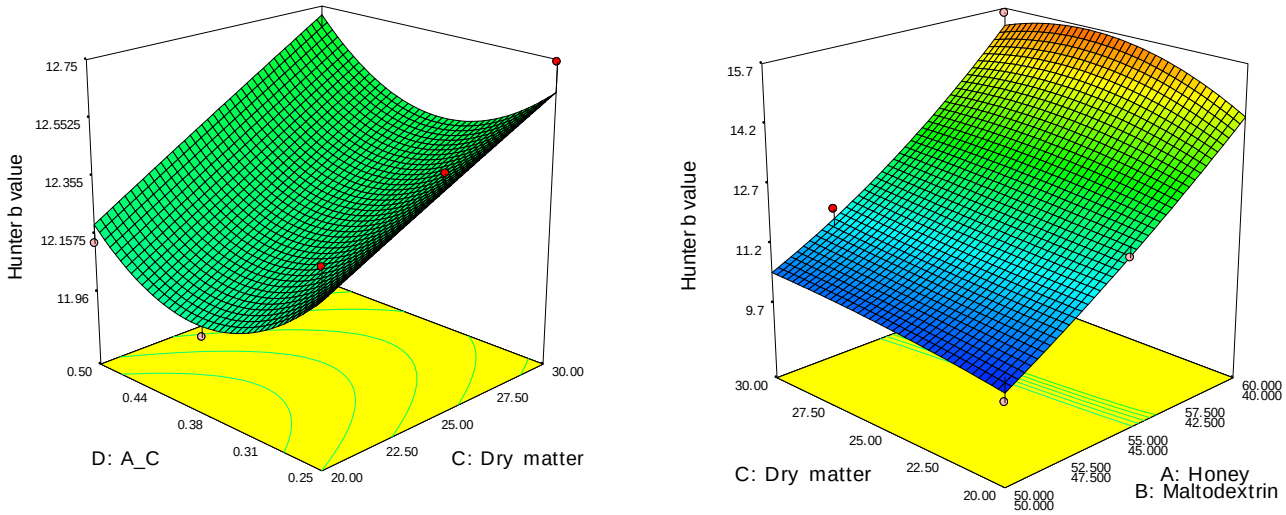


Figure 3. Calculated effect of feeding solution composition (a) dry matter and anti-caking agent content, (b) dry matter content and the ratio of honey to maltodextrin on Hunter b value of honey powder

The optimization of spray drying process in honey powder production was applied for selected ranges of the ratio of honey to maltodextrin in mixture, dry matter content of feeding solution and the amount of anti-caking agent in the end product as mixture and process variables, respectively. Desirability function was developed for the mixture and process parameters of feeding solution to obtain the optimum quality honey powder. When desirability function approach was applied, the optimum feeding solution parameters were defined as the ratio of honey to maltodextrin of 58:42, dry matter content of feeding solution of 30% and the amount of anti-caking agent in end product of 0.25%. At this process

conditions, Carr Index value, yellowness (Hunter b value) and production yield were calculated as 24.60, 13.7 and 74.3%, respectively.

4. CONCLUSION

The flowability, production yield and yellowness (Hunter b value) of honey powder produced in spray dryer from 28 different feeding solutions were investigated in this study. The high solid content of feeding solution caused an increase in flowability, yield and yellowness of honey powder. The flowability and yellowness of honey powder were also increased with increasing concentration of honey in feeding solution. However the production yield of honey powder was maximized at 55% honey concentration in dry basis. The optimum composition of feeding solution was determined as 30% dry matter, 0.25% anti-caking agent, 58% honey and 42% maltodextrin content in dry basis.

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MATHEMATICAL MODELING IN FOOD ENGINEERING

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ABSTRACT

Mathematical modeling is applied to computationally evaluate a process for design and optimization purposes. Mathematical models are either observation (depending upon the availability of experimental data) or physics based describing the transport phenomena (formulation of continuity, energy and momentum equations with initial and boundary conditions) governing the process. Empirical observational models are limited for their predictive abilities, but they might be useful for well-defined systems in the presence of extensive experimental data. Physics based mathematical models, on the other hand, are more practical for process evaluation and design purposes. Essential aspects of a food process model are to define physical, chemical and/or biological changes to develop mathematical background of the process with appropriate assumptions and to solve and validate the model. In this study, mathematical modeling approaches are summarized focusing on heat transfer and fluid dynamics. For this purpose, numerical approaches and CFD methodologies bringing new innovative approaches into modeling are described with various examples. In addition, significance of model validation is explained with traditional and innovative techniques.

Keywords: Mathematical modeling, heat transfer, fluid dynamics, CFD, validation

1. INTRODUCTION

Wikipedia (http://en.wikipedia.org/) defines “simulation” as the imitation of a real thing or process. Ultimate goal of a simulation is to create alternative plans and answer what-if questions for a process without laborious and time-consuming experiments. Based on this definition, food process simulation is described to represent a process using mathematical models as a quick way of evaluation for possible design and optimization purposes. Mathematical models are either observation or physics based. Observational models depend

upon the availability of experimental data, and they are limited for predictive capabilities. Hence, `mathematical model` term should be used for physics based models, and a model is required to simulate a physical process where the relationships between inputs and outputs of the process are represented (Wedzicha and Roberts, 2006). Since frequent computations are required due to possible variations in input parameters, mathematical models have started becoming practical for process evaluation, design and optimization with the availability of high-power PC's (Datta, 2002).

2. MATHEMATICAL MODELING IN FOOD ENGINEERING

Mathematical modeling of food processing operations is based on physical fundamental mechanisms governing a given process and has a benefit of providing a basic understanding of the process (Singh and Vijayan, 1998) and started with the pioneering work of Texieira et al. (1969) where optimization of nutrient retention in thermal processing of conduction heated foods were carried out using a finite difference numerical methodology. In a mathematical model, with the given physical fundamentals, processes are described with a set of differential equations, and modeling involves solution of these equations. This requires to determine temperature distribution for heat transfer, concentration of certain component for mass transfer and changes in velocity, pressure and velocity field distributions for momentum transfer. Reaction kinetics might also play a significant role in modeling If destruction of a certain nutrient, or variation in textural-sensory attributes, or formation of a certain food safety problem (e.g., formation of acrylamide in french fries) is the objective. Therefore, food process modeling is regarded to be an interdisciplinary approach involving engineering fundamentals with chemistry, reaction kinetics and predictive microbiology (Datta and Halder, 2008). Essential aspects of a model are to (Singh and Vijayan, 1998):

- state the problem with its rationale and define physical, chemical or biological changes,
- develop mathematical basis with appropriate assumptions,
- convert the model into a numerical scheme if necessary,
- conduct experiments or use literature data for required thermal-physical properties, and
- validate the model.

Following equations, obtained by applying conservation of mass, energy and Fourier's laws on a differential volume in cartesian coordinates, are given for a complete heat transfer modeling where conduction and convection is involved with heat generation.

Continuity equation:

$$\frac{\partial u_x}{\partial x}+\frac{\partial u_y}{\partial y}+\frac{\partial u_z}{\partial z}=0 \tag{1}$$

Energy equation:

$$\left(\rho\cdot c_p\right)\cdot\left(\frac{\partial T}{\partial t}+u_x\cdot\frac{\partial T}{\partial x}+u_y\cdot\frac{\partial T}{\partial y}+u_z\cdot\frac{\partial T}{\partial z}\right)=k\cdot\left(\frac{\partial^2 T}{\partial x^2}+\frac{\partial^2 T}{\partial y^2}+\frac{\partial^2 T}{\partial z^2}\right)+Q \tag{2}$$

Momentum equation in x-direction:

$$\rho\cdot\left(\frac{\partial u_x}{\partial t}+u_x\cdot\frac{\partial u_x}{\partial x}+u_y\cdot\frac{\partial u_x}{\partial y}+u_z\cdot\frac{\partial u_x}{\partial z}\right)=-\frac{\partial P}{\partial x}+\frac{\partial}{\partial x}\left(\mu\cdot\frac{\partial u_x}{\partial x}\right)+\frac{\partial}{\partial y}\left(\mu\cdot\frac{\partial u_x}{\partial y}\right)+\frac{\partial}{\partial z}\left(\mu\cdot\frac{\partial u_x}{\partial z}\right)+\rho\cdot g_x \tag{3}$$

Momentum equation in y-direction:

$$\rho\cdot\left(\frac{\partial u_y}{\partial t}+u_x\cdot\frac{\partial u_y}{\partial x}+u_y\cdot\frac{\partial u_y}{\partial y}+u_z\cdot\frac{\partial u_y}{\partial z}\right)=-\frac{\partial P}{\partial y}+\frac{\partial}{\partial x}\left(\mu\cdot\frac{\partial u_y}{\partial x}\right)+\frac{\partial}{\partial y}\left(\mu\cdot\frac{\partial u_y}{\partial y}\right)+\frac{\partial}{\partial z}\left(\mu\cdot\frac{\partial u_y}{\partial z}\right)+\rho\cdot g_y \tag{4}$$

Momentum equation in z-direction:

$$\rho\cdot\left(\frac{\partial u_z}{\partial t}+u_x\cdot\frac{\partial u_z}{\partial x}+u_y\cdot\frac{\partial u_z}{\partial y}+u_z\cdot\frac{\partial u_z}{\partial z}\right)=-\frac{\partial P}{\partial z}+\frac{\partial}{\partial x}\left(\mu\cdot\frac{\partial u_z}{\partial x}\right)+\frac{\partial}{\partial y}\left(\mu\cdot\frac{\partial u_z}{\partial y}\right)+\frac{\partial}{\partial z}\left(\mu\cdot\frac{\partial u_z}{\partial z}\right)+\rho\cdot g_z \tag{5}$$

The viscous dissipation term appearing on the right hand side of the energy equation is neglected since it is significant for high-speed flows where its magnitude is comparable to that of conduction term. The term Q (W/m^3) in the energy equation represents internal heat generation. Microwave, radio-frequency and ohmic heating processes are examples of this case.

A general approach to solve the given equations with applied initial and boundary conditions is to use numerical solutions or a computational fluid dynamics (CFD) approach. Numerical solutions (finite difference and finite element) make discrete mathematical approximations of time, spatial variations and boundary conditions defined by partial differential equations

where governing partial differential equations are transformed into a series of difference equations and solved. Since the solution requires a discrete number of points (so-called grid) as a consequence of discretization, the result becomes an approximation rather than an exact solution (Nicolai et al., 2001) opposite to the case of exact solutions (so-called analytical solutions). The error in the approximations is reduced by increasing the number of discretized points in both space and time at the expense of computational time (Nicolai et al., 2001). While finite difference methods have been widely used especially for regular shaped geometries, more complex finite element methods performed better for irregular geometries. Stability, convergence and consistency are significant issues in numerical solutions. The solution is said to be unstable if errors (round-off, truncation and discretization errors) introduced at one time step grows unboundedly in the following steps; conversely, the solution is stable if these errors die out (Clausing 1969). Consistency is the property that truncation errors approach zero as spatial and time increments approach zero. A finite difference representation is consistent if truncation errors tend to approach to zero with increasing number of differential elements. Consistency and stability are required conditions for convergence, and a numerical method is convergent if the solution approaches the exact solution as both time and space increments are reduced (Palazoğlu and Erdoğdu, 2009). Besides finite difference and finite element methods, CFD methodology is another powerful numerical tool widely used in food processing with sophisticated modeling requirements. Availability of CFD programs relieves researchers from difficulty of code writing to carry out the numerical solutions (Datta, 2002). In CFD methodology, continuity, momentum (Navier-Stokes equations) and energy equations are numerically solved. Computational procedure is mostly based on finite volume method which combines the flexibility of finite element with the execution speed of finite difference method (Nicolai et al., 2001).

As indicated by Nicolai et al. (2001), governing fundamental physics of food processing operations have been well understood, and numerical approaches should be used except for some simplified cases:

- regarding process involves a solid object conforming to a regular geometry (i.e. slab, cylinder or sphere) during the entire process (without any significant changes in the physical geometry),
- governing heat transfer mechanism is conduction where heat is transferred by a molecular diffusion process (there are exceptions of analytical solutions for convection heat transfer with simplified and appropriate assumptions)

- initial temperature distribution is uniform,
- Surrounding medium temperature with required parameters of the given boundary condition is constant, and
- Thermal and physical properties are constant and isotropic.

Obtaining analytical (exact) solutions involving a non-zero volumetric heat generation (Q) term or with non-uniform initial temperature distribution and variable thermal properties is also possible, but the results might be mathematically complicated. Therefore, for the conditions non-conforming to the above stated simplified cases, it would be better to apply numerical solutions or CFD methodologies. For the cases with volumetric heat generation term (as in the case of microwave, radio frequency and ohmic heating), numerical methods or CFD solutions are also preferred since modeling for these cases also involve additional complexities (i.e., temperature dependent thermal and physical properties). With given assumptions, analytical solutions can be obtained using different solution techniques, such as separation of variables, Green functions and Laplace transform. Separation of variables has been widely used in obtaining analytical solutions, and this method is based on expanding a function in terms of Fourier series. This is applied when the governing equation and differential equations representing boundary and initial conditions are homogeneous and linear, and dependent variable, temperature, is assumed to be the product of independent variables (time and location). For the cases of non-homogeneous conditions, superposition techniques are applied to split the problem into simpler problems. The first step in obtaining the analytical solution for the above given simplified cases is to choose orthogonal coordinate system of which coordinate surfaces will coincide with boundary. For example, cartesian coordinate system is used for rectangular bodies while spherical coordinate system is used for spherical shapes, and cylindrical coordinate system is for cylindrical ones.

The simplest case, assuming that there is no fluid motion or bulk flow involved with no heat generation term, with constant and isotropic thermo-physical properties is:

$$\nabla^2 T = \frac{1}{\alpha} \cdot \frac{\partial T}{\partial t} \qquad (6)$$

where α (thermal diffusivity) is $\alpha = \frac{k}{\rho \cdot c_p}$ and ∇^2 is the Laplacian of temperature in rectangular (cartesian), cylindrical and spherical coordinate systems.

Analytical (exact) solution of Eqn. 6 for convection boundary across the surface and symmetry condition at the center with a constant uniform initial temperature distribution is:

$$\frac{T(x,t)-T_\infty}{T_i-T_\infty}=\sum_{n=1}^{\infty}\left[C_n(x)\exp\left(-\mu_n^2\frac{\alpha\cdot t}{L^2}\right)\right] \tag{7}$$

where μ_n and $C_n(x)$ and are given for regular shaped geometries of slab, cylinder and sphere, respectively:

Slab:

$$C_n(x)=\frac{2\cdot\sin(\mu_n)}{\mu_n+\sin(\mu_n)\cdot\cos(\mu_n)}\cdot\cos\left(\mu_n\cdot\frac{x}{L}\right)$$
$$N_{Bi}=\mu\cdot\tan(\mu) \tag{8}$$

Cylinder:

$$C_n(x)=\frac{2\cdot J_1(\mu_n)}{\mu_i\cdot\left[J_0^2(\mu_n)+J_1^2(\mu_n)\right]}\cdot J_0\left(\mu_n\cdot\frac{x}{L}\right)$$
$$N_{Bi}=\mu\cdot\frac{J_1(\mu)}{J_0(\mu)} \tag{9}$$

Sphere:

$$C_n(x)=\frac{2\cdot\left[\sin(\mu_n)-\mu_n\cdot\cos(\mu_n)\right]}{\mu_n-\sin(\mu_n)\cdot\cos(\mu_n)}\cdot\frac{\sin\left(\mu_n\cdot\frac{x}{L}\right)}{\mu_n\cdot\frac{x}{L}}$$
$$N_{Bi}=1-\frac{\mu}{\tan(\mu)} \tag{10}$$

where J_0 and J_1 are the first kind, 0^{th} and 1^{st} order Bessel functions, L is half-thickness for slab and radius for cylinder and sphere, x is the distance from the center $(0\le x\le L)$, and N_{Bi} is the Biot number $\left(\frac{h\cdot L}{k}\right)$. For the case of convection heat transfer is involved, momentum equations with continuity equation, in addition to the energy equation, come into the picture, and numerical solutions or CFD methodologies are preferred instead of analytical solutions.

Boundary conditions usually encountered in heat transfer problems are prescribed surface temperature, prescribed heat flux and convection boundary condition. For the case of fluid flow, no-slip condition at the walls is applied. No-slip boundary condition states that velocity of fluid, in contact with a solid wall, is equal to the wall velocity. In addition, free-slip conditions, constant velocity profiles at the inlets and pressure outlets can be defined for solution. Inlets might also involve turbulence intensities depending upon the nature of the flow. Required thermal and physical properties for modeling purposes can be easily found in

the literature. Thermal conductivity (k), specific heat (c_p) and density (ρ) are required parameters in modeling conduction heat transfer processes. In freezing and thawing processes, enthalpy is used since specific heat becomes infinitely high at initial freezing or thawing point. For modeling convection, viscosity of fluid product is needed to solve the momentum equations. Additional properties required for modeling infrared, ohmic, microwave and radio frequency heating processes are radiative properties of food products (emissivity, spectral transmittance and reflectance), dielectric properties and electrical conductivity. In microwave and radio-frequency heating, dielectric properties of food products, dielectric constant (ε) and relative dielectric loss$(\varepsilon")$, are taken into consideration while electrical conductivity (σ) is required for ohmic heating. These three parameters are strong functions of temperature. Heat transfer coefficient, in addition to thermal and physical properties of food products, is another parameter required for the effect of convective boundary condition. It depends on thermo-physical properties of medium, shape, dimensions, surface temperature and surface roughness of the food product and velocity and turbulence of fluid flow. Shrinkage problem is also encountered during thermal processing of foods due to the changes in the protein structure leading to moisture loss. In some cases, it is also important to include the shrinkage in the models since the physical dimensions can be quite effective in the results.

As explained, an accurate modeling of a food processing operation is a complex task and requires number of properties with some reasonable assumptions. Sensitivity analysis is sometimes needed to make judgment for acceptability of an applied assumption (Wang and Sun, 2003). Experimental validation of the mathematical models is also required to be sure of the meaningful computational results.

3. MODEL VALIDATION

Numerical models and CFD solutions have the ability of solving complex problems, but their results should be validated with experimental data. Experimental validation is a difficult task, and it might be also time consuming. In some cases, analytical solutions can also be used to validate the results of numerical solutions when the models are run for simulation of simpler problems. For the case of CFD solutions, Datta (2002) suggests checking the convergence of the solution, using common sense about the physics of the process, checking the results of a

simpler problem (for this case, analytical solution results can also be applied) and comparing the results with the experimental data. For conduction heat transfer, model validation seems to be rather easy with the suggested ways since using analytical solutions or measurement of temperature at a certain location of the product is a rather simple task. However, for convection processes, more sophisticated technologies should be applied. For example, understanding and measurement of flow field is significant to validate and improve upon the developed mathematical models for extruders, aseptic processing systems, mixers, air impingement systems and cold air blast freezers. For this case, particle image velocimetry (PIV) can be applied. Magnetic resonance imaging (MRI) is another useful technique to measure temperature changes and provide direct measurement of flow field maps. Another method of determining the flow field maps is the use of positron emission particle tracking technique. Measuring surface temperature in food processing studies is also not easy. For this purpose, liquid crystal termography methods and infrared tomography can be applied. Another way of validating the models is the use of time-temperature indicators.

4. CONCLUSIONS

Mathematical modeling approaches for food processing simulation were summarized in this study by focusing on heat transfer and fluid dynamics. As explained, numerical methods have their own complexities and advantages over the analytical solutions while the CFD methodologies bring new innovative approaches (as in the case of determining heat transfer coefficient automatically) into the picture. In conclusion, mathematical models for simulation studies can be accepted as a perfect way of creating alternative plans and answering what-if questions for a given food processing operation without laborious and time-consuming experiments.

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VOLATILE COMPOUNDS EVALUATION OF TRITICALE (*TRITICOSECALE WITTMACK*) FLOUR BLEND DOUGH IN FERMENTATION PROCESS

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ABSTRACT:

The most important sensory characteristics that describe the flour products are taste and aroma. Aroma development in bread crumb has been found to be dependent on yeast concentration, mixing stage and fermentation time. The aim of this research was to investigate the effect of fermentation time and temperature on volatile compounds in dough made from flour blend (triticale, hull-less barley, rye, rice, and maize). Dough was made from flour blend, sugar, salt, yeast and water, according to the recipe and technology which are used in wheat bread making. An investigation of volatile compounds was done using solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS). Before the fermentation dough was mixed for 6 minutes at 32 °C; for 8 and 10 minutes at 25 °C. Volatile compounds were analyzed on the 10^{th}, 20^{th} and 30^{th} minutes of dough fermentation at fermentation temperatures 30, 35 and 40 °C. In the fermentation process of flour blend dough there were detected 16 volatile compounds. Identified volatile compounds belong to alcohols, aldehydes, acids, terpens and terpenoids. Part of identified volatile compounds such as hexanal and D-limonene that were detected in dough fermentation originates from flour blend. Total peak area of volatile compounds increased extending dough fermentation time and temperature.

Keywords: dough fermentation, triticale, solid-phase microextraction, volatile compounds

1. INTRODUCTION

Triticale (*Triticosecale wittmack*) is the first man-made cereal produced by crossing wheat (*Triticum spp.*) and rye (*Secale ceral* L.). The nutritional value of triticale is close to wheat

and rye, and some triticale-based food can be purchased at health food stores (Salmon et al, 2002; Hosseinian and Mazza, 2009). Triticale is mainly used as an ingredient in animal feeding, but also on a smaller scale as a food ingredient, for example in bread making or as a replacement for soft wheat in biscuits, cakes and cookies (Sabovics et al, 2010; Rakha et al, 2011).

The yeast used for bread manufacturing is *Saccharomyces cerevisiae*, often referred to as simply baker's yeast. It converts the fermentable sugars present in the dough into carbon dioxide and ethanol as the main products. The fermentation intensity depends on the temperature, the form of the yeast and the availability of fermentable sugars in the flour, including maltose produced by starch hydrolysis (Hutkins, 2006).

Taste, smell and the flavour are undoubtedly the most important attributes determining the quality of bread or baked cereals in general and one of the most important attributes influencing the acceptance of the consumer (Hansen and Schieberle, 2005). Flavour is usually the result of the presence, within complex matrices, of many volatile and non-volatile components possessing diverse chemical and physicochemical properties. Whereas, the non-volatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Gatfield, 1988; Urbach, 1997). Salim-ur-Rehman et al (2006) reported that although yeasts have primary leavening role in sourdough fermentation, lactic acid bacteria (LAB), with trophic and non-trophic relationships, produce important flavour components. Aroma compounds in typical wheat bread are 3-methylbutanal, 2,3-butandione, 3-methylbutanol, acetic acid, 1-octen-3-one, 1-hexanol, 1-propanol, 2-methyl-1-butanol, 2-phenylethanol, ethyl acetate etc. (Schieberle, 1996; Poinot et al, 2008), but in bread what is made by sourdough composition of aroma compounds are propanol, pentanol, ethyl acetate, heptanal, acetic acid, ethanol, 2-pentylfurane, acetaldehyde, 3-methyl-1-butanal etc. (Hansen and Schieberle, 2005; Guerzoni et al, 2007). Also, the ingredients and various technological processes (dough fermentation and the baking process) can play a vital role in the development of characteristic flavour of the bread (Hansen and Schieberle, 2005).

For volatile compound investigation in dough fermentation process can be used a solid-phase microextraction (SPME) fiber. Solid-phase microextraction is a relatively new sample preparation technique (Arthur and Pawliszyn, 1990) before analysis through gas

chromatography (Hook et al, 2002). SPME shows some advantages over other widely used techniques: solvent-free and rapid sampling, low cost, and higher sensitivity (Pawliszyn, 1997). Three different types of fiber can be used to determine the volatile compounds. Carboxen/Polydimethylsiloxane showed the best extraction efficiency for volatile analysis (Ruiz et al, 2003).

The aim of this research was to analyze the composition of volatile compounds during triticale flour blend dough fermentation.

2. MATERIALS AND METHODS

Experiments were carried out at the Faculty of Food Technology, Latvia University of Agriculture. The data are reported as means of measurements made in triplicate, where each sample was obtained from a separately prepared batch of dough for each formulation.

2.1. Materials

Triticale, rye and hull-less barley crops cultivated at the Priekuli Plant Breeding Institute (Latvia) were used in the current study. Triticale, rye and hull-less barley used for study were grounded in a laboratory mill *Hawos* (Hawos Kornmühlen GmbH, Germany) obtaining whole grain fine flour. Rice and maize flour were purchased from Joint Stock Company *Ustukiu Malunas* (Lithuania). For this research there was used the flour blend that contained 60% – whole grain triticale, 15% – whole grain hull-less barley, 15% – whole grain rye, 5% – rice and 5% – maize flour. Ingredients, such as sugar, salt, water and yeast were included in dough formulation in order to improve sensory properties and keep quality of bakery products.

2.2. Sample preparation

For dough sample preparation there were used flour blend (250 g), dried yeast (7.8 g), sugar (4.5 g), salt (3.8 g) and water (170 ml). Dough was mixed using three mixing times 6, 8 and 10 minutes and dough temperature 32, 25 and 25 °C respectively. After mixing the dough samples were analyzed after 10, 20 and 30 minutes of yeast fermentation using three fermentation temperatures 30, 35 and 40 °C.

2.3. Detection and identification of volatile compounds

For detection of volatile compounds there was used 50.0±0.1 g of mixed flour blend dough, the sample was weighted into a 250 mL glass container equipped with aluminium lid with 1 mm diameter hole in the middle for SPME fibre. The container was placed in a water bath *Clifton Food Range* (Nickel-electro Ltd, UK) at water temperature 30±1, 35±1 and 40±1 ^{o}C for fermentation process (Figure 2).

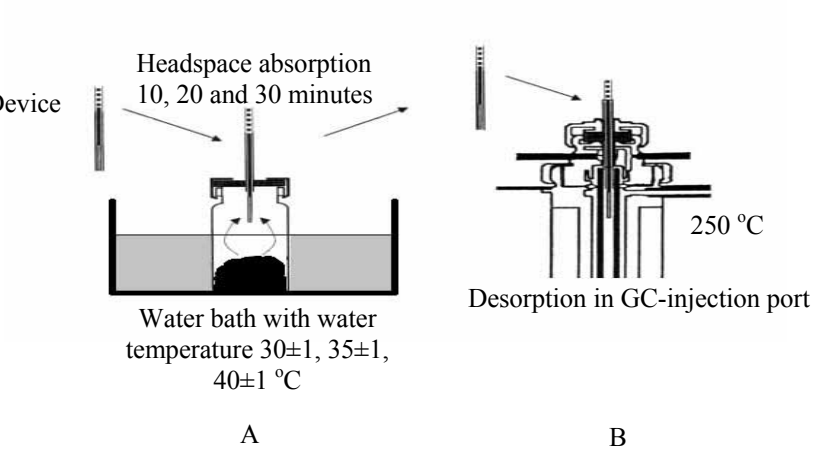

Figure 2. Volatile compound adsorption (A) and desorption (B) process.

Volatile compounds were determined from flour blend dough samples using SPME in a combination with gas chromatography/mass spectrometry (GC/MS). The SPME fibre was coated with a thin polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The film thickness is 85 μm with bipolar polarity (Supelco, Inc., USA). The SPME extraction time was done for different duration of dough fermentation: the first - extraction time is 10 min at 30±1, 35±1 and 40±1 ^{o}C without pre-incubation, the second – 20 min and the third – 30 min in the same temperature.

Volatile compounds from fiber were thermally desorbed in the injector of GC/MS. Separation of volatiles was carried out in the *Elite-Wax* (PerkinElmer, Inc., USA) capillary column (60 m x 0.25 mm i.d., DF 0.25 μm). The details of the program used in GC–MS analysis are following: the initial temperature was 40 ºC, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C min^{-1} and from 160 ºC to 210 °C at a rate of 10 °C min^{-1} with a hold time for 15 min. The total run time was 47 min for a sample. Mass spectrometer in Electron impact Ionization mode was set on 70 eV as the electron energies, while the ion source temperature was set to 250 °C and inlet line temperature was set to 250 °C. Injections were performed in split mode (2:1) and helium (He) was used as carrier gas at a constant flow of 1 ml min^{-1}. Acquisition parameters in full scan mode: scanned m/z 40-300. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98.

3. RESULTS AND DISCUSSION

Cereal grains contain important flavour precursors, such as amino acids, fatty acids and phenolic compounds, which produce various flavours during processing – mixing, fermenting and baking (Hansen and Schieberle, 2005). All volatile compounds that were found in dough, made from triticale flour blend, fermented for 10, 20, and 30 minutes at various temperatures are shown in Table 1, 2, and 3.

Analysing the volatile compounds after 10, 20 and 30 minutes of triticale flour blend dough fermentation at various temperatures overall there were identified 9 alcohols, 3 acids, 2 ketones, 1 aldehyde, and 1 terpene. The peak areas of detected 15 volatile compounds increased in dough fermentation process, but peak area of D-limonene decreased. The highest peak area of D-limonene was detected in the sample which was mixed 6 minutes at dough temperature 32 °C and fermented 10 minutes at 40 °C temperature (0.57×10^6) (Tab.1), but the lowest in the sample which was mixed 8 minutes at dough temperature 25 °C and fermented 10 minutes at 30 °C temperature (0.06×10^6) (Tab.2). The decreasing of D-limonene could be explained by the fact, that this compound can oxidize easily in moist air to produce carveol, carvone, and limonene oxide (Karlberg et al, 1992). Carvone peak area in all samples increased from 0.58×10^6 to 1.36×10^6 (Tab. 1, 2, 3).

The highest peak area value (684.43×10^6) among all detected volatile compounds had 4-penten-2-ol (alcohol), what was indentified in the sample which was mixed 6 minutes at dough temperature 32 °C and fermented 10 minutes at 40 °C temperature (Tab.1), but the lowest peak area value (0.06) was detected for D-limonene (terpene) in the sample which was mixed 8 minutes at dough temperature 25 °C and fermented 30 minutes at 30 °C temperature (Tab. 2).

Alcohols can be formed in the metabolism of yeast when are produced long-chain and complex alcohols, but aldehydes and ketones can be formed from alcohols. Acetic acid is formed in dough yeast fermentation process from yeast and gives acidic and vinegar flavour. Yeast secondary metabolism can form 3-methyl-1-butanol, which gives malty flavour to dough. According to Schieberle (1996) the amount of flavour compounds formed in dough can be affected by yeast amount and activity, fermentation time and fermentation temperature.

TABLE 1: Peak area* ($\times 10^6$) of volatile compounds in flour blend dough mixed 6 minutes at dough temperature 32 °C influenced by fermentation temperature and time.

Volatile compounds	Fermentation temperature 30 °C			Fermentation temperature 35 °C			Fermentation temperature 40 °C		
	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Alcohols									
4-amino-1-pentanol	45.90	110.22	178.22	45.93	138.21	257.18	80.63	159.99	300.81
4-penten-2-ol	327.00	503.20	517.17	374.85	556.11	575.17	450.83	573.67	684.43
2-methyl-1-propanol	10.25	16.41	19.15	13.45	19.85	23.17	16.43	23.14	25.98
(Z)-2-penten-1-ol	0.44	0.76	0.79	0.53	0.78	0.80	0.55	0.88	0.95
3-methyl-1-butanol	72.40	151.40	164.02	92.95	167.57	185.30	114.73	180.48	208.42
1-pentanol	1.90	3.25	3.93	2.49	3.56	4.31	3.36	3.80	4.70
3-methyl-1-pentanol	12.56	27.94	50.59	14.81	28.95	56.82	20.00	49.81	63.59
(E)-3-nonen-1-ol	0.44	0.72	0.80	0.48	0.78	0.82	0.53	0.88	0.94
Phenylethylalcohol	0.20	0.49	0.55	0.24	0.55	0.76	0.41	0.59	1.36
Acids									
Acetic acid	0.42	0.93	1.08	0.61	1.03	1.27	0.75	1.21	1.33
2-methylpropanoic acid	0.33	0.43	0.53	0.37	0.49	1.11	0.51	0.90	1.51
2-(aminooxy)-propanoic acid	0.16	0.16	0.24	0.18	0.19	0.27	0.21	0.27	0.43
Ketones									
3-hydroxy-2-butanone	3.10	8.18	13.13	4.24	9.40	14.25	4.74	11.64	14.85
Carvone	0.79	0.90	1.22	0.93	0.94	1.27	1.15	1.19	1.32
Aldehyde									
Hexanal	0.57	1.07	1.25	0.60	1.52	1.64	1.21	1.74	1.87
Terpene									
D-limonene	0.54	0.21	0.18	0.52	0.31	0.16	0.57	0.37	0.19
Total peak area	**477.02**	**826.26**	**952.84**	**553.17**	**930.27**	**1124.30**	**696.61**	**1010.57**	**1312.67**

**Results are given as mean (n = 3)*

TABLE 2: Peak area* ($\times 10^6$) of volatile compounds in flour blend dough mixed 8 minutes at dough temperature 25 °C influenced by fermentation temperature and time.

Volatile compounds	Fermentation temperature 30 °C			Fermentation temperature 35 °C			Fermentation temperature 40 °C		
	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Alcohols									
4-amino-1-pentanol	35.31	74.27	103.68	37.24	98.69	165.29	49.52	140.08	275.64
4-penten-2-ol	214.22	282.78	376.55	302.23	477.19	523.10	348.53	528.88	635.76
2-methyl-1-propanol	7.30	8.76	15.08	12.05	17.20	22.02	15.77	21.96	23.76
(Z)-2-penten-1-ol	0.41	0.69	0.76	0.46	0.72	0.79	0.53	0.86	0.92
3-methyl-1-butanol	62.17	81.84	92.19	79.87	146.63	178.68	91.31	175.58	202.67
1-pentanol	2.15	3.41	4.01	2.48	4.23	5.54	3.39	4.47	5.80
3-methyl-1-pentanol	10.73	22.47	42.24	12.66	23.75	54.99	18.57	41.00	59.95
(E)-3-nonen-1-ol	0.37	0.51	0.61	0.41	0.61	0.72	0.49	0.82	0.91
Phenylethylalcohol	0.23	0.51	0.58	0.29	0.62	0.89	0.45	0.70	1.45
Acids									
Acetic acid	0.35	0.68	0.85	0.57	0.91	1.18	0.71	1.08	1.20
2-methylpropanoic acid	0.27	0.36	0.43	0.30	0.42	0.66	0.46	0.84	1.33
2-(aminooxy)-propanoic acid	0.17	0.20	0.26	0.20	0.22	0.35	0.24	0.38	0.51
Ketones									
3-hydroxy-2-butanone	2.46	5.17	6.87	2.77	6.90	10.04	3.13	7.15	10.53
Carvone	0.58	0.71	1.07	0.79	0.82	1.15	1.06	1.17	1.27
Aldehyde									
Hexanal	0.52	1.03	1.18	0.59	1.44	1.57	1.17	1.71	1.81
Terpene									
D-limonene	0.33	0.18	0.06	0.36	0.18	0.08	0.39	0.21	0.10
Total peak area	**337.56**	**483.57**	**646.42**	**453.27**	**780.55**	**967.05**	**535.72**	**926.89**	**1223.61**

**Results are given as mean (n = 3)*

TABLE 3: Peak area* ($\times 10^6$) of volatile compounds in flour blend dough mixed 10 minutes at dough temperature 25 °C influenced by fermentation temperature and time.

Volatile compounds	Fermentation temperature 30 °C			Fermentation temperature 35 °C			Fermentation temperature 40 °C		
	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Alcohols									
4-amino-1-pentanol	36.87	77.30	117.94	43.54	100.72	173.94	62.99	148.57	281.33
4-penten-2-ol	231.08	292.74	395.94	349.87	548.83	567.06	355.96	555.03	645.99
2-methyl-1-propanol	8.32	9.14	16.16	12.53	17.48	22.71	16.17	22.58	24.39
(Z)-2-penten-1-ol	0.54	0.83	0.85	0.56	0.86	0.89	0.67	0.94	1.02
3-methyl-1-butanol	63.08	95.80	120.89	84.97	164.51	182.68	102.07	178.97	206.04
1-pentanol	3.51	3.62	5.61	4.00	8.99	9.38	4.39	9.11	9.90
3-methyl-1-pentanol	11.34	26.31	48.53	14.14	27.28	55.72	19.07	46.01	61.48
(E)-3-nonen-1-ol	0.40	0.54	0.72	0.46	0.73	0.80	0.51	0.85	0.93
Phenylethylalcohol	0.34	0.55	0.78	0.38	0.84	1.29	0.58	0.90	1.59
Acids									
Acetic acid	0.37	0.69	1.06	0.60	0.97	1.23	0.73	1.19	1.30
2-methylpropanoic acid	0.31	0.39	0.50	0.35	0.46	0.93	0.48	0.88	1.49
2-(aminooxy)-propanoic acid	0.22	0.25	0.33	0.26	0.28	0.42	0.33	0.53	0.64
Ketones									
3-hydroxy-2-butanone	2.70	5.84	8.97	3.35	9.19	11.36	3.45	11.20	11.76
Carvone	0.77	0.91	1.29	0.86	0.96	1.30	1.11	1.22	1.36
Aldehyde									
Hexanal	0.76	1.39	1.66	0.78	1.62	1.70	2.03	2.28	3.05
Terpene									
D-limonene	0.52	0.19	0.10	0.48	0.21	0.11	0.52	0.28	0.12
Total peak area	**361.12**	**516.49**	**721.31**	**517.14**	**883.95**	**1031.52**	**571.05**	**980.54**	**1252.40**

**Results are given as mean (n = 3)*

All of the detected volatile compounds can add flour blend dough specific aroma: 2-methyl-1-propanol produce whiskey odour, 4-penten-2-ol – fruity, 1-pentanol – sweet, 3-hydroxy-2-butanone – like butter or yogurt, 1-hexanol – freshly mown grass and 3-methyl-1-pentanol - earthy, green odour (Schieberle, 1996; Kulp et al, 2003).

Assessing the data, the increasing of fermentation time and temperature significantly ($p<0.05$) influence volatile peak areas. Thus, increasing the fermentation time and temperature, it was observed that volatile compounds and total volatile compounds areas sum were higher.

CONCLUSION

In all fermented dough samples totally were detected 16 volatile compounds: 9 alcohols (4-amino-1-pentanol, 4-penten-2-ol, 2-methyl-1-propanol, (Z)-2-penten-1-ol etc.), 3 acids (acetic acid, 2-methylpropanoic acid, 2-(aminooxy)-propanoic acid), 2 ketones (3-hydroxy-2-butanone, carvone), 1 aldehyde (hexanal) and 1 terpene (D-limonene).

Increasing of fermentation time and temperature significantly ($p<0.05$) influence volatile peak areas. Thus, increasing the fermentation time and temperature it was observed that volatile compounds and total volatile compounds areas sum were higher.

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UV-C TREATMENT OF VERJUICE

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ABSTRACT:

Verjuice is a green grape juice that is obtained directly by squeezing of unripened grapes. It is generally produced and consumed in Mediterranean and Southeastern Anatolia region of Turkey and shown as an alternative to lemon juice and vinegar because of its specific odor and sour taste. Verjuice is usually produced at home and possess a short shelf life. On the other hand, thermal treatment of verjuice results in off flavour and color.

In this project, verjuice is treated with UV-C irradiation i.e. non thermal treatment by using a continuous flow UV-C Reactor System. The system consists of seven UV-C lamp emitting light at 254 nm, one of them is replaced at the centre and other lamps are placed throughout the annular quartz glasses. The juice is circulated in the annular flow region where a path lenght is less than 1 cm by a peristaltic pump. Two different types of unripened grapes as Sultana and Yediveren are freshly squeezed in laboratory conditions and filtered in order to obtain more clear verjuice samples. Juice samples are circulated through the continuous flow UV-C system 5 times at the lowest flow rate of the system (54 ml/min, 116.5 J/mL). Before and after the treatment, some quality parameters such as pH, brix, titradable acidity (%), absorbance, color, turbidity and ascorbic acid amount are analysed for both type of verjuice samples. Total aerobic count, yeasts and moulds are counted before and after all treatments by plating on petri dishes prepared by appropriate medium. All of the physical, chemical, optical and microbiological experiments for both Sultana and Yediveren verjuice samples are followed during six months period for evaluation of their shelf life.

Keywords: UV-C irradiation, verjuice, continuous flow UV reactor, shelf life

UV-LED Technology for Non Thermal Treatment of Liquid Foods

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ABSTRACT:

UV-C irradiation performs effective sterilization excluding the use of heat to inactivate microorganisms. It has been used to disinfect water, air, surfaces and foods such as fruit juices, liquid egg, milk and fresh produce. Generally of UV disinfection systems operate with low- or medium-pressure mercury lamps which are large in size and fragile, require high amounts of energy to operate and have a short lifespan of approximately 4000- 10000 h. Moreover they contain mercury which is an important environmental contaminant. Therefore, a safer, less expensive and more durable UV disinfection devices are required. The new UV-LED technology has been launched as an alternative to traditional UV irradiation systems. Advantageous UV-LEDs are known to be shock resistant, more efficiently use electricity, and have a lifetime exceeding 100000 h. Additionally, having a very compact design make them available for the sterilization of small or narrow spaces. Most importantly, UV-LEDs do not contain toxic substances (mercury) or pollutants. Hence, these safer devices are now being investigated for water disinfection. Recent results indicated the same inactivation efficiency of UV-C LEDs with low-pressure mercury lamps. This work compares the available UV-C irradiation with the new UV-C LED technology and assess the applicability of this new technology in food industry.

Keywords: low-pressure mercury lamps, UV-LED technology, UV irradiation, disinfection

1. INTRODUCTION

Thermal pasteurization is the best known technique in order to remove pathogens, reduce the number of spoilage microorganisms and inactivate the enzymes which decrase the quality of the products. However, use of high temperatures may cause some quality problems such as

colour, taste and flavour defects (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso and Ortega-Rivas, 2007; Choi and Nielsen, 2004).

Increased trend towards fresh-like products forced the researchers to investigate alternative processing techniques (Tahiri, Makhlouf, Paquin and Fliss, 2006; Basaran-Akgul, Churey, Basaran and Worobo, 2009). One of the alternative method to thermal pasteurization is UV-C radiation. Inactivation mechanism of UV-C irradiation is based on the absorption of UV photons by the genetic material and the formation of dimers which inhibit the transcription and replication of the cell (Oguma, Katayama and Ohgaki, 2002; Bolton, Linden and Asce, 2003; Koutchma, 2009). Antimicrobial effect of UV-C light is very well known and this technique is used for disinfection of fruits surfaces, hospital equipments, water resources etc. (Begum, Hocking-Ailsa and Miskelly, 2009; Pan, Vicente, Martinez, Chaves and Civello 2004; Bintsis, Tzanetaki and Robinson, 2000; Nigro, Ippolito and Lima, 1998).

Generally low or medium pressure mercury vapor lamps at 254 nm wavelength are used for UV-C irradiation. However these lamps contain mercury which is known to be toxic for both environment and human body. Additionally UV reactors need to be designed according to the shape of the lamps. These lamps are mostly large in size and take up too much space. Moreover, UV lamps are not resistant to shock and have a low life span (approximately 4,000-10,000 hours). Therefore, it is necessary to design new sterilization equipments in various sizes which do not contain toxic substances and have low energy consumption rate. Use of UV-LEDs for sterilization and disinfection purposes is a new approach. They have a very compact, shock-resistant and robust design. They also can be used for sterlization of narrow spaces and allow saving space due to their small sizes. UV-LEDs do not need a warm-up time in contrast to traditional UV-C lamps. Hence they consume less energy. It was also reported that UV-LEDs have relatively longer life time exceeding 100,000 hours. Most importantly, they do not contain any toxic substances which are harmful for human health and the environment. They are capable of emitting UV light at multiple individual wavelengths. Besides, it is possible to use the combination of different UV-LEDs emitting light at different wavelengths. In literature it was indicated that UV-LEDs emitting light at 275 nm resulted in much higher microbial inactivation (Bowker, Sain, Shatalov and Ducoste, 2011). This is due to the fact that protein absoprtion spectrum reaches the maximum near 280 nm and thus enzymes become more sensitive to inactivation at these wavelengths. Furthermore it is known that UV light at UV-A wavelength (320-400 nm) inhibits the protein synthesis by damaging cell membranes and has better penetration property (Chevremont, Farnet, Coulomb and

Boudenne, 2012a). Considering all these circumstances it is more effective to use UV-LED systems which allow the use of LEDs at different wavelengths so as to enhance the enzyme inactivation.

2. UV-C IRRADIATION

2.1 Theoretical background

Ultraviolet light is a part of electromagnetic spectrum with ranges between 100-400 nm (Miller, Jeffrey, Mitchell and Elasri, 1999; Bintsis, Tzanetaki and Robinson, 2000; Begum, Hocking-Ailsa and Miskelly, 2009).

The spectrum can be divided into four groups based on wavelengths. The subgroups are;

- UV-A (320 to 400 nm)
- UV-B (280 to 315 nm)
- UV-C (200 to 280 nm)
- UV-V (100 to 200 nm)

The highest germicidal effect of UV light was reported to be observed between 250 and 270 nm (Tran and Farid, 2004; Guerrero-Beltran and Canovas, 2005). Koutchma (2009) and Oteiza, Giannuzzi and Zaritzky (2010) declared that the most efficient inactivation can be obtained at 253.7 nm due to the maximum absorption of UV photons by the genetic material at this specific wavelength.

Low or medium pressure mercury vapor lamps were reported to be used for many years in UV light treatment (Koutchma, 2009).

The mechanism of UV-C irradiation is based on the absorption of UV photons by DNA or RNA pyrimidine bases. Incident light causes the formation of dimers on the same DNA strand which inhibit the transcription and replication of the cell.

2.2 Applications

UV-C light treatment was reported to be effective on the inactivation of bacteria, protozoa, algea and viruses. This process has been used to disinfect water effluent, potable water and water for swimming pools for many years (Begum, Hocking-Ailsa and Miskelly, 2009). It is also used for surface disinfection of foods, decontamination in hospitals, pharmaceutical industry and public buildings (Begum, Hocking-Ailsa and Miskelly, 2009; Pan, Vicente, Martinez, Chaves and Civello, 2004; Nigro, Ippolito and Lima, 1998).

US Food and Drug Administration (US FDA) has approved the use of UV-C light as a germicidal agent for the disinfection of fruit juices (US FDA 2000). There are many applications of UV-C irradiation on different fruit juices (Torkamani and Niakousari, 2011; Gabriel, 2012; Azhuvalappil, Fan, Geveke and Zhang, 2010; Koutchma, Keller, Chirtel and Parisi, 2004; Choudhary and Bandla, 2012).

2.3 Advantages and disadvantages

UV-C light processing is a physical treatment which does not leave chemical residues (EPA, 1999; Canitez, 2002). Also it is effective against most type of microorganisms (Bintsis, Tzanetaki and Robinson, 2000).

However, lack of penetration in highly absorptive media, reduced effectiveness in existence of organic matters and in presence of suspended particles are the disadvantages of the system. Moreover, it was reported that UV-C light treatment has no effect on the enzymes which influence the fruit juice quality, such as pectinmethylesterase (PME), polyphenoloxidase (PPO) and peroxidase (POD) (Tran and Farid 2004; Noci, Riener, Walkling-Ribeiro, Cronin, Morgan and Lyng, 2008).

3. UV-LED TECHNOLOGY

3.1 Theoretical background

Use of UV light emitting diodes (UV-LEDs) is a relatively new method to generate UV light. UV-LEDs are created by connecting p-type and n-type semiconductors that move electrons into positively charged holes between these two materials. Light is generated when the electrons and holes recombine at a junction. The wavelength of light depends on the type of material that is used for those semiconductors (i.e. indium gallium nitride for light in the visible range, and aluminium gallium nitride and aluminium nitride for UV range) (Bowker, Sain, Shatalov and Ducoste, 2011).

3.2 Applications

There are many applications of GaN-based blue, green, and white light emitting diodes (LEDs) like backlighting of mobile phones, laptops and TVs, street lighting, and car headlight (Techneau, 2010). UV-LEDs are also used for;

- Water disinfection (UV-C, UV-A)

- Sensing (UV-C, UV-A)
- UV curing (UV-B, UV-A)
- Medical treatment (e.g. treatment of psoriasis, UV-B)
- Tanning (UV-B)
- Lithography (UV-A)
- Non-line-of-sight communication (UV-C)
- Sterilization (e.g. medical, UV-C)

In literature there are limited numbers of studies related to the use of UV-LEDs for water disinfection purpose. Chatterley and Linden (2010) reported that most of those data were available for LEDs emitting light at UV-A range. However, UV-C LEDs were also indicated to be preferred for this purpose (Li, Hirota, Yumoto, Matsuo, Miyake and Ichikawa, 2010; Chevremont, Farnet, Coulomb and Boudenne, 2012a; Chevremont, Farnet, Sergent, Coulomb and Boudenne, 2012b; Bowker, Sain, Shatalov and Ducoste, 2011; Würtele et al, 2011; Hamamoto et al, 2007). Moreover combination of UV-A and UV-C LEDs was used in some studies (Aoyagi et al, 2011; Chevremont, Farnet, Coulomb and Boudenne, 2012a).

UV-A radiation mechanism is based on the inactivation of microorganisms by damaging proteins and producing hydroxil and oxygen radicals which destroy cell membrane and other cellular components. On the other hand UV-C radiation causes the formation of cyclobutane thymine dimers by directly affecting the microbial DNA and inactivates microorganisms without any intermediate steps (Chatterley and Linden, 2010). Although DNA damage caused by UV-C radiation can be repaired by the enzyme photolyase, there is no possibility to repair the damage to bacterial membranes by UV-A radiation.

Chevremont, Farnet, Coulomb and Boudenne (2012a) showed that coupling UV-A and UV-C could be paired by using the germicidal effect of UV-C and greater penetrating ability of UV-A. They also found that use of coupled wavelenghts 280/365 nm and 280/405 nm caused total dissappearance of fecal enterococci, total coliforms and fecal coliforms in the effluent (Chevremont, Farnet, Coulomb and Boudenne, 2012a). Besides lack of possibility to repair the damage in the bacterial membranes occured after UV-A exposure increased the efficiency of microbial inactivation (Chevremont, Farnet, Coulomb and Boudenne, 2012a).

3.3 Comparison of UV-LEDs and traditional UV lamps

Table 1 shows the main differences between UV-LEDs and traditional UV lamps. According to that the mercury-vapor lamps contain mercury, which is toxic to the environment as well as

to the human body. On the other hand a UV-LED is an environment-friendly sterilizer. It does not contain any toxic substances so it does not have harmful effects (Mori et al, 2007). Additionally, traditional UV lamps are fragile, non-durable to shock, less energy efficient and large in size. Sterilizers using UV lamps must be designed according to the shape of the lamps which take up a lot of space. It was also speculated that these lamps have a short life span of approximately 4000-10000 hours (Chevremont, Farnet, Coulomb and Boudenne, 2012a). Hence, the cost of a UV device equipped with mercury lamps were reported to be relatively higher (Hamamoto et al, 2007). Moreover low pressure mercury lamps are known to emit nearly monochromatic UV light at a wavelength of 254 nm whereas UV-LEDs offer the possibility to use preferred wavelenght (Crawford et al, 2005).

TABLE 1. Differences between UV-LEDs and traditional mercury vapor UV lamps

UV-LEDs	**Traditional Mercury Vapor UV Lamps**
Do not contain toxic substances	Contain mercury
Robust design	Fragile
Compact size	Take up a lot of space
Longer life time	Short life span
Use different light source emitting UV light at different wavelengths (UV-A, UV-B, UV-C)	Emit monochromatic UV light at 254 nm (UV-C)

In contrast to traditional UV lamps, UV-LEDs were shown to have a robust design, compact size, higher energy efficiency, reduced frequency of replacement due to long life time of nearly 100000 hours since they do not need a warm-up time (Chevremont, Farnet, Sergent, Coulomb and Boudenne, 2012b; Vilhunen, Rokhina and Virkutyte, 2010). Furthermore using UV-LEDs will provide the opportunity to develop small, space-saving sterilization equipments. UV-LED devices can sterilize small and narrow spaces due to their compact size (Mori et al, 2007). Therefore, design of a new sterilization equipment of low energy consumption which is in various shapes and sizes without using harmful substances is possible using UV-LEDs.

Another advantage of UV-LEDs can be the possibility to compose a UV-LED array at different wavelengths in order to enhance the germicidal effect. Such a system will hold for the potential to design sterilization units which target a wide range of pathogens or the main pathogens specific for the product of concern (Chatterley and Linden, 2010).

CONCLUSION

UV-LED technology is a promising method for nonthermal processing of liquid foods since it has many advantages over conventional UV-C lamps. It is first essential to develop a static or bench top apparatus which is constructed by using UV-LEDs in order to compare the results of the studies in the literature performed by conventional UV-C lamps with those of the new system. However, it is known that power outputs of LEDs at the germicidal wavelengths (250-260 nm), where UV absoprtion of DNA reaches the maximum, are low. Despite the disinfectant property reduces with increasing wavelength, power output shows an increase. Therefore it is worth investigating whether an increase in the power output using UV-LEDs at higher wavelengths compensate for the reduction in the germicidal efficiency.

There are a few studies in the literature related to use of this new technology for disinfection purpose. The water disinfection is the only applied area among these limited studies. Hence, there is a gap in the literature in terms of disinfection of liquid foods. More needs to be done on this area.

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EFFECT OF PACKAGING TECHNOLOGY ONTEXTUREAND COLOUR OFA PORTUGUESE TRADITIONAL BLOOD SAUSAGE FROM *MONCHIQUE* REGION – *MORCELA DE ARROZ*

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ABSTRACT:

Texture and colour stability arevery important characteristics to delineate shelf lifeof meat products such as*Morcela de Arroz* (MA) - a traditional cooked blood and rice sausage from *Monchique,* South of Portugal. It is currently commercialized without packaging. The objective of this study was to evaluate the best packaging(AIR, vacuum (VAC) or MAP 80% CO_2:20% N_2) to extend the shelf life of MA. Samples were drawn from 3 manufacturing batches ina local industry. Samples were analysed in triplicate, before packaging (day 0) and after 8, 15, 22, 30 and 44 days of storage at 4ºC.Moisture was determinedusing a Mettler LP16-M infrared scale. Texture profile analysis was determined with a LFRA 1500 Texture Analyser with a load cell of 1.5 kg. Refrigerated samples wereleftfor 1 h at (20.0 ±0.1 ºC)before analysisto homogenise temperature. Samples were double cycle punctured (10 measures/sample) with a cylindrical steel probe (2.0 mm diameter; cross-speed of 1mms^{-1};depth of 18 mm). The parameters retained were: hardness and extensibility of the casing, consistency, adhesiveness, adhesive force, cohesiveness.Colour wasmeasured with a Dr Lange colorimeter (CIE $L^*a^*b^*$; D_{65}) with a 10 mm diameter window.10 measures were made at each sampling moment.Moisture was significantly reduced in the AIR samples from day 15 to the end of the storage period (44days). ConsequentlyL^* was significantly lower in the AIR samples comparatively to VAC and MAP from the 8th day of storage on. AIR had significantly lower ($p<0.05$) a^*b^* values during the most part of the storage

period. The texture parameters ofAIR weresignificantly different ($p<0.05$) during the most part of the storage period, since dryinghad a strong influence on these parameters. Comparatively toVAC, MAP revealed asignificantly better ($p<0.05$) extensibility of the casing and significantlylower ($p<0.05$) adhesiveness and cohesiveness. From these results, the MAP packaging proved to be the better choice for the Portuguese MA.

Keywords Blood sausage; traditional meat product; modified atmosphere packaging; vacuum packaging; textural parameters; colour

MONITORING THE EFFECT OF SOLIDS CONCENTRATION AND TEMPERATURE ON COAGULATION AND CUTTING TIME OF MILK GELS USING NIR SPECTROMETRY

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ABSTRACT:

The cheese cutting time depends on factors such as milk composition and gelation temperature and the accuracy of the prediction is a significant factor to improve final product quality and consistency. In this study a complete randomized factorial design with 3 replicates was used to determine the effect of solids concentration (10 and 15%) and temperature (30 and 40 ºC) on coagulation and cutting times of reconstituted skim milk gels. The coagulation process was simultaneously monitored using an inline light backscatter sensor operated at 880 nm, a small amplitude oscillatory rheometer and visually-derived coagulation indexes.The changes occurred in the protein structure during milk coagulation were correlated to the signal changes derived from the optical sensor and the light backscatter ratio increased as aggregation and gel assembly proceeded. The increase in temperature produced a significant ($P<0.05$) decrease in the optical time parameters (t_{max}, t_{2max} and t_{2min}), which wasattributed to changes in the rate of the casein hydrolysis and the aggregation of the casein micelles. The increase in solids concentration caused a significant ($P<0.05$) increase in the same optical parameters as a result of the saturation of the enzyme by the substrate. However, the increase of those optical milk coagulation indicators was correlated with a significant reduction in the rheological cutting time at 30 Pa. These results demonstrated that the parameters generated by the optical sensor can describe properly the milk coagulation process and the rheologically-determined cutting time at different temperatures and in presence of different solids concentration.

Keywords: milk coagulation, optic sensor, light backscatter, rheology, cutting time, solids concentration.

1. INTRODUCTION

In cheese manufacture cutting time (t_{cut}) selection depends on rheological and microstructural properties of gels, such as coagulum firmness and rearrangement capability that, in turn, depend on milk composition, milk pretreatment and coagulation factors,. For this reason, t_{cut} selection greatly affects moisture, yield, and quality of cheese and whey fat losses. In practice, gel is usually cut after a predetermined reaction time or upon the operator's judgment based on subjective evaluation of textural and visual gel properties, but the efficacy of that methods could be questionable (Castillo, 2006a). The lack of a rigorous t_{cut} characterization and the usual changes in milk protein content are forcing modern cheese plants to standardize the protein content of milk in an attempt to control coagulation, gel firming, curd syneresis, cheese yield, and product quality.

Due to the importance of real-time estimation of curd firming and prediction ofcutting time on the final cheese quality, numerous devices have been developed to study and control phenomena occurring during coagulation on plant and/or laboratory scale. O'Callaghan et al. (2002) reviewed the various systems developed to monitor coagulum formation and Castillo (2006a) did a comprehensive classification of the methods for the prediction of cutting time. At plant scale, an ideal monitoring system should evaluate curd firmness, as distinct from gelation, operate on-line in the process vat, be non-intrusive, non-destructive and compatible with hygiene requirements (Klandar et al., 2007). Changes of the optical properties of milk during coagulation have permitted the development of several devices, more particularly since fibre-optics became widespread. A fiber optic sensor technology used to measure light backscatter (CoAguLite®; Payne et al., 1990) has been demonstrated to be one of the most promising inline, nondestructive methods for monitoring milk coagulation and has become commercially available for inline monitoring of cheese production.Direct light backscatter measurements, such as those performed at 880 nm by the inline sensor CoAguLite, have the advantage that the measurements do not depend on a certain path length as compared to indirect light scatter measurements based in relative transmission (Optigraph). In that light backscatter (LB) device, light from a light-emitting diode (LED) is transferred to the milk through a fiber, and the light backscattered from the milk is transmitted through an adjacent fiber to an optical detector.The LB profile increases sigmoidallyas a consequence of milk coagulation and contains information about the gel assembly (aggregation of casein micelles and gel firming) (Fig. 1). The LB ratio profile has a latent period (Fig. 1 I) during which

enzymatic reactions predominate with no detectable change in backscatter intensity. The LB ratio increases as particle size increases during gel matrix formation due to casein micelle cross-linking. As a result, a sigmoidal period can be distinguished (Fig. 1 II) during which aggregation reactions predominate, as well as an asymptotic period (Fig. 1 III) during which cross-linking proceeds at an ever-decreasing rate while curd firming is developing(Payne and Castillo, 2007).

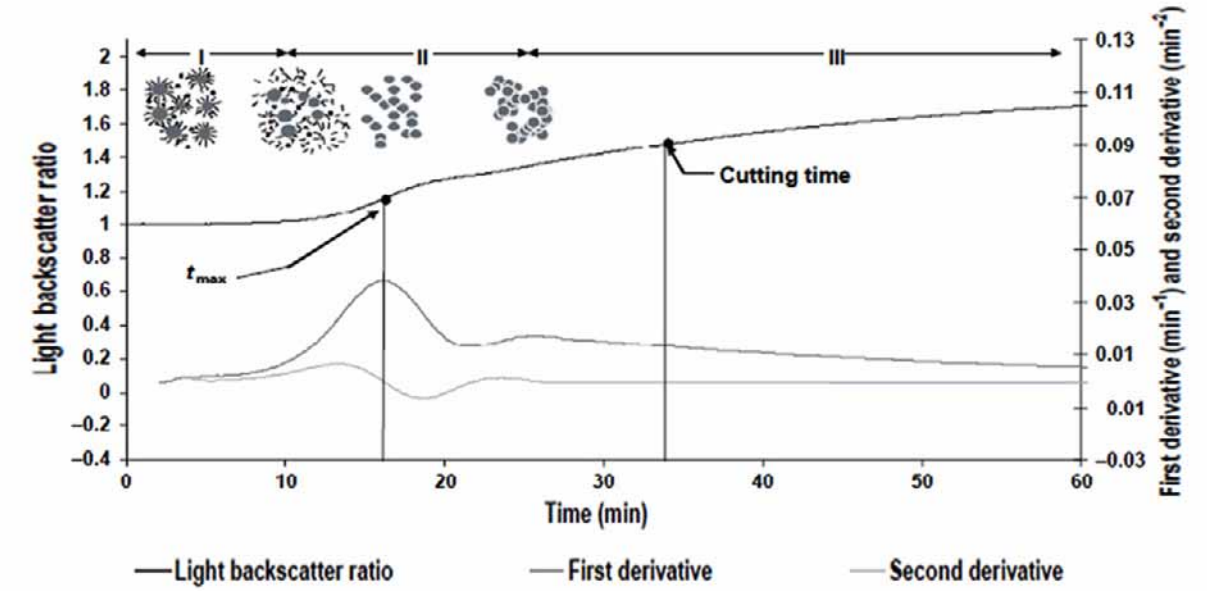

FIGURE 1: Light backscatter profile with first and second derivatives as function of time. (I) latent period, (II) sigmoidal period, (III) asymptotic period (Payne and Castillo, 2007). Cutting time and optical parameter t_{max} are defined in the text.

As a consequence of the sigmoidal shape of the LB ratio profile, a unique parameter is obtained: the time from enzyme addition to the inflection point of the LB ratio profile, t_{max} (Fig. 1). The light backscatter parameter$t_{max}$was strongly correlated (R^2 = 0.9913)to the Berridge clotting time (Castillo et al., 2000) and the following prediction equation was developed by Payne et al. (1993)to predict cutting time: $t_{cut} = \beta t_{max}$. The coefficient β is selected in the plant to replicate the cheese maker's judgment of cutting time. On the other hand, it was estimated that an average of 78% of κ-casein was hydrolysed at t_{max}, (Castillo et al., 2003a) and a strong correlation between the rheological gelation time(t_{gel}) and the light backscatter parametert_{2min}, was found, so it was considered that the parameter t_{2min} corresponded the beginning of the gel firming process (Castillo, Lucey and Payne, 2006).

The effect of protein and temperature on cutting time prediction ingoats' milk using an optical reflectance sensor was studied by Castillo et al. (2003b), but in that study the cutting time was evaluated by a subjective method which could affect the precision of the results. Nowadays, there are no studies simultaneously evaluating the effect of variation in solids concentration and temperature in both light backscatter parameters and the rheological parameters of milk gels, and therefore the objective of this work was monitoring the effect of solids concentration and temperature on coagulation and rheologically-derived cutting time of milk gels using NIRlight backscatter.

2. MATHERIALS AND METHODS

2.1 Experimental designand statisticalanalysis

A completely randomized factorial design with two factors: temperature (30, 40ºC) and solids concentration (10, 15%) was used to studythe effect of solids concentration and temperature on coagulation and cutting time of milk gels. The study was performed randomly and each treatment had three replications.All data was processed and analyzed using *"Statistical Analysis System"* (SAS version 9, 2004, SAS Institute Inc., Cary, NC, USA). Pearson correlation coefficients were determined with the *correlation* (*CORR*) procedure, while the analysis of variance (ANOVA) was performed using the *General linear model* (*GLM*) procedure. Least squares means (LSM) and significance of treatments were calculated using type IV sum of squares. LSM values were considered to be statistically different when $P < 0.05$. Linear regression of severaldifferent linear models were tested on collected data forpredicting gelation and cutting time, using the GLM procedure of SAS, with the independent variables in combination with dependent variables generated fromthe light backscatter profile.

2.2 Enzyme and calcium

Recombinant chymosin 100% (CHY-MAX extra; EC 3.4.23.4, isozyme B, 600 IMCU mL^{-1}) supplied by Chr. Hansen Inc. (Chr. Hansen Barcelona, Spain) was used to induce milk coagulation. The enzyme was stored refrigerated at 4°C and added at a concentration of 100 μL kg^{-1} milk at the beginning of the trials. A solution of 525 g anhydrous calcium chloride per liter was used ($CaCl_2 \cdot 2H_2O$; PanreacQuimica S.A., MontcadaiReixac, Barcelona, Spain) to enhance milk coagulation. An amount of 0.3 mL of the calcium chloride solution was added per liter of prepared milk.

2.3 Testing procedure

In order to reduce the inherent composition variability of fresh milk, low heat skim milk powder (Chr. Hansen, Barcelona, Spain) with ~34% protein was used. The corresponding amount of powder milk was dissolved in distilled water at a temperature of ~43°C for 30 min using a heating plate with continuous stirring. The average protein content of the reconstituted milk resulted in 3.6% ± 0.05 and 5.4%± 0.06protein for samples containing 10 and 15% solids, respectively.The reconstituted milk was tempered to the target coagulation temperature using a water bath. Once the milk reached the target temperature, the enzyme was added and stirred thoroughly with a spatula during 20 s. Immediately, two aliquots of ~80 mL were placed in the optical sensor vessels and another aliquot of ~40 mL was placed in the rheometer and the respective data acquisition software were launched.

2.4 Rheological parameters determination

The milk coagulation process was monitored through small amplitude oscillatory rheology (SAOR) by using a ThermoHaake rheometer RS1 (Thermo-Haake GmbH, Karlsruhe, Germany) equipped with a concentric-cylinders sensor (Z34). The tests were done by applying a deformation of 3%, which is within the region of linear viscoelasticity for rennet milk gels (Zoon et al. 1988) and a frequency of 1 Hz. The parameters identified were the elastic module or storage module (G'), the viscous module or loss module (G'') and tanδ(tanδ = G''/G'). Gelation time (t_{gel}) was defined as the time when the gels had a G' = 1 Pa.

2.5 Determination of NIR light backscatter parameters

The equipment used to determine the near infrared (NIR) light backscatter profile at 880 nm during milk coagulation was designed at the University of Kentucky (Fig. 2). A detailed description of it was presented in the work of Tabayehnejad et al. (2011). The device has two vats of 98 mL (Fig. 2a) capacity to monitor coagulation in two samples simultaneously and make accurate comparisons. Fig. 2b shows the setup of the complete laboratory measurement system. The circulating water between the water bath and the outside of the coagulation vats regulated the temperature of milk samples in the vats.

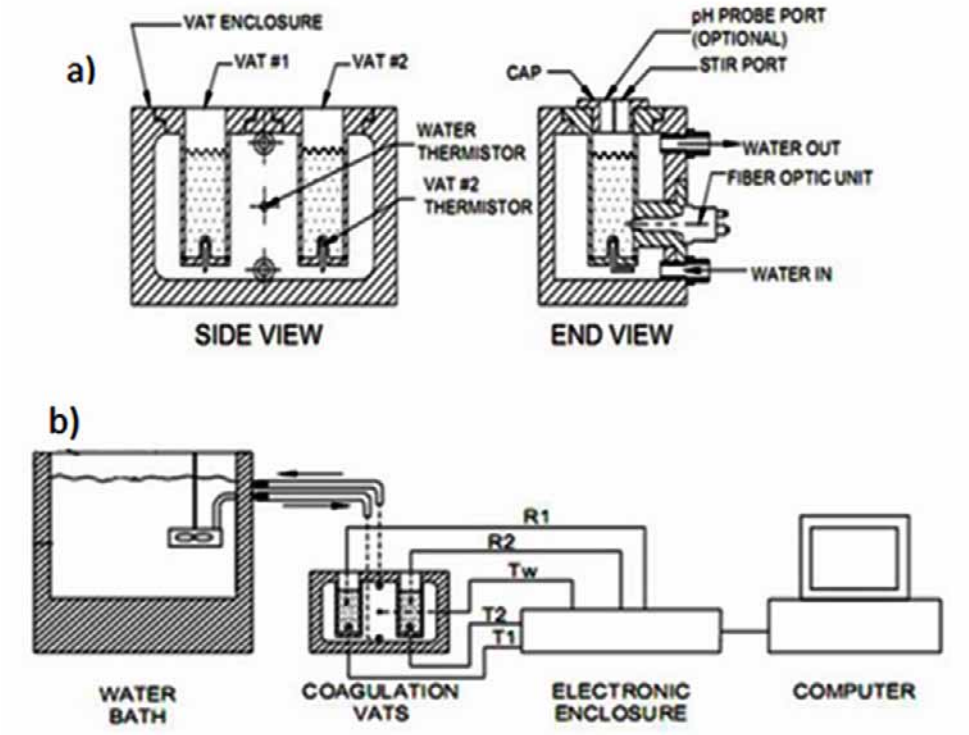

FIGURE 2: Schematic of the coagulation measurement apparatus used to measure near infrared light backscatter during milk coagulation (Tabayehnejad et al., 2011).

This sensor transmitted near infrared light at 880 nm through two 600 µm diameter fibers. One fiber transmitted infrared radiation into the milk sample while the other fiber transmitted the radiation scattered by the milk particles to a silicon photo-detector. For calibration, the optic sensor was zeroed by excluding light and adjusting the output voltage to 1 V. The initial voltage response (V_0) was calculated by averaging the first ten data points after correction for 1 V offset. The optical data generated in the vats were collected at intervals of 6 s. The light backscatter ratio (R) was calculated by dividing the voltage output from the detector by the average of the first ten voltage data points collected after the enzyme addition, according to the procedure described by Castillo et al. (2000).As described by Castillo et al. (2006b), an algorithm was used to calculate the first (R') and second (R'') derivatives of the light backscatter ratio profile andseveral optical time parameters were defined by the maxima and minima of the derivatives. The elapsed times from enzyme addition to the first maxima of the first and second derivatives were defined as $t_{max}$and t_{2max}, respectively, while the time to the first minimum of the second derivative was defined as t_{2min}. Onecomplementary optical parameter was defined as R_{max},which corresponded to the value of R at t_{max}.

2.6 Statistical analysis

In this study, the statistical analyses was performed using the statistical program SAS (SAS® 9.2.3., 2009). The analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of SAS, while Pearson correlation coefficients, r, were obtained by the correlation (CORR) procedure. Least-square means (LSMEANS) and significance of each treatment were computed using type IV sum of squares. The least square mean method predicts differences between all treatments. In this experiment the means were considered to be significantly different at $P<0.05$. To predict several dependent variables as t_{gel}, t_{cut30}, t_{cut60}, linear regressions (GLM procedure) and maximum R-square (Maximum R^2 procedure) were used. The combination of the independent variables (solids and temperature) and dependent variables from the light backscatter equipment make predicting possible.

3. RESULTS AN DISCUSSION

As it is shown in figure 3, the light backscatter ratio increased during coagulation and the rate of increase diminished after t_{max}, namely, at the beginning of the aggregation reaction.

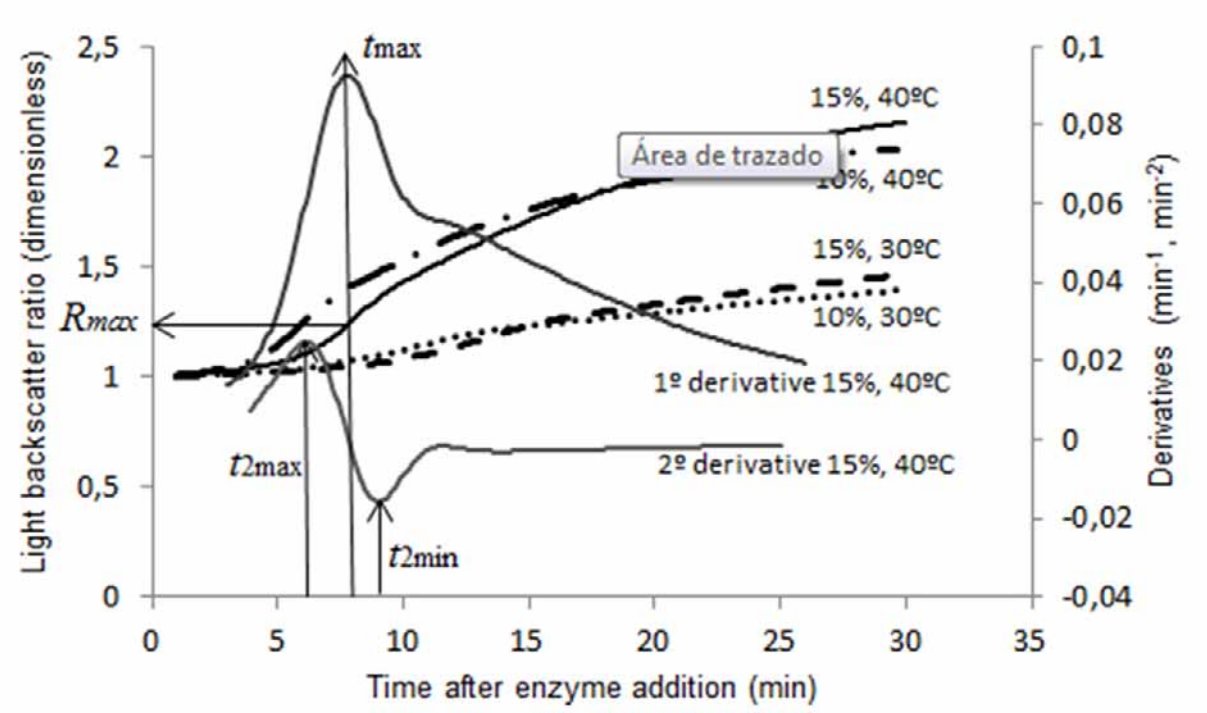

FIGURE 3: Effect of temperature and solids concentration on the light backscatter ratio profile. Optical time parametersexplained in the text.

The change in light backscatter varied with coagulation temperature and solids concentration, being greater the effect of temperature. An ANOVA was conducted to determine the main sources of variation in the dependent variables (Table 1).

TABLE 1. Analysis of variance and *F* statistics for dependent variables[a]

	Model		Main factors		Interaction
			S (%) (DF=1)	*T* (ºC) (DF=1)	*SxT* (DF=1)
Parameters	R^2	F	F	F	F
t_{max}	0.951	51.46^{c}	20.49^{b}	127.8^{c}	6.10^{a}
t_{2max}	0.931	35.93^{c}	23.79^{b}	78.70^{c}	5.29^{ns}
t_{2min}	0.957	59.00^{c}	13.78^{b}	159.3^{c}	3.88^{ns}
R_{max}	0.991	303.7^{c}	56.08^{c}	841.0^{c}	14.1^{b}
t_{gel}	0.969	82.94^{c}	3.490^{ns}	243.5^{c}	1.80^{ns}
t_{cut30}	0.990	268.0^{c}	188.6^{c}	527.2^{c}	88.3^{c}
t_{cut60}	0.992	345.5^{c}	319.5^{c}	574.0^{c}	143^{c}

[a]Number of observations *N* = 12; *T*, temperature; *S*, percentage of solids; S*xT*, solids x temperature interaction; R^2, determination coefficient; *F*, ANOVA *F*-statistic; DF, degree of freedom; $^{a}P<0.05$, $^{b}P<0.001$, $^{c}P<0.0001$; ^{ns}, not significant. Dependent variables explained in the text.

Highly significant temperature effects were observed (P < 0.0001)for all the evaluated dependent variables,while the effect of solids concentration was found to be significant for all variables except t_{gel}. The effect of the interaction *SxT* was significant on the variables t_{max}, t_{cut30} *and* t_{cut60}.

3.1 Effect of temperature on milk coagulation

The least square mean analysis is shown in table 2. The increase in the temperature produced a significant decrease (P < 0.05) in all light backscatter and rheological parameters. The increase in temperature from 30 to 40°C produced a decrease in the parameter t_{max} by ~36%; likewise the gelation time (t_{gel}) and the rheological cutting time at 30 Pa ($t_{G'30}$) were reduced by 45 and 60%, respectively. The temperature accelerates both the enzymatic hydrolysis and the aggregation reaction of milk coagulation (McMahon & Brown, 1984; van Hooydonk& van den Berg, 1988). Thus, the observed significant decrease in time parameters was attributed to the effect of temperature on both κ-casein hydrolysis and destabilized casein

micelles aggregation, although the effect of temperature on casein micelle aggregation is much more pronounced as compared to that on hydrolysis.

TABLE 2. Influence of main effects (solids concentration and temperature) on optical and rheological parameters[a,b]

	Solids concentration (%)		Temperature (°C)	
	LSM		LSM	
Parameter	10	15	30	40
t_{max}	9.63[a]	11.5[b]	12.8[a]	8.24[b]
t_{2max}	7.35[a]	9.22[b]	9.99[a]	6.58[b]
t_{2min}	11.6[a]	13.3[b]	15.3[a]	9.60[b]
R_{max}	1.19[a]	1.22[b]	1.15[a]	1.26[b]
t_{gel}	16.4[a]	15.3[a]	20.5[a]	11.3[b]
t_{cut30}	39.4[a]	23.2[b]	44.9[a]	17.7[b]
t_{cut60}	62.3[a]	28.7[b]	67.9[a]	23.0[b]

[a]Least squares means (LSM) with same letters are not significantly different ($P<0.05$); number of replications = 3; number of observations, N = 12. [b]Dependent variables explained in the text.

3.2 Effect of solids concentration on milk coagulation

The optical parameters increased significantly with increasing solids concentration. The results suggest that, at constant enzyme concentration, the saturation of the enzyme by the substrate is inducing a significant increase of optical time parameters (t_{max}, t_{2max}, t_{2min}) when the solid concentration increases (Castillo et al., 2003b). It was observed a significant decrease in the rheological cutting time at 30and 60 Pa (t_{cut30} and t_{cut60}) by ~41 and ~54% respectively, with the increase in solids concentration. It was probably due to the increase in the aggregation rate because of the increased number of collisions between the casein micelles. Several authors have found that the rate of curd firming increased with increasing protein concentration (van Hooydonk& van den Berg, 1988; Guinee et al. 1997).

Using the maximum R^2 procedure of SAS several predictive models were obtained: the rheological gelation time (t_{gel}) with a R^2 of 0.9964, the rheological cutting time at 30 (t_{cut30})with R^2 of 0.9951 and the rheological cutting time at 60 Pa (t_{cut60}) with R^2 of 0.9951.

4. CONCLUSIONS

Use of NIR spectrometry to measure changes in light backscatter was foundto be an accurate and objective method for monitoringthe progress of milk coagulation with changing levels of

total solids in milk. Parameters generated from the light backscatter profile were a function of coagulationrate, which varied withdifferent temperatures and in presence of different solids concentration.The rheologically-determined cutting time could be estimated properly based on the NIR sensor response.

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DEVELOPMENT OF NOVEL MICROPARTICLES FROM LIGNIN

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ABSTRACT

This research aims to produce novel microparticles from lignin, in response to sustainability and obesity concerns within the food chain. Sustainability concerns have seen growing interest in the development of alternative supplies of food ingredients from materials that are usually discarded as part of waste streams processing. Furthermore, current concerns surrounding obesity indicate an ongoing need for reduced energy density ingredient and food systems. As with currently utilisedmicroparticulated ingredient systems, lignin may have the ability to modify the rheological and textural properties of food systems and thus may have potential as a sustainable particulated filler material, compensating for changes to food microstructure when components such as sugar or fat are removed. In the current work, food grade solvent extraction and solvent evaporation techniques were explored for their ability to produce lignin particles with desirable properties for use in food systems: notably, a particle diameter of 0.1-40μm, spherical shape and stability against aggregation. These techniques involve precipitating a dispersed phase containing the lignin in a continuous phase and subsequent isolation of the dispersed phase solvent through removal, ultimately producing a suspension of hardened, precipitated microparticles. Various processing and composition parameters such as the temperature and concentration of surfactant were also investigated as a means of controlling particle properties. This presentation will aim to highlight the key results obtained and outline the recommended approaches to produce novel microparticles suitable for use in a range of food systems.

Keywords: microparticles, lignin, filler, solvent extraction, solventevaporation, surfactant

INTRODUCTION

Microparticulate ingredients have wide-ranging use in the food industry. For example, microcapsules, produced using technology derived from the formation of microparticles

(Freitas, Merkle, & Gander, 2005), are used for the controlled delivery of drugs into the body (Hurtado-Lopez & Murdan, 2005). By mimicking the lubrication properties of fat droplets in a dispersed phase, through their small size and homogeneity, microparticles have also been used as fat mimetics in emulsion based foods (Gómez, 2008; Setser & Racette, 1992; Singer, 1996). Furthermore, microparticles have been used as bulking agents, to replace solids in low-calorie and low-fat foods (Humphreys, 1996). Finally, microparticles, generally in the form of micro-rods, have been used as foam and emulsion stabilisers (Campbell, Stoyanov, & Paunov, 2009; Wege, Kim, Paunov, Zhong, & Velev, 2008).

Microparticles for the food industry must be prepared from materials with suitable functionality in food and mouth, safety, availability and cost. Size less than 80μm, softness and spherical shape are suggested to be critical features for particles added to foods (Tyle, 1993). Microparticles used as fat mimetics are insoluble. However, with diameters of 0.1-3 μm, the particles are not detected individually, but rather as a smooth, creamy, fluid product that coats the mouth in a similar way to fat. In this way, fat droplets in emulsion-based foods can be replaced by equivalent phase volumes of microparticle fat mimetics. For example, Simplesse®, a fat mimetic made from microparticulated gelled whey protein, mimicks the creaminess of fat in foods such as ice cream, frozen desserts, cheese, dressings, sauces, soups and baked goods (Gómez, 2008; Setser & Racette, 1992; Singer, 1996).

There is great potential for lignin to be used for the preparation of microparticulate ingredients for the food industry. Lignin, an aromatic heteropolymer found in the woody stems of hardwoods and softwoods and in all types of vascular plants (Sarkanen & Ludwig, 1971; Wool, 2005) is water insoluble and also relatively hydrophobic(Lora & Glasser, 2002). Thus microparticulation has been identified as an appropriate means by which refined lignin can be incorporated into food systems. Importantly, lignin is non-caloric and since it is consumed naturally as part of some plants, it is also assumed to be non-toxic. Furthermore, lignin is a high purity byproductof increasingly popularlignocellulosicbiorefining and is therefore likely to be in both abundant and relatively cheap supply in the future. Thus we suggest lignin could be well utilised as a food ingredient, on the basis that a form suitable for incorporation into foods can be produced.

A technique based on antisolvent precipitation presents an appropriate route for the preparation of lignin microparticles. In this technique, an organic solution containing the polymer of interest is added to an aqueous solution under shear. Through removal of the organic solvent – by either evaporation or extraction – polymer precipitation occurs and

hardened microparticles are produced. For example, Patel, Bouwens&Velikov(2010), Zhong& Jin (2009) and Parris, Cooke & Hicks (2005) sheared ethanol solutions containing dissolved zein into water, and then removed the ethanol to produce zein micro or nanoparticles. We suggest that the solubility and hydrophobicity properties of zein may be similar to those of lignin and therefore that methods used to prepare zein microparticles may be useful for the preparation of lignin microparticles. Finally, numerous parameters can be altered when using the antisolvent precipitation method. These include mixing type and speed, polymer concentration, volume ratio of the dispersed and continuous phases, temperature, composition of the phases, the rate of solvent removal and the use of emulsifiers and stabilisers.

Temperature may influence the rate of evaporation of the solvent during microparticle formation. Yang, Chia & Chung (2000) and Yang, Chung, Bai& Chan (2000) suggest that temperatures close to or above the boiling point of the solvent increases the rate of solvent evaporation from the surface of the polymer dispersion during the preparation of PLGA microparticles. The faster evaporation reduces the shearing of the dispersion that can occur before precipitation of solid microparticles, producing larger microparticles with larger particle size distributions. Stark & Gross (1994) suggest that smaller microparticles are formed if higher temperatures are used during the precipitation process. However, the maximum temperature is limited by the boiling point of the system and also by the solution properties of the polymer. In fact, Asrar& Ding (2006) recommended that the temperature should remain at least 20°C below the normal boiling point of the organic solvent. This indicates that higher temperatures during shearing may reduce particle size, but only if the temperature is below the boiling point of the system.

The use of emulsifiers and stabilisers during microparticle manufacture influences particle size and stability. Emulsifiers may enable the size of dispersed phase droplets to be better controlled (Asrar & Ding, 2006), while stabilisers can prevent aggregation due to particle interactions or drying (Patel *et al.*, 2010). Asrar& Ding (2006) found cellulose derivatives, particularly methylcellulose to be useful emulsifiers for the preparation of lignin microparticles. Sansdrap&Moes(1993) used hydroxypropyl methylcellulose to reduce the size of nifedipine microspheres. Finally, Stark & Gross (1994) suggest thatcarboxymethyl cellulose, gum Arabic, sodium caseinate, lecithin, DATEM-esters, polysorbates and sodium dodecyl sulfate (SDS) can all be used to effectively stabilise zein microparticles. Thus there

are a variety of emulsifiers and stabilisers that may be useful for the production of lignin microparticles.

The main focus of this study is to examine the influence of process and compositional parameters on the size, shape and size distribution of lignin microparticles. These parameters include the temperature of the aqueous phase during shearing and the addition of surfactant.

MATERIALS AND METHODS

Materials

Lignin was extracted from chipped *Salix purpurea* and used in the form of extraction liquor containing 4.68 ± 0.04% solids.

Sodium dodecyl sulfate (SDS) (BDH, VWR International Ltd.) was used as received.

Methods

Lignin extraction

Lignin was extracted from chipped *S. purpurea* by heating the feedstock with 60% aqueous ethanol to 195°C in a pressurised vessel and holding the mixture at this temperature for 132 minutes. The extraction liquor containing the lignin was recovered by filtering the mixture through glass wool to remove the wood chips. The extraction liquor was then stored at 5°C until use.

Microparticle preparation

Microparticles were prepared using a method based on antisolvent precipitation (Patel *et al.*, 2010). To study the influence of preparation conditions on morphology and size distribution, 30 mL samples of lignin extraction liquor (20 °C) were added to 75 mL distilled water (with and without SDS)(4-60°C), under continuous stirring (875 rpm) using a magnetic stirrer. The resulting dispersions were stirred at 875 rpm for the first hour, followed by 21 hours of stirring at 500 rpm to enable slow evaporation of ethanol from the sample. The resulting suspensions were immediately analysed for particle size. To obtain dry powder samples, the dispersions were concentrated by centrifugation at 30,000 rpm for 1 hour (Sorvall WX Ultra 100 Centrifuge, Thermo Scientific, NZ), air-dried at 30°C overnight and ground to powder using a mortar and pestle.

Particle size analysis

Particle size analysis was carried out using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK). A refractive index of 1.6 (Donaldson, 1985) and absorption of 0.01 (based on

analysis of fit of the light scattering data) were used by the instrument to calculate the particle size distributions.

The particles with diameter smaller than 1 μm were also tested using a ZetasizerNano ZS (Malvern Instruments Ltd., UK) to further distinguish between smaller particles.

Scanning electron microscopy

The morphology of dried lignin microparticles was characterised using SEM (FEI Quanta 200). The dried samples were sputter coated with a thin layer of gold and observed microscopically.

RESULTS AND DISCUSSION

Effect of temperature

To determine the effect of temperature on microparticle size and shape, the distilled water aqueous phase was cooled or heated to 4, 20, 40 or 60°C immediately prior to the addition of the organic phase, which was always added at a temperature of 20°C. In Figure 1, the size distribution of the microparticles as a function of aqueous phase temperature is shown.

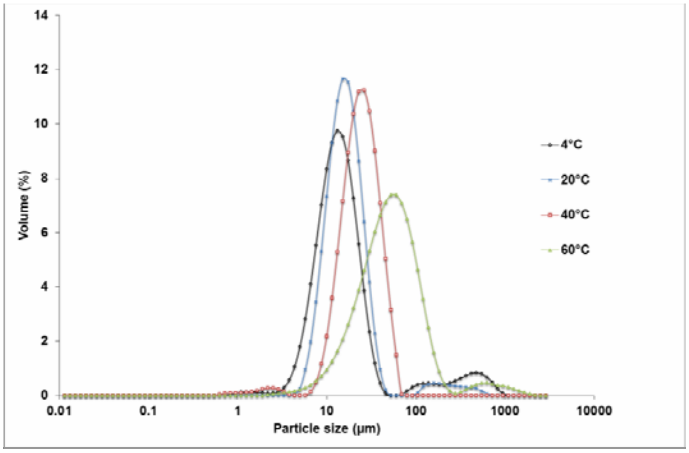

Figure 1. Particle size distributions of microparticle suspensions prepared using aqueous phase temperatures of 4, 20, 40 and 60°C.

Microparticles prepared at all four temperatures exhibit a major peak in particle size between approximately 10-100 μm. With the exception of microparticles prepared at 40°C, at most temperatures there is also a smaller volume of particles in the 100-1000 μm size range. As the aqueous phase temperature increases, the peaks seem to shift to the right, indicating an increase in particle size with temperature.

The increase in particle size with increasing aqueous phase temperature was also illustrated by differences in colour of the microparticulate suspensions. While all suspensions were brown,

the suspension of particles prepared at 4°C had a lighter brown and more milky appearance than the other suspensions, which became increasingly darker and more watery with increasing temperature. The presence of visible particulate material in the suspensions also increased at higher temperatures. Lighter colour and milkiness indicate the existence of smaller particles, since if concentration is constant between samples, smaller particles tend to scatter light more than larger particles (Swaisgood, 2008). Irrespective of appearance, in all samples, rapid sedimentation of the particles occurred due to the large particle size and lack of stability.

Figure 2 shows scanning electron microscopy (SEM) images of the microparticles prepared at 4, 20, 40 and 60°C. The images show that while microparticles prepared at 4°C are quite distinct particles that are small and close to spherical, the particles become increasingly less spherical, larger and less distinct with increasing temperature. At 60°C, the image shows large continuous masses of material.

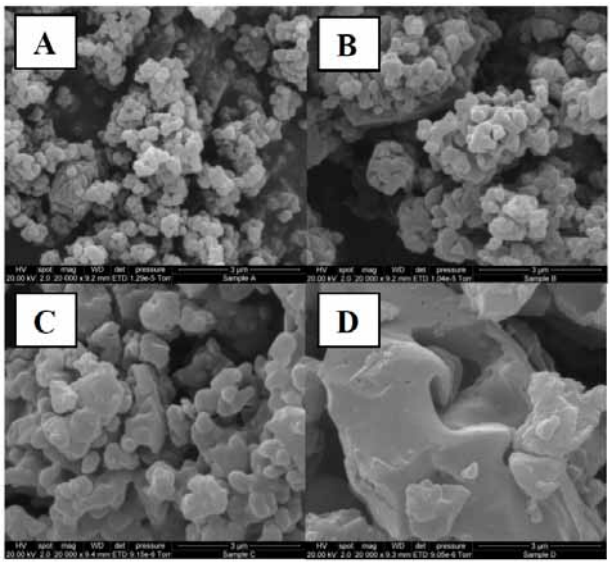

Figure 2. SEM images of the dried, crushed lignin microparticles prepared by shearing 30 mL of lignin extraction liquor into 75 mL aqueous solutions at temperatures of (A) 4°C, (B) 20°C, (C) 40°C and (D) 60°C.

The morphological changes could be the result of aggregation with at higher temperatures. Alternatively, the changes may occur in response to the thermal behaviour of lignin, such as an increase in lignin fluidity with temperature. Higher fluidity may cause lignin to coalesce much easier and thus form larger particles. However, this would need to be investigated further by studying the thermal behaviour of the lignin used in the current work.

Effect of surfactant

To determine the effect of surfactant addition on the morphology of lignin microparticles, aqueous phases containing 0-1% (w/v) of the small molecule anionic surfactant SDS were used. The size distributions of the prepared microparticles are presented in Figure 3. A decrease in particle size upon SDS addition to the aqueous phase is clearly shown by the leftward shift of the peak. Microparticles prepared using SDS concentrations of 0.0625-0.75% SDS exhibit a major, narrow peak in particle size at around 0.1 μm. This indicates that there is very little advantage in using any higher concentrations of SDS. In fact, for microparticles prepared at an SDS concentration of 1%, there is a small shoulder of particles in the 1-100 μm size range. This indicates that using too much SDS can cause an increase in particle size. It is not immediately apparent why this increase in size occurs.

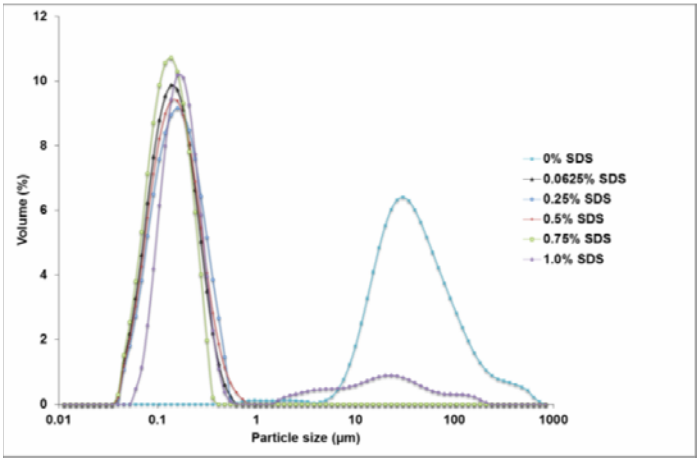

Figure 3. Particle size distributions of microparticle suspensions prepared using SDS concentrations of 0, 0.0625, 0.25, 0.5, 0.75 and 1% SDS at 60°C

The SEM images in Figure 4 largely confirm the small impact of changes in SDS concentration on particle size, since all particles seem to be of similar size and shape. Even the microparticles prepared using an SDS concentration of 1% (D) appear to be very similar to those produced using 0.25 and 0.75% SDS. Thus the larger particle size for these microparticles may be due to aggregation in suspension.

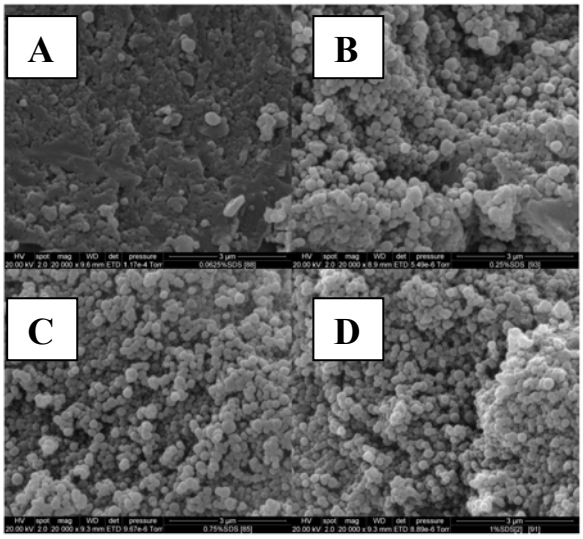

Figure 4. SEM images of dried, crushed lignin microparticles prepared by shearing 30 mL lignin extraction liquor into 75 mL aqueous solutions of (A) 0.0625%, (B) 0.25%, (C) 0.75% and (D) 1% SDS at aqueous phase temperatures of 60°C

Microparticles prepared using an SDS concentration of 0.0625%, however, appear to be less distinct and spherical than particles prepared using 0.25-1% SDS. It is possible that at 0.0625% SDS, there is an insufficient concentration of surfactant to provide effective surface coverage and thus stabilisation during drying, hence the small size measured in suspension yet more fused appearance once dried. Table 1 presents surface charge measurements for microparticles prepared using various concentrations of SDS. As the SDS concentration increases, surface charge increases, indicating that SDS provides stabilisation by coating the particle surface. For concentrations of 0.5 and 0.75% SDS, however, the surface charges were very similar. This suggests that at these higher concentrations, the particles are fully coated with SDS and thus more stable to drying, whereas at lower concentrations the particles are not fully coated. Therefore, it is recommended that an SDS concentration of 0.25-0.75% is used for the preparation of lignin microparticles.

TABLE1. Surface charges of microparticles prepared using various concentrations of SDS

Sample details	Zeta-potential (mV)
0% SDS, aqueous phase at 4°C	-5.1
0.0625% SDS, aqueous phase at 60°C	-38.4
0.5% SDs, aqueous phase at 60°C	-56.7
0.75% SDS, aqueous phase at 60°C	-57.8

CONCLUSION

In this study, lignin microparticles were manufactured using a method based on antisolvent precipitation. An organic solution of lignin, obtained by aqueous ethanol extraction from shrub willow, was added to distilled water under simple stirring. Slow removal of ethanol by evaporation allowed the lignin to precipitate into hardened microparticles.

Results show that the temperature of the water phase influences particle morphology, with lower temperatures producing smaller particles that are more distinct and spherical. Increases in aggregation or lignin fluidity with temperature are two mechanisms recommended for further investigation with respect to these results.

Addition of the small molecule surfactant SDS to the aqueous phase dramatically reduced particle size at an aqueous phase temperature of 60°C. SDS concentrations of 0.25-0.75% (w/v) are recommended for the production of lignin microparticles using the current method.

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INFLUENCE OF TECHNOLOGICAL OPERATIONS IN THE DISSOLVED OXYGEN CONTENT OF WINES

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ABSTRACT:

Oxygen plays a crucial role in oenology. The oxygen leads to changes in the chemical and sensory profile of wines. On one hand, the impact of moderate oxygen exposure of red wine has a positive effect in its colour, aromatic bouquet and mouth-feel properties. On the other hand, oxygen has a negative effect on white wine's quality, as well as the sensory and compositional levels.

The purpose of this study was to quantify the dissolved oxygen in red and white wine, during different cellar operations such as racking, tartaric stabilization, filtering and bottling. The techniques that contribute most to the enrichment of oxygen in wine are: cold tartaric stabilization (2.29 mg/l, white wines), bottling (1.38 mg/L red wines and 1.11 mg/L white wines) and bag-in-box filling (2.47 mg/L red wines; 2.22 to white wines).

Having identified the most critical technical operations in winemaking, preventive measures will be taken to reduce the dissolved oxygen content of wines, and thus prevent its depreciation.

Keywords: wine, oxygen, oxidation, dissolution, technological operations

1. INTRODUCTION

Oxygen exists as a gaseous element representing 20.9% of earth atmosphere, under standard temperature and pressure, and it is indispensable for the majority of the living organisms.

The oxygen plays a crucial role in enology. Louis Pasteur (1873) had already noted that oxygen could be a great enemy of wine or wine making.

Oxygen can dissolve in wine at several stages during winemaking, bottling and aging. It plays a beneficial role during the winemaking process: in promoting yeast multiplication, in the maturation and stabilization of wine colour, in the removal of the reduced compounds taste, and attenuation of the vegetable character, etc. However, when the wine is exposed to excessive amounts of oxygen, it can have negative effects such as the promotion of the growth of unfavourable microorganisms in wine (acetic bacteria, *Brettanomyces*), the degradation of colour and the production of organoleptic defects, etc.

The oxidation in white wine causes important sensory alterations in the colour and in the aroma. The browning is characterized by progressive darkening which can be globally characterized by absorbance at 420 nm (Singleton, 1987). The oxygen induces a loss of freshness and fruitiness, and develops an unpleasant oxidized character, which is generally described as non-desirable flavours of “honey-like”, “boiled-potato”, “cooked vegetable”, “farm-feed” and “woody-like” (Escudero et al., 2002; Silva Ferreira et al. 2002, 2003; Lopes et al; 2009, Karbowiak et al., 2010).

Phenolics compounds are the main oxidation substracts. Various researchers have studied the impact of oxygen exposure on the polyphenolic composition of red wines (Atanasova et al. 2002, Toit et al. 2006; Wirth et al, 2010). Some controlled and continuous O_2 is introduced into red wine (micro-oxygenation) to improve wine quality, such as: colour stability and intensity, softening of astringent tannins and decreases reductive and vegetative aromas.

During winemaking, the different unit operations may integrate some oxygen from the atmosphere into the wine. The operations such as pumping, transport, racking, centrifugation, stabilization, filtration and bottling have been studied by various authors (Vidal *et al.*, 2001, 2003, 2004a, b; Castellari et al. 2004; Valade et al. 2006, 2007).

Various researchers have monitored the dissolved oxygen of wine at bottling and during storage and the influence of packaging in wine conservation (Escudero, et al. 2002; Vidal et al.2004a, 2004b, Vidal & Moutounet, 2006; Valade et al. 2007, Lopes et al. 2009; Wirth et al. 2010; Ghidossi et al. 2012).

The Esporão SA is one of the most important wineries in Portugal. The purpose of this study was to quantify the dissolved oxygen in red and white wine, during different technical operations such as racking, tartaric stabilization, filtering and bottling, to identify the critical points (the unit operations) that contribute most to the integration of oxygen in wine and to establish preventive and corrective measures.

2. MATERIAL AND METHODS

2.1 Wines

The white or red wines analyzed were produced during 2010 vintage in Esporão S.A. (Alentejo) from different grapes varieties (Arinto, Antão Vaz, Perrum; Moreto, Trincadeira e Castelão, Touriga Nacional, Cabernet Sauvignon, among other). Each grape variety fermented individually, followed by the usual winemaking processes. Later, the wines were mixed to achieve the most interesting blend. All studied wines were obtained by blending the wines obtained from individual varieties.

2.2 Wine technical operation

Red and white wines were submitted to specific technical operation (table 1) with systems, equipment and procedures commonly used in winery. The increase of dissolved oxygen (O_{diss}) was calculated as the difference between the oxygen concentration measured before (O_{bef}) and immediately after (O_{aft}) each technical operation [$O_{diss} = O_{aft} - O_{bef}$].

Table 1 – Description of oenological treatments

Red wine	n	**White wine**	n
Racking (bottom-bottom)	113	Protein stabilization (bentonite addition)	5
Tartaric stabilization (metatartaric acid addition)	12	Continuous tartaric stabilization (cooling method)	12
Tangential filtration	46	Filtration (membrane cartridges, nominal porosity 1-0,45 μm)	29
Bottling (glass bottles)	168	Bottling (glass bottles)	28
Packaging (bag-in-box, 5L)	26	Packaging (bag-in-box, 5L)	9

n = number of analysed wines

2.3 Measurement of dissolved oxygen

The measurement of dissolved oxygen was carried out before and immediately after the technical operation using a portable oxímeter Crison OXI 45 P.

2.4 Statistical procedures

i) Data of the increase of dissolved oxygen is expressed as the average and standard deviation of measurements performed for the different wines.

ii) The influence of technical operations was analyzed by non-parametric procedures by the paired-samples T-test (before and after operation), with the Wilcoxon signed ranks test, using the SPSS Statistics software (version 17.0).

3. RESULTS AND DISCUSSION

The level of dissolved oxygen at a given time in the wine depends on the dissolution and consumption kinetics. When applied to a wine movement (pumping, filling, stirring) the kinetics of oxygen dissolution is higher than the consumption by the wine´s compounds. Dissolved oxygen reacts with the constituents of the wine and disappears gradually, reaching very low levels.

After racking, the evolution of dissolved oxygen consumption in the wine was monitored over time (Figure 1). It was found that the kinetics of consumption varied depending on the type of wine, having been noted that the white wine kinetics were slower than the red wines kinetics. Red wines consume oxygen faster than white wines because of its constitution of phenolic compounds. In the case of red wines, in just four days phenolic compounds consumed more than half of dissolved oxygen. For white wines, it took more than thirty days. Increasing temperature increases the kinetics of dissolved oxygen consumption.

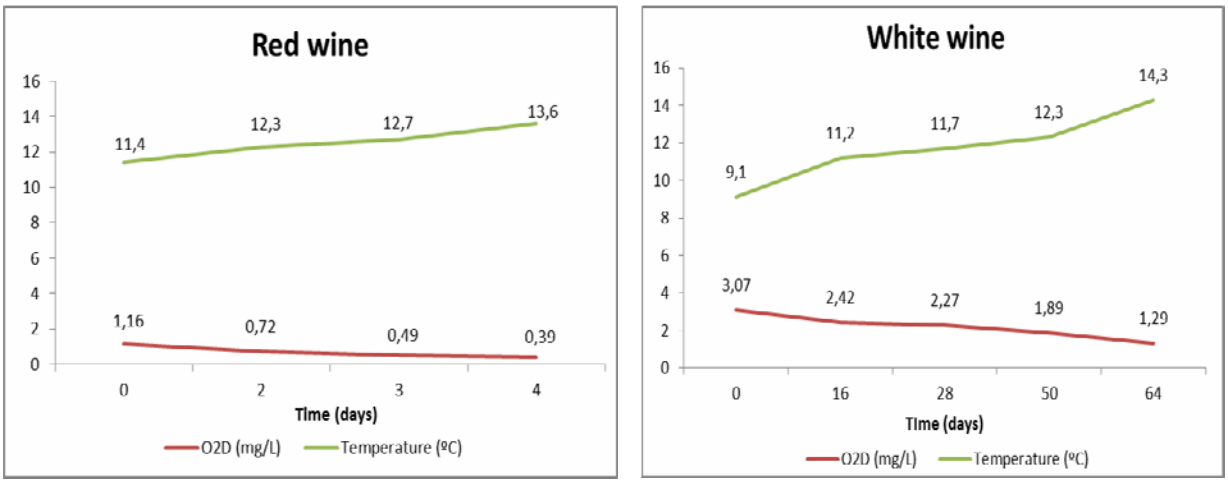

Figure 1 – Evolution of dissolved O_2 (O_2D) in wine after racking: a) red wine b) white wine

The monitoring of oxygen concentrations before and after specific technical operations provided data on the levels of enrichment of the winery and its variability.

Statistical analysis performed using nonparametric paired samples revealed that the enrichment in dissolved oxygen is significant for all operations performed (table 2).

Table 2 – The average content of dissolved oxygen (mg/L), before and after each technical operation, p-value of Wilcoxon signed ranks test

	Technical Operation	**Before technical operation (mean ± SD)**	**After technical operation (mean ± SD)**	**Increase of O_2D (mean ± SD)**	**p-value** (Wilcoxon test)
Red wine	Racking	0.19 ± 0.12	0.65 ± 0.34	0.46±0,31	<0.001
	Tartaric stabilization	0.28 ± 0.25	0.38 ± 0.26	0.10±0,06	0.002
	Tangential filtration	0.14 ± 0.09	0.49 ± 0.33	0.35±0.35	<0.001
	Bottling (glass bottles)	0.49 ± 0.42	1.87 ± 0.93	1.38±0.75	<0.001
	Packaging (bag-in-box)	0,98 ± 0.28	3.45 ± 0,86	2.47±0.93	<0.001
White wine	Protein stabilization	1.22 ± 0.85	1.35 ± 0.89	0.13±0.05	<0.05
	Cold tartaric stabilization	0.60 ± 0.37	2.89 ± 1.80	2.29±1.78	<0.01
	Filtration (membrane cartridges)	0.20 ± 0.17	0.71 ± 0.16	0.51±0.18	<0.001
	Bottling (glass bottles)	0.67 ± 0.24	1.78 ± 0.42	1.11±0.47	<0.001
	Packaging (bag-in-box)	0.65 ± 0.22	2.87 ± 0.96	2.22±0.77	<0.01

Increase of O_2D = Difference [$O_{\text{after operation}}$- $O_{\text{before operation}}$]

Racking increased the dissolved oxygen content by on average of 0.46 mg / L, exceeding that observed by Vidal et al. (2001) and Castellari et al. (2004). The oxygen enrichment in the racking occurs essentially at the beginning and end of the transfer, due to the dissolution of air contained in the pipes and tanks. The racking should be performed bottom to bottom of the tanks, and it uses inert gas in the pipes and tanks before starting the transfer process (Vidal et al. 2004a, Valade at al. 2006). For the tartaric stabilization of red wine, metatartaric acid was added just prior to bottling. The homogenization of the wine was achieved with nitrogen. The addition of the metatartic acid lead to an increase of 0.10 mg/L of dissolved oxygen, which is a reduced amount when compared to the cold tartaric stabilization. The important enrichment

that occurred during cold tartaric stabilization of the white wines, was on average 2.29 mg/L (table 2), and these results are consistent with data found by other authors (Vidal et al. 2003, 2004a; Castellari et al. 2004). The sharp temperature drop caused an increase in the oxygen dissolution rate, therefore it is important to protect the wine from the oxygen in the operation.

The protein stabilization of white wine by the addition of bentonite showed a small enrichment in dissolved oxygen. It was noted that this operation did not contribute a lot to the increase of dissolved oxygen in the wine; similar to what was observed for the tangential filtration of red wines, in which the dissolved oxygen was lower than that reported by Vidal et al. (2001, 2004a). The filtration by membrane cartridges increased the dissolved oxygen content mainly due to air inlets in the circuit, in particular through the pipe connections, the pump and filter body. The dissolved oxygen in wine is higher than that reported in literature (Vidal et al. 2001, 2004a; and Castellari et al. 2004), these authors noted average values of 0.10 mg/L and 0.06 mg/L respectively. This is due to the length (200 m) and the diameter of the tubing as well as the turbulent flow, which influences the kinetics of oxygen dissolution.

Unlike the results of Vidal et al. (2004a, b) filling in bag-in-box (BIB) has contributed to a greater enrichment in dissolved oxygen than bottling. This is due to a problem with an inertized filling machine used to fill BIB containers. To reduce the risk of wine oxidation in BIB, the concentration of SO_2 was increased up to a maximum of 50 mg/L, (one mg/L oxygen oxidizes 4 mg/L SO_2), so that the SO_2 protects the wine from oxidation and browning (Ghidossi et al, 2012).

Bottles were filled using a filling machine inertized with nitrogen. However, this operation was responsible for some increase in dissolved oxygen content, both in white wine and in red wine. These results are in agreement with those previously described (Vidal et al. 2004a,b; Vidal and Moutounet 2006). The filling is the main critical point for wine enrichment in oxygen.

4. CONCLUSION

The rationalization of the operating conditions and the reasonable use of neutral gases, especially at the beginning and the end of operation, are necessary to control and decrease significantly the oxygen pick up in all the operations.

The most important enrichments occur especially during the operations of cold tartaric stabilization and during the bottling and packaging, specially if no protection is taken to protect the wine from the oxygen in the air.

Controlling the oxygen ingress during filling (bottle or BIB) is an important way of improving the quality and the shelf-life of wine.

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USE OF VEGETABLE WASTE TO DEVELOP INNOVATIVE BIODEGRADABLE PACKAGING**

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ABSTRACT

The use of agriculture-derived biopolymers appears as an interesting alternative to synthetic plastics for food packaging. Extrusion-cooking is playing an important role in processing biodegradable packaging material, thus it could result particularly suitable to process complex matrixes such as vegetable solid wastes added with cereal flours, for the development of new packaging materials.

The aim of this research was to study an innovative biodegradable packaging using vegetable waste coming from asparagus processing, properly treated.

Results showed that the optimal drying conditions of asparagus wastes were reached at 70°C, since the obtained dried material becomes brittle (breaking strength < 5 N/cm^2) and, after milling process, a powder with a granulometry suitable to subsequently extrusion-cooking processing (<200 microns) was obtained. The operating conditions of extrusion-cooking processing to get a material with plastic flow and good texture involved a barrel temperature less than 100 °C; low screw speed (< 200 rpm) and high liquid feed content (~ 60 %).

Keywords: asparagus waste; biodegradable packaging; extrusion-cooking; cereal flour; texture; drying; milling.

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1. INTRODUCTION

The wide use of petroleum-derived plastics and their negative impact on the environment prompted the research for biodegradable materials obtained from renewable resources (Denavi, 2009). The use of agriculture-derived biopolymers, such as proteins and polysaccharides, appears as an interesting alternative to synthetic plastics for some applications, especially those with a short life-time, such as food packaging, and generates new uses of higher added value for agriculture products and by-products. Extrusion plays an important role in processing biodegradable packaging material (Fang and Hanna, 2001). Wheat and rice straws - widely available in many parts of the world - can be combined with other materials and processed by extrusion into raw materials for industrial uses (Averousa and Boquillon, 2004). Extrusion is a process which combines several unit operations including mixing, cooking, kneading, shearing, shaping and forming and results particularly suitable to process complex matrixes such as vegetable solid wastes added with starch, for the development of new packaging materials that could result as an interesting alternative to petroleum-derived plastics.
The aim of this research was to study an innovative biodegradable packaging using vegetable waste coming from asparagus processing.

2. MATERIAL AND METHODS

2.1 Drying of asparagus wastes

Asparagus wastes (thickness and width 1 cm and length 3 cm) are dried using a climatic room Binder mod. KBF 240 (Tuttlingen, Germany) using the following process parameters: 60; 70 and 80°C (drying temperature); dryer air flow 100 m^3/h; drying time 25-40 hr. For each drying cycle the temperature and relative humidity (80% RH) were kept constant until cooling started; then they were lowered to 30°C and 60% RH, respectively. Experimental drying curves were built taking samples at regular intervals and submitted to moisture analysis. The moisture content was determined placing samples in an oven (ISCO mod. NSV 9090, Italy) at 105°C until constant weight. The dried wastes were grinded for 8 min at 200 rpm through a laboratory mill (GM 200 Grindonmix Retsch Haan, Germania). Granulometry analysis was carried out sifting 100 g waste powder by 5 sieves (Giuliani Tecnologie, Torino, Italy) with

mesh of 500, 355, 250, 180 and 100 microns. The traced granulometry curves reported thickness of grains in abscissa and percentage of product, *i.e.* the materials that passed through each sieve, in ordinate.

2.3 Extrusion experiments

For extrusion experiments was used a formula containing 50% cereal flour and 50% asparagus waste. The extrusion experiments were carried out using a co-rotating twin-screw extruder Clextral model BC21 (Ferminy, France). The screw geometrical features were the following: length 900 mm and diameter 25 mm (L/D = 36:1) and distance between shafts 21 mm. Material feed rate was kept constant at 9.1 kg/hr (dry weight) and proportioned by a volumetric gravity feeder. For all experiments, the first seven zones of extruder were kept at 20; 25; 30; 35; 45; 50 and 60°C respectively, whereas the last two zones were adjusted at the temperature reported in the experimental plan (Table 1). The liquid fraction (water and plasticizing agent, ratio 1:1) was pumped by volumetric pump (Watson Marlow Digital 5055, UK) to the first zone of extruder and the liquid feed content was 2.8; 3.7 and 4.6 L/h. According to the equation (1) the specific mechanic energy (SME) was estimated:

$$SME = \frac{speed\ of\ \ screw\ \ (run)}{speed\ of\ \ screw\ \ (rate)} \times \frac{motor\ torque\ \ (run)}{100} \times \frac{motor\ power\ \ (rated)}{\mathrm{Pr}oduction\ \ capacity} \quad (1)$$

The used die had a spherical shape (diameter 100 mm) with a rectangular hole (width of 3 cm and height of 0.3 cm). At the exit of the die, the extrudates were manually cut into strips (10 cm length, 3 cm width and 0.3 cm thickness) using a knife. The extrudates were dried over a night at 40°C in a vacuum oven (Termaks, TS-8265, Norway). All extrusion experiments were performed at least in triplicate. According to Central Composite Design (CCD) (Box, Hunter and Hunter, 1978), a factorial design of three variables (temperature profile, screw speed and liquid feed content) and three levels of values, was used. Eleven tests for each type of extruded matrix (Table 2) with different combinations of process variable values were obtained (Box, Hunter and Hunter, 1978).

2.4 Analyses

Water activity was evaluated at 25 °C by an AquaLab CX-2 instrument (Decagon Devices, Inc.). Cohesiveness of fresh extrudates is determined using an Instron mod. 3343 (Instron

Ltd., High Wycombe, UK). Each sample was cut to cylinder shape (1 cm height and 2.5 cm diameter) and submitted to double uniaxially compression of 30% of sample height (3 mm from contact point of sample) by cylindrical probe with a descendent speed of 150 mm/min. Flexure test of dried extrudates was carried out by Instron restraining the end of sample strip through two clamps and breaking the middle of sample by a parallelepiped probe (the thickness of contact surface with extrudate was 1 mm^2 and descendent speed was 10 mm/min). The run of the probe was set at 25 mm (i.e. the space from the beginning of the run and the sample surface). The results are elaborated by software Merlin ver. 9.

Coded values	*Barrel temperatures of last two zones (°C)*	*Screw speed (rpm)*	*Liquid feed content (%)*
+1	110	200	60
0	100	150	50
-1	90	100	40

TABLE 1. Coded and actual values of variables.

Treatments	*Coded values*			*Processing variables*		
	X1	X2	X3	X1 (°C)	X2 (rpm)	X3 (%)
1	-1	-1	-1	90	100	40
2	-1	0	1	90	150	60
3	-1	1	0	90	200	50
4	0	-1	1	100	100	60
5	0	0	0	100	150	50
6	0	1	-1	100	200	40
7	1	-1	0	110	100	50
8	1	0	-1	110	150	40
9	1	1	1	110	200	60
10	0	0	0	100	150	50
11	0	0	0	100	150	50

X1: Barrel temperatures of last two zones (°C); **X2**: Screw speed (rpm); **X3**: Liquid feed content (%)

TABLE 2. Experimental factorial design.

2.5 Statistical analysis

Data were submitted to statistical analysis using Statsoft software vers.10 (Statsoft, Tulsa, USA). The analysis was carried out in two steps: the first involved a stepwise regression analysis to identify the relevant variables and the second used a multiple regression analysis

(Standard Least Square Fitting) to fit a second order mathematical model, according to the following polynomial equation:

$$y = B0 + \Sigma Bi\chi i + \Sigma Bii\chi ii2 + \Sigma Bij\chi i\chi j \quad (2)$$

where y is the dependent variable (moisture content, cohesiveness; a_w and flexibility), B0 is a constant value, χi and χj are the independent variables (barrel temperature, screw speed and liquid feed content) in coded values and Bi, Bii and Bij are the regression coefficients of the model. Variables with a significance lower than 95% ($p>0.05$) were left out of the equation. To describe both individual and interactive effects of the independent variables of extrusion-cooking processing on moisture content, cohesiveness; a_w and flexibility, iso-response surfaces were developed (Box, Hunter and Hunter, 1978).

3. RISULTS AND DISCUSSION

Table 3 showed kinetic constants and correlation coefficients related to drying curves of asparagus waste calculated during constant rate period. Contrarily as expected, samples dried at 70 °C presented the highest values of kinetic constants; in fact they lost 67 % moisture during treatment, while samples dried at 80°C lost 35 % moisture in the same time (data not shown). The strange behaviour of sample dried at the highest temperature could be due, probably, to the quick reaching of critical value of sample water activity at which the dehydration process becomes very slow (Srikiatden and Roberts, 2007).

Drying temperature (°C)	k_0	r^2	p-level
60°C	-7.32	0.95	0.001
70°C	-11.28	0.97	0.001
80°C	-6.24	0.96	0.001

TABLE 3. Kinetic constants related to drying curves of waste asparagus.

Breaking strength values (N/mm^2) of asparagus waste dried at 60; 70 and 80°C as a function of drying time are reported in Figure 1. All samples showed no univocal trend during drying because of heterogeneity of matrix and, consequently, their moisture content. Depending on their composition and molecular weight, solid food may exhibit a large variety of rheological properties, from brittle glassy solids, to sticky materials (like sugars just above Tg) or elastic "rubbers" or leather-like materials (like high molecular weight carbohydrates or proteins over a large range of temperatures above Tg) (Bonazzi and Dumoulin, 2011). In any case, samples that showed low breaking strength values, at the end of drying treatment, were those dried at

70°C (Figure 1); this means that these samples resulted more brittle and then more suitable to milling processing. Figure 2 shows the granulometry curves of asparagus wastes powder dried at 60; 70 and 80°C. In this research, the diameter of waste powder ranged between 200 and 300 micron was considered optimal, because this range is similar to granulometry of cereal flours. All samples showed a granulometry less than 100 micron, but the powder of asparagus waste dried at 70 °C had the highest percentage (26 %) of required granulometry range.

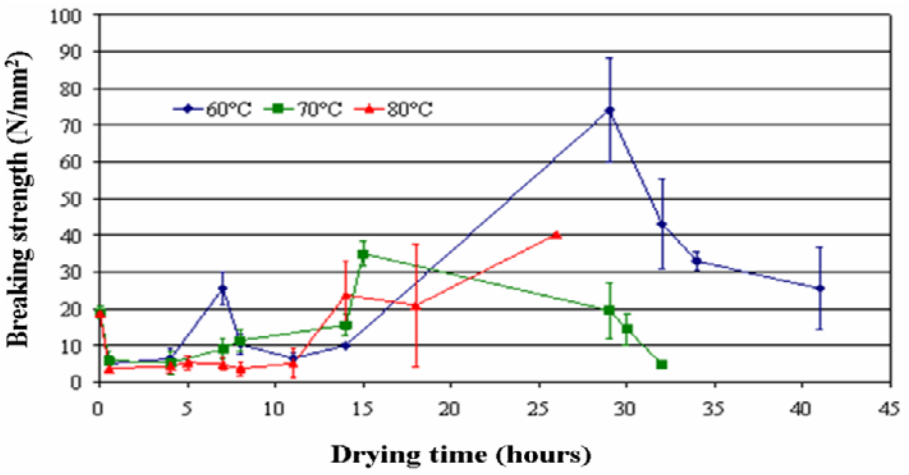

Figure 1. Breaking strength values (N/mm^2) of asparagus wastes dried at 60; 70 and 80°C as a function of drying time.

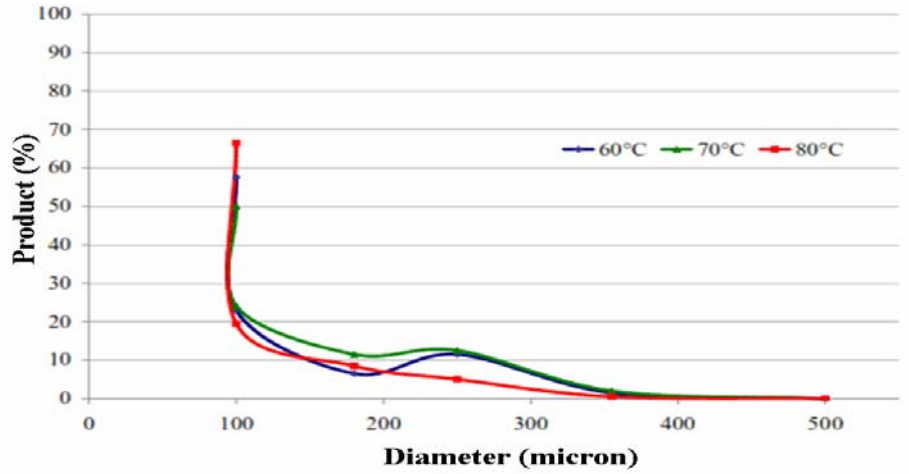

Figure 2. Granulometry curves of powder asparagus wastes dried at 60; 70 and 80°C.

Figure 3 shows the SME values obtained during extrusion of blend made up of dried powder as a function of liquid feed content and screw speed. SME has been demonstrated to affect the rheological properties of molten and glass transition of the extrudate (Welti-Chanes Barbosa-Cánovas, and Aguilera, 2002). The maximum values of this system parameter were obtained at low values of liquid feed content and at high screw speed (Figure 3). The increase of SME

with decreasing of liquid feed content confirms the plasticizer effect of water, besides that of plasticizing agent, which decreased shear stress in extruder (Ilo, Tomschik, Berghofer and Mundigler, 1996). Moreover, the increase of screw speed at low liquid feed content, determined an increase of SME due to shear rate that change, probably, the apparent viscosity of dough. In fact, an increase of motor torque and pressure values were observed at the same operating conditions (data not shown).

The effects of barrel temperature, liquid feed content and screw speed on moisture content of un-dried extruded material are reported in Figures 4 and 5.

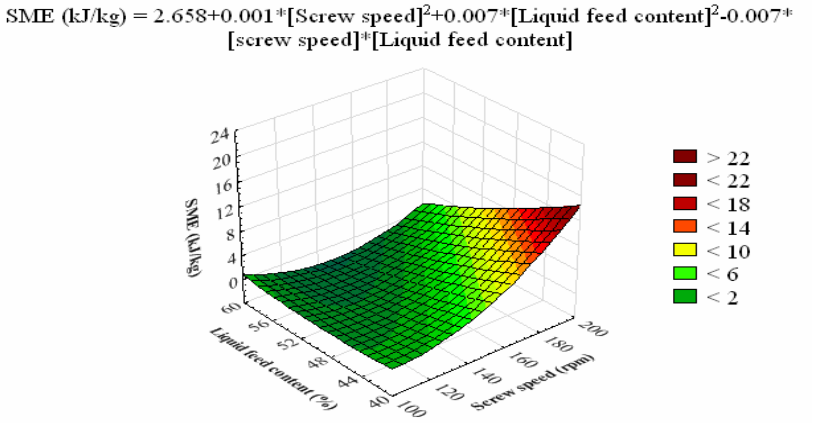

Figure 3. SME (kJ/kg) values as a function of liquid feed content and screw speed.

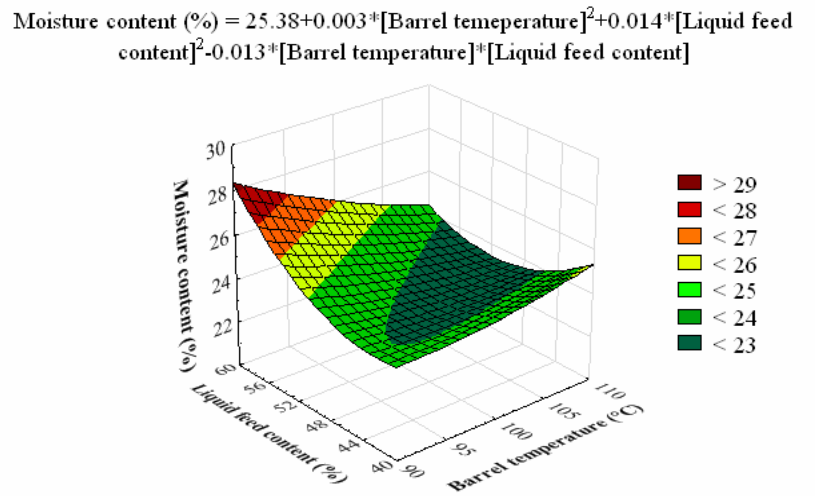

Figure 4. Moisture content (%) of **undried** extrudates processed at different liquid feed content and barrel temperature values.

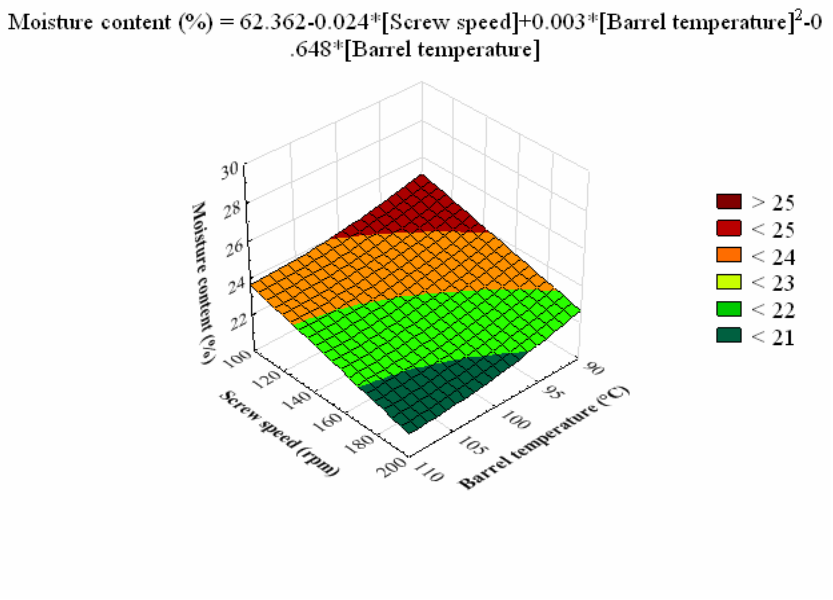

Figure 5. Moisture content (%) of **undried** extrudates processed at different screw speed and barrel temperature values.

As expected, the decrease of liquid feed content caused a decrease of moisture of extrudates. Also the increase of barrel temperature and screw speed determined a decrease of moisture content. This could be attributed to greater water evaporation from material, which was caused by high heating due to increasing of both barrel temperature and friction force (at the highest screw speed). The effect of operating conditions did not result significant on a_w values of un-dried extrudates (p-level > 0.05), which ranged between 0.69-0.73. In Figure 6 is reported the cohesiveness of un-dried extruded material as a function of barrel temperature and liquid feed content. Partial equation and iso-response surface showed a negative interaction term between barrel temperature and liquid feed content. This means that the increase of barrel temperature at low liquid feed content determined a decrease of cohesiveness while at high values of water and plasticizing agent an opposite behaviour was observed. At the highest values of liquid feed content, the increase of processing temperature could be favour starch gelatinization and solubilization of plasticizing agent, which could be help the structure formation of extrudate. A_w value of dried extruded material decreased with increasing liquid feed content (Figure 7). The increase of liquid feed content involved an increase of plasticizing agent amount, since this last had a humectant effect, it decreased the available water into extrudates. A prominent effect of liquid feed content and, consequently of plasticizing agent, was also observed for flexibility of dried extruded material, which increased with increase of liquid feed content (Figure 8). The worst results in term of low flexibility were observed at the lowest values of barrel temperature and liquid feed content.

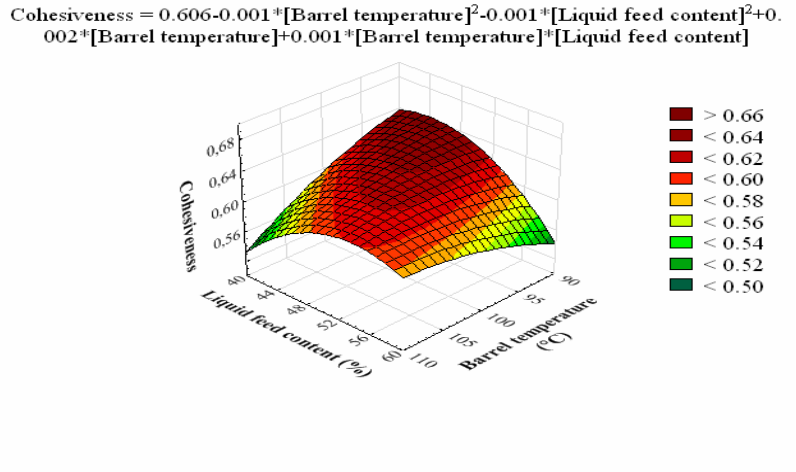

Figure 6. Cohesiveness of **undried** extrudates processed at different liquid feed content and barrel temperature values.

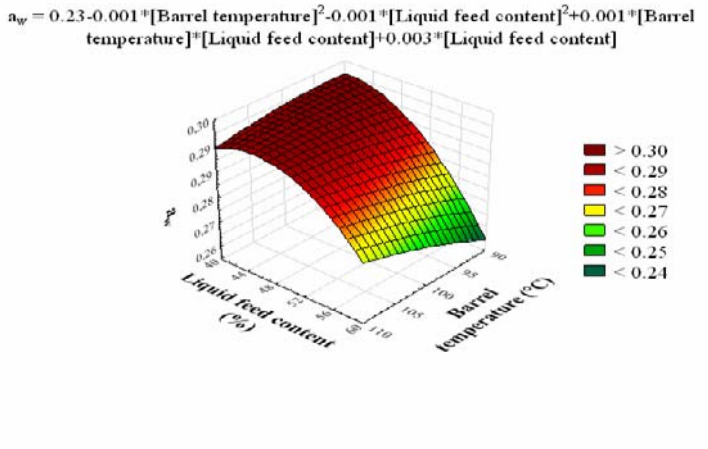

Figure 7. A_w of **dried** extrudates as a function of barrel temperature and liquid feed content.

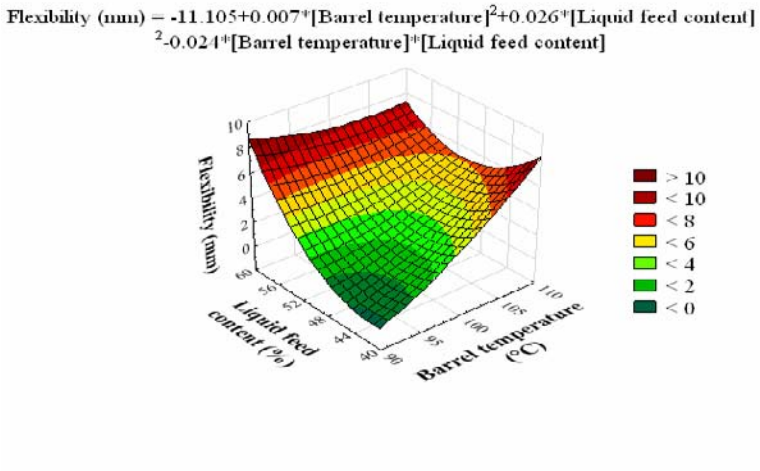

Figure 8. Flexibility (mm) values of **dried** extrudates processed at different liquid feed content and barrel temperature values.

4. CONCLUSION

The results obtained in this preliminary research showed that optimal drying conditions of asparagus wastes were reached at 70°C: dried material becomes brittle (breaking strength < 5 N/cm^2) and, after milling process, a powder with a granulometry <200 microns was obtained. The operating conditions of extrusion-cooking processing to get a material with plastic flow involved a barrel temperature less than 100 °C; low screw speed (< 200 rpm) and high liquid feed content (~ 60 %). In conclusion it is possible affirm that the extruded material could be suitable to produce biodegradable packaging even if other investigation should be make about barrier properties and thermal analysis.

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PRODUCTION OF PHYSICALLY AND CHEMICALLY STABLE SUB-MICRON OIL-IN-WATER EMULSIONS BY ULTRA-HIGH PRESSURE HOMOGENIZATION AND SODIUM CASEINATE AS EMULSIFIER

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ABSTRACT

Pre-emulsions containing 1 and 5% of sodium caseinate (SC) and 20% oil (15% sunflower + 5% olive) were obtained by colloidal mill (CM, 5000 rpm for 5 min), conventional homogenization (CH, 15 MPa) and ultra-high pressure homogenization (UHPH, 100-300 MPa). Emulsions were characterized for their microstructure, physical properties including stability to creaming and oxidative stability under light (2000 lux/m^2). The droplet size distributions of the CM emulsions showed a large size of ~ 6 µm, whereas, emulsions produced via CH and UHPH homogenization showed a much smaller size range (~ 0.59 and 0.137 µm, respectively). An increase in the protein content from 1 to 5% resulted in a decrease in the particle size (d3.2 and d4.3) in all emulsions; however, these differences were only significant in CM emulsions. Emulsions containing 10 and 30% oil content and 5% of sodium caseinate were also produced. Increasing the oil concentration from 10 to 30% tended to decrease the d3.2 in CM emulsions, but the particle size in CH and UHPH emulsions was affected neither by the oil concentration nor by the pressure applied. CM and UHPH emulsions treated at 100 MPa showed a flow Newtonian behaviour (n≈1) with no change after increasing the protein concentration to 5%. Newtonian behavior was also observed in CH emulsions and UHPH emulsions treated at 200 and 300 MPa containing 1% protein content with a complete change to shear thinning behavior as the protein concentration increased to 5%. All CM emulsions and UHPH emulsions containing 10% oil exhibited a Newtonian behavior; however, shear thinning behavior was observed in all CH emulsions and UHPH emulsions containing 30% oil content. Creaming stability was improved using the CH and UHPH treatments compared to those treated by CM, especially when high protein and oil concentrations were used. However, the creaming rate was faster in CH emulsions and UHPH

emulsions treated at 100 MPa in comparison to those treated at 200 and 300 MPa. Oxidative stability was improved when UHPH was applied; whatever the oil content was, whereas CM and CH emulsions suffered a significant increase in the oxidation. Increasing the protein concentration in UHPH emulsions resulted in emulsions more stable to oxidation, especially in emulsions treated at 300 MPa.

Keywords: Ultra-High Pressure Homogenization (UHPH), sodium caseinate, submicron emulsions, physical stability and oxidative stability.

1. INTRODUCTION

Sub-micron emulsions have a number of unique functional attributes that have led them to be utilized within an increasing number of industrial products, including foods, pharmaceuticals, cosmetics, personal care products and chemicals.

The preparation of submicron emulsions requires a high energy input. Preparation techniques such as ultrasonic or high-pressure homogenization are vital, with high pressure homogenization generally preferred due to better effectiveness and more homogeneous droplet size distributions (Washington and Davis, 1988). Ultra-high pressure homogenization (UHPH) is a technology which has demonstrated its potential benefit in the food industry as an alternative to conventional technologies, such as heat treatments. UHPH within a range of pressures of 100-400 MPa is capable of producing stable submicron emulsions during storage by breaking down the oil droplets to the nano-/submicron scale (< 1 μm) with a narrow size distribution (Floury et al., 2004).

Emulsifiers, i.e., casein and caseinates, have the ability to form and stabilize emulsions by being absorbed to the oil-in-water interface during homogenization, reducing the interfacial tension by an appreciable amount, thus preventing droplet coalescence from occurring during homogenization (Dickinson, Murray, and Stainsby, 1988; Dickinson, 2001). Casein forms a thicker interfacial layer (10 nm) in oil-in-water emulsion droplets, which may explain why caseinate-stabilized oil-in-water emulsions have been found to exhibit increased oxidative stability compared to whey protein isolate-stabilized emulsions (Hu, McClements, and Decker, 2003).

Although a great deal of research has been focused on the physical stability of submicron emulsions, very little research has focused on the oxidative stability of these emulsions. The objective of this work was to study the physical and oxidative stability of emulsions produced

from vegetable oils and sodium caseinate (SC) with different formulations of protein and oil concentrations and processed by UHPH comparing with those produced by colloidal mill and conventional homogenization.

2. MATERIAL AND METHODS

2.1 Emulsions preparation

Sodium caseinate dispersions containing 1 and 5% (w/w) of protein were prepared using decalcified water by agitating using a Frigomat machine (Guardamiglio, Italy) with two different blenders at room temperature to avoid foam formation. Protein dispersions were stored overnight at 4 ºC to allow protein hydration. After rehydration, the SC dispersions with different concentrations and oil were equilibrated at 20 ºC before mixing.

Colloidal mill (CM) emulsions were prepared by mixing the above SC dispersions with an oil mix (3 sunflower oil : 1 olive oil) using the CM homogenizer (E. Bachiller B. S.A, Barcelona, Spain) at 5000 rpm during 5 min at room temperature. CM emulsions were treated by UHPH (100-300 MPa) at inlet temperature of 25 ºC using a Stansted high-pressure homogenizer (Model/DRG number FPG 11300:400 Hygienic Homogenizer, Stansted Fluid Power Ltd., UK) with a flow rate of 120 L/h. The homogenizing chamber was cooled with a cooling jacket containing cold water at 5 °C, in order to slow down the rise of temperature. The conventional homogenization was performed using APV Rannie Copenhagen Series Homogenizer (Model number 40.120H, single stage hydraulic valve assembly, Copenhagen, Denmark) with inlet temperature of 60 ºC at 15 MPa single stage (CH emulsions).

Each emulsion was carefully collected and stored at 4 °C for 10 days under light (2000 lux/m^2) and analyzed for the oxidative stability. The physical stability parameters were analyzed immediately after emulsion preparation. Sodium azide (0.1% w/w) was added to the final emulsions in order to prevent microbial growth in the samples which were used to assess the physical characteristics. The experiment was duplicated.

2.2 Physical and oxidative analyses

The particle size distribution, d3.2 and d4.3 indices, and the specific surface area (SSA, m^2/ml) of the emulsions were obtained using a particle size analyzer (Beckman Coulter LS 230 Laser, USA). Rheological measurements were performed using a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany) using a con (1º, 60 mm diameter) and plate geometry probe at 25 °C. Flow curves were fitted to the

Ostwald de Waele rheological model. To examine the changes in emulsion microstructure, emulsion samples were observed by transmission electron microscopy (TEM), preparing samples as described by Cruz et al. (2007). Physical stability was assessed in conventional and UHPH emulsions, measuring the d4.3 values at the top or at the bottom of the emulsion tubes stored at room temperature during 9 days and under the same conditions for comparison. The oxidative stability was assessed by analyzing hydroperoxide formation (Hu et al., 2003).

2.3 Statistical analysis

The statistical analysis was performed using SAS System ® v9.2 (SAS Institute Inc., Cary, NC, USA) using a General Lineal Model with repeated measures in order to obtain the descriptive statistics, mean and standard deviation. For all statistical tests, a nominal significance level of 5 % ($p < 0.05$) was applied. Tukey adjustment was performed for multiple comparisons of the means.

3. RESULTS AND DISCUSSION

3.1. Effect of SC concentration on emulsion characteristics

3.1.1. Particle size distribution

Table 1 gives the mean diameters (d3.2 and d4.3) and SSA of emulsions containing different amounts of SC (1 and 5%) and treated by colloidal mill, conventional homogenization and UHPH at different pressures (100, 200 and 300 MPa), averaged from at least four measurements. The droplet size distributions of the CM emulsions showed a large size of ~ 6 μm, whereas, emulsions produced via CH and UHPH homogenization showed a much smaller size range (~ 0.59 and 0.137 μm) and higher SSA, due to the much higher shear forces during homogenization. The high particle size observed in CM emulsions could be attributed to the incapability of the homogenizer to create particles with small sizes and to the droplet re-coalescence as shown in Figure 1 A. Perrier-Cornet, Marie, and Gervais, (2005) reported that the differences in droplet sizes in emulsions could be directly correlated to the energy density, which lies between 10^4 and 10^6 W m^{-3} for the rotor/stator processes (Karbstein & Schubert, 1995) and near 10^{12} W m^{-3} for the high-pressure jet.

An increase in the protein content from 1 to 5%, in general, resulted in a decrease in the particle size (d3.2 and d4.3) in emulsions, which may be due to the better availability of this surfactant to stabilize droplets during break-up. However, these differences were only

significant in CM emulsions. Generally speaking, increasing the protein concentration from 1 to 5% tended to increase the SSA in UHPH-treated emulsions, but not in CM and CH emulsions. This increase in protein concentration would also increase the coverage of oil droplets thereby inhibiting the droplet aggregation and forming a smaller droplet, a fact that was confirmed by the TEM images of 1% protein content (Fig. 1 C-E), in comparison to those of 5% protein content (Fig. 1 H), where a clearly visible decrease in the aggregation could be noticed. The d4.3 parameter allows coalescence and flocculation processes to be detected with more sensibility than the d3.2 value. A significant decrease in the d4.3 was observed in CM and UHPH emulsions as the protein content increased to 5%, suggesting a reduction in the flocculation processes when 5% of protein was used, especially in the case of CM emulsions.

TABLE 1. Mean ± standard deviation of particle size distribution indices, d3.2 and d4.3, specific surface area (m^2/ml) and rheological characteristics (flow and consistency indices) for O/W emulsions prepared by colloidal mill, conventional homogenization (15 MPa) and UHPH at various homogenization pressures (100, 200 and 300 MPa) with 20% (w/w) of sunflower and olive oil plus sodium caseinate (1 and 5%).

Pressure (MPa)	Protein content (%)	Particle size distribution			Rheological behavior	
		d3.2 (µm)	d4.3 (µm)	Specific surface area SSA (m^2/ml)	Consistency coefficient (K) mPa × s	Flow behavior index (n)
CM	1	6.701 ± 0.368^{a}	20.29 ± 4.528^{a}	0.920 ± 0.029^{f}	0.0015 ± 0.0003^{f}	1.093 ± 0.039
	5	5.648 ± 0.115^{b}	13.80 ± 1.712^{b}	1.111 ± 0.036^{f}	0.0121±0.0006^{c}	1.009 ± 0.016
15	1	0.616 ± 0.041^{c}	1.216 ± 0.103^{c}	9.955 ± 0.784^{e}	0.0017 ± 0.0001^{f}	0.994 ± 0.007
	5	0.564 ± 0.113^{c}	1.421 ± 0.216^{c}	9.387 ± 0.386^{e}	0.0457 ± 0.0090^{ab}	0.732 ± 0.052
100	1	0.200 ± 0.056^{d}	0.283 ± 0.050^{d}	24.56 ± 0.935^{d}	0.0022 ± 0.0003^{e}	0.976 ± 0.019
	5	0.117 ± 0.009^{de}	0.140 ± 0.003^{f}	53.98 ± 1.292^{a}	0.0252 ± 0.0030^{cd}	0.905 ± 0.027
200	1	0.145 ± 0.022^{de}	0.229 ± 0.019^{e}	44.45 ± 1.003^{c}	0.0037 ± 0.0019^{e}	0.910 ± 0.091
	5	0.112 ± 0.008^{de}	0.137 ± 0.011^{f}	58.31 ± 1.742^{a}	0.0308 ± 0.0087^{bc}	0.836 ± 0.073
300	1	0.129 ± 0.002^{de}	0.206 ± 0.006^{e}	45.59 ± 1.579^{bc}	0.0031 ± 0.0005^{e}	0.948 ± 0.017
	5	0.116 ± 0.008^{e}	0.124 ± 0.008^{f}	51.52 ± 4.089^{ab}	0.0463 ± 0.0048^{a}	0.845 ± 0.023

$^{a-h}$ Different letters at the same column indicate significant differences *($P < 0.05$)* between treatments.

3.1.2. Rheological behaviour

Table 1 shows the consistency coefficient (K) value, which corresponds to the viscosity when the fluid is Newtonian, and the flow behavior index (n≈1 indicates Newtonian behaviour). All CM emulsions showed a Newtonian flow behaviour (n≈1), which may be attributed to the low particle-particle interactions (Samavati, Emam-djomeh, Mohamedifar, Omid, and Mehdinia, 2012). Increasing the protein concentration to 5% in CM emulsions resulted in a significant increase in the viscosity without affecting the Newtonian behavior. Similar trend was observed for CH emulsions, where an increase in the K value was found when the protein concentration was increased to 5%, but in this case the emulsion flow behavior was completely changed to shear thinning (n=0.732), as a result of the high degree of aggregation, as can be observed in Figure 1 F, which may be a result of the excessive protein amount in the medium.

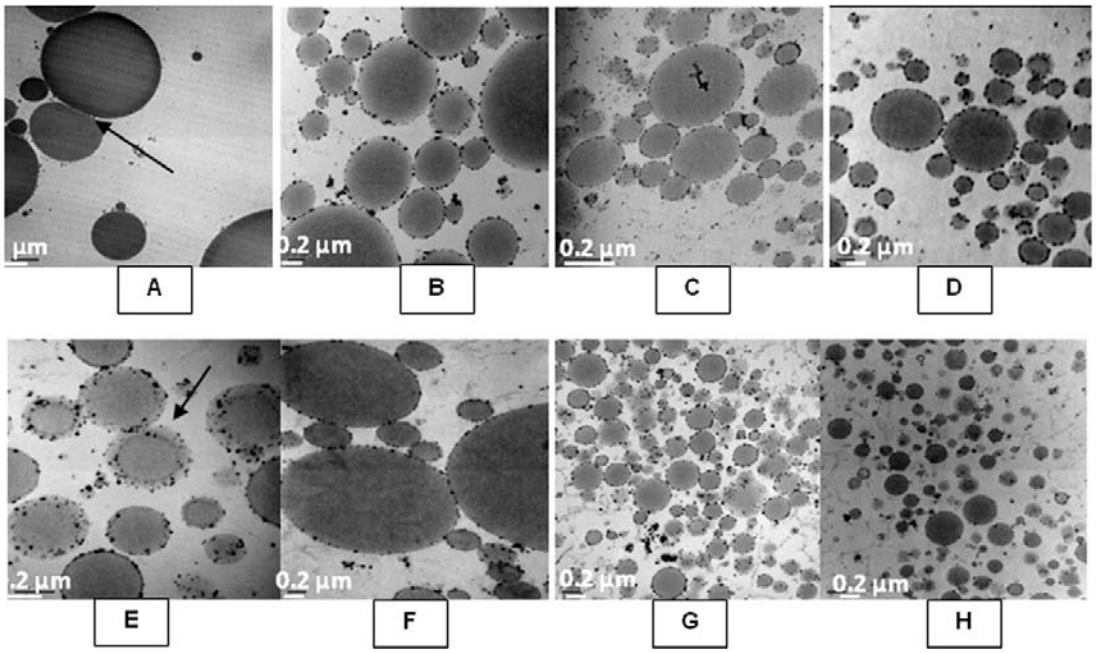

Figure 1. TEM images of O/W emulsions containing sodium caseinate 1 % (A-E) and 5 % (F-H), stabilized by colloidal mill (A) ×3000, conventional homogenization (B,F) ×50000, and by UHPH emulsions at 100 (C,G) and 200 MPa (D,H) ×50000 and (E) ×100000.

Concerning UHPH emulsions, increasing the protein concentration significantly increased the K value with a slight change from Newtonian (100 MPa) to shear thinning in emulsions treated at 200 and 300 MPa. The significant higher viscosities observed in UHPH emulsions, especially those treated at 200 and 300 MPa, could be attributed to the reduced droplet size and to the increased number of fat globules in these emulsions, which increases the

hydrodynamic interactions between the droplets (Pal, 2000), and to the increased adsorption of SC at the surface, which may increase the emulsion viscosity (Hayes, Lefrancois, Waldron, Goff, and Kelly, 2003).

3.1.3. Creaming stability

As a direct consequence of the larger droplet size in the CM emulsions, these creamed within just a few hours after preparation. In contrast and generally, the CH and UHPH emulsions showed no or slow creaming rate and remained structurally stable over the period of 9 days. The creaming phenomena was found to be faster in all CH emulsions, where significant differences were observed in the d4.3 value between the top and the bottom of the emulsions after 9 days of storage at room temperature: 2.428 and 1.926 μm, and 0.961 and 0.417 μm for the top and the bottom of CH emulsions containing 1 and 5% of SC, respectively. On the other hand, the creaming rate was reduced to a great extent in the UHPH emulsions, mainly in those treated at 200 and 300 MPa containing high protein concentration (5%), in which the differences between the top and the bottom were not noticeable: 0.148 and 0.134 μm, and 0.145 and 0.131 μm for emulsions treated at 200 and 300 MPa, respectively. However, UHPH emulsions treated at 100 MPa and those treated at 200 and 300 MPa containing 1% of protein exhibited creaming phenomena. The creaming observed in these UHPH emulsions may be due to the limited protein available to cover the new created surface, in the case of UHPH emulsions containing 1% of protein, or the excess protein in the medium, only in emulsions treated at 100 MPa containing 5% protein, which results in bridging flocculation of particles, as can be observed in the TEM images (Fig. 1).

The reason for the high creaming stability of UHPH emulsions treated at 200 and 300 MPa containing high protein concentrations is the observed decrease in the droplet diameters (Lee, Lefèvre, Subirade, and Paquin, 2009). It has been shown that when the particle sizes are smaller than 100 nm, creaming would be greatly reduced and aggregation becomes a dominant mechanism for emulsion instability (McClements, 2005). The low particle sizes in emulsions also increase the emulsion viscosity, as shown in Table 1, limiting the movements of the oil particles and then lowering the creaming rate.

3.1.4. Oxidative stability

Hydroperoxide content variations of emulsions treated by CM, CH and UHPH and containing different protein concentrations are shown in Figure 2. At the first day of storage, all samples

showed a low level of oxidation with no significant differences between treatments, except for emulsions treated at 300 MPa containing 5% of SC, which presented the lowest levels of hydroperoxides. As the time increased to 10 days, all CM and CH emulsions suffered a significant increase in oxidation. Among UHPH emulsions, only those treated at 100 and 200 MPa containing low protein concentration also suffered oxidation, whereas low hydroperoxide content was observed in UHPH emulsions containing high protein concentrations, especially those treated at 300 MPa, in which the lowest degree of oxidation was found. The ability of SC to form a thick interfacial layer around droplets at high protein concentrations as shown in the TEM image (Fig. 1 E), may protect the emulsion from oxidation.

It can be observed that lipid oxidation occurred to a higher extent in the CM and CH emulsions as compared with the UHPH fine emulsions. At a fixed oil concentration, total droplet surface increases as each droplet diameter decreases, and therefore the rate of lipid oxidation is expected to increase (Nakaya, Ushio, Matsukawa, Shimizu, and Ohshima, 2005). However, this tendency can be modified, or even inverted, as in our case, owing to the specific characteristics of the emulsion and the protective ability of the interface against oxidation. Nakaya, Ushio, Matsukawa, Shimizu, and Ohshima, (2005) suggested that the location of emulsifier molecules at the O/W interface may influence the mobility of the lipid molecules and may consequently improve oxidative stability. They estimated that the actual concentration of emulsifier on smaller droplets was 10 times higher than that on the larger droplets, and consequently the concentration of unsaturated oil in a smaller droplet becomes lower, and therefore lipids in the emulsion become more stable against oxidation.

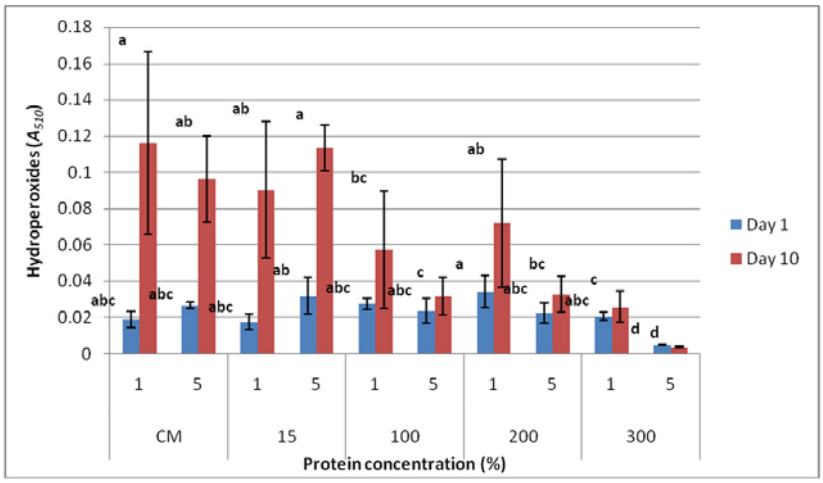

Figure 2. Oxidative stability under light during 10 days of storage of O/W emulsions prepared by colloidal mill, conventional homogenization (15 MPa) and UHPH at various homogenization pressures (100, 200 and 300 MPa) with 20% (w/w) of sunflower and olive oils plus sodium caseinate (1 and 5%).

3.2. Effect of oil concentration on emulsion characteristics

In order to study the effect of oil concentration on emulsion characteristics, the better processing conditions achieved in the first part of this study were applied. Emulsions containing 10 and 30% oil content and 5% of SC were produced using CM, CH and UHPH (200 and 300 MPa).

3.2.1. Particle size distribution

Droplet size indices, d3.2 and d4.3, and SSA for emulsions containing 5% of SC and different oil concentrations (10 and 30%) are shown in Table 2. CM emulsions, at the oil concentrations used, had the largest particle size (d3.2 and d4.3) followed by emulsions stabilized by CH, and the minimum droplet size was observed in emulsions stabilized by UHPH, but no differences could be found between the UHPH treatments. The oil concentration significantly affected the particle size (d3.2) in CM emulsions, in which increasing the oil concentration from 10 to 30% tended to decrease the d3.2.

TABLE 2. Mean ± standard deviation of particle size distribution indices, d3.2 and d4.3, specific surface area (m^2/ml) and rheological characteristics (flow and consistency indices) for O/W emulsions prepared by colloidal mill, conventional homogenization (15 MPa) and UHPH at various homogenization pressures (200 and 300 MPa) with 5 % (w/w) of sodium caseinate plus sunflower and olive oils (10 and 30 %)

[a-h] Different letters in the same column indicate significant differences *(P < 0.05)* between treatments.

Pressure (MPa)	Protein conc. (%)	Particle size distribution			Rheological behavior	
		d3.2 (μm)	d4.3 (μm)	Specific surface area SSA (m^2/ml)	Consistency coefficient (K) mPa × s	Flow behavior index (n)
CM	10	6.109 ± 0.663[a]	15.56 ± 2.324[a]	0.907 ± 0.197[c]	0.005 ± 0.0007[d]	0.988 ± 0.009
	30	5.264 ± 0.521[b]	11.79 ± 2.901[a]	1.148 ± 0.114[c]	0.024 ±0.0021[c]	1.004 ± 0.008
15	10	0.517 ± 0.042[c]	0.978 ± 0.151[c]	11.67 ± 0.962[b]	0.010 ± 0.0021[c]	0.858 ± 0.018
	30	0.623 ± 0.031[c]	1.099± 0.108[b]	9.855 ± 0.575[b]	0.209 ± 0.1042[b]	0.609 ± 0.067
200	10	0.107 ± 0.006[d]	0.126 ± 0.005[c]	56.64 ± 2.096[a]	0.005 ± 0.0010[d]	0.998 ± 0.017
	30	0.104 ± 0.006[d]	0.125 ± 0.008[c]	57.89 ± 3.071[a]	2.864 ± 1.280[a]	0.339 ± 0.052
300	10	0.094 ± 0.007[d]	0.113 ± 0.006[c]	64.43 ± 4.989[a]	0.005 ± 0.0010[d]	1.011 ± 0.008
	30	0.102 ± 0.017[d]	0.119 ± 0.021[c]	59.85 ± 10.05[a]	7.383 ± 4.412[a]	0.252 ± 0.039

Applying the CH and UHPH treatments significantly decreased the d3.2 and d4.3 values, and increased the SSA, but no significant differences were observed between the oil concentrations at the same treatment, indicating that the particle size in UHPH emulsions was affected neither by the oil concentration nor by the pressure applied (200 and 300 MPa).

Normally, at constant energy density (e.g. emulsification pressure), particle size rises with increasing oil content. Some experiments by high-pressure valve homogenization (Phipps, 1985; Tesch, Gerhards, and Schubert, 2002) or ultrasound emulsification (Abismail, Canselier, Wilhelm, Delmas, and Gourdon, 1999) have confirmed this trend. At constant emulsifier concentration, and as the oil content increases, there may be an insufficient amount of protein present to completely cover the new droplets during high pressure homogenization. An inadequate amount of protein in the aqueous phase could cause some aggregation of fat globules, as reported by Tomas, Paquet, Courthaudon, and Lorient, (1994). The results obtained in the present study indicate that 5% of SC is sufficient to stabilize the emulsions containing these oil concentrations.

3.2.2. Rheological behaviour

Low viscosities and Newtonian behavior were observed in CM emulsions, because of the low interaction between particles, but high consistency coefficient (K) and shear thinning behavior or pseudo-plasticity were observed in CH emulsions. The shear thinning behavior was more notable when the oil concentration increased from 10 to 30%, which indicates the high degree of aggregation at high oil concentrations. Although no significant differences in the d4.3 value were found in CM emulsions (Table 2), which allows coalescence and flocculation processes to be detected, the probability for aggregation of these emulsions could be confirmed by the significant increase of the K value and the high shear thinning behavior when oil content was increased from 10 to 30%. Shear thinning behavior is observed in flocculated emulsions because of deformation and breakdown of the aggregates as shear stresses increase.

Applying the UHPH treatments in emulsions containing 10% of oil resulted in a Newtonian flow behavior; however, increasing the oil concentration to 30% tended to a huge increase in the viscosity and to a complete change toward shear thinning behavior. San Martin-González, Roach, and Harte, (2009) showed that high pressure homogenization is able to develop a gel-like structure in micellar casein stabilized emulsions containing 30% oil and 2 - 3.5% casein content emulsions when homogenized between 20 and 100 MPa. The authors hypothesized

that homogenization results in the exposure of hydrophobic sites within the micelle core, which allows micelle-coated oil droplets to interact with neighboring particles, creating an elastic three-dimensional structure that becomes fairly strong at a threshold casein concentration.

3.2.3. Creaming stability

As can be observed in Figure 3 (A, B), although creaming could be detected in all CH emulsions by determining the d4.3 values obtained at the top and the bottom of the CH emulsions tubes, the CH emulsions were more stable against creaming in comparison to CM emulsions, which were separated in a few hours, with high stability in CM emulsions containing 30% oil. The d4.3 values measured at the top and the bottom of UHPH-treated emulsions indicated the high physical stability of these emulsions against creaming whatever the oil concentration was (Fig. 3 C-D).

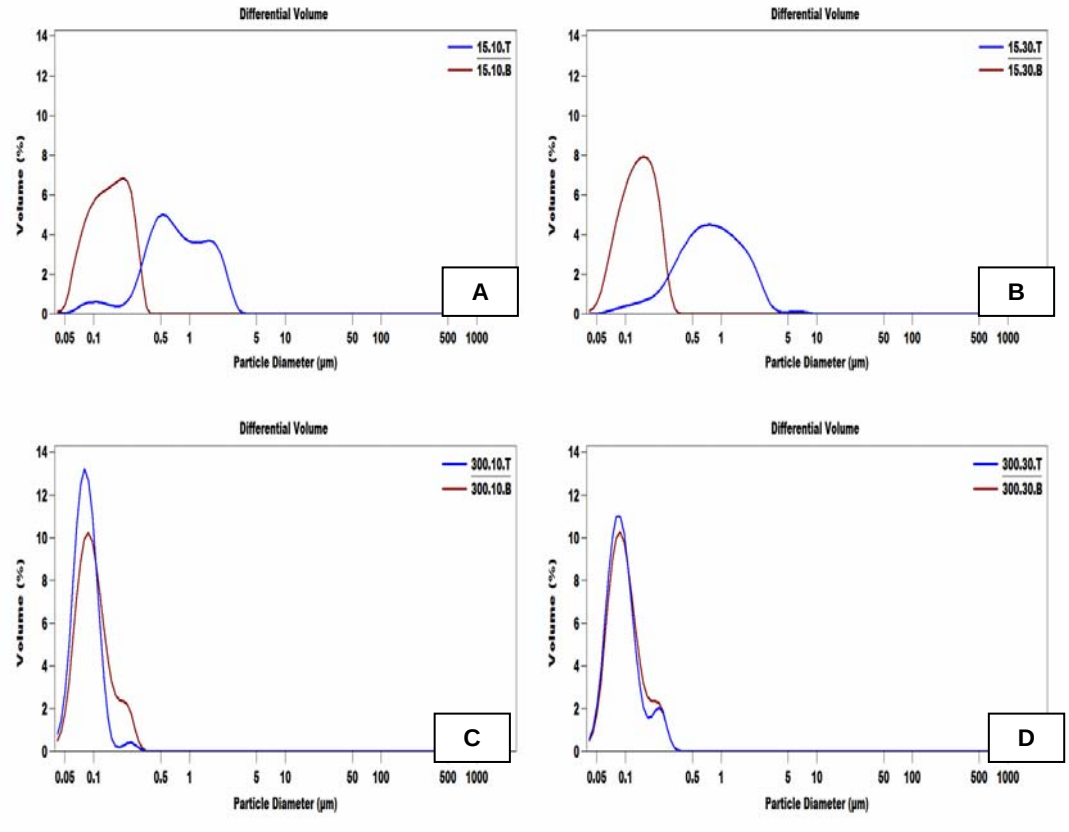

Figure 3. Physical stability to creaming at room temperature during 9 days of storage determining the d4.3 value at the top or at the bottom of O/W emulsions prepared by conventional homogenization (15 MPa) and UHPH (300 MPa) with 20% (w/w) of sunflower and olive oil plus sodium caseinate 1% (A, C) and 5% (B, D).

3.2.4. Oxidative stability

All emulsions contained almost the same amount of hydroperoxides at the first day of storage (A_{510}=0.045), with no significant differences between treatments. After 10 days of storage, CM emulsions were the emulsions with the highest levels of hydroperoxides (A_{510}=0.154) and the most oxidized emulsions, followed by the CH emulsions (A_{510}=0.077), and the lowest levels of hydroperoxides were obtained in UHPH emulsions (A_{510}=0.020).

4. CONCLUSIONS

From the abovementioned results, it can be concluded that high physical stability was achieved in UHPH-emulsions containing up to 30% oil content treated at 200 and 300 MPa using 5% of protein compared with CM and CH emulsions. UHPH treatments improved the oxidative stability of emulsions, especially when 5% of sodium caseinate was used.

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ULTRASONIC AND MEGASONIC PROCESSING OF FOODS

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ABSTRACT:

This manuscript will briefly discuss the fundamentals of ultrasound technology, i.e., the effects of ultrasound at low and high frequencies, including stable and unstable sound-induced cavitation, microstreaming, biochemical stress responses and effects achieved through standing waves. Furthermore, it will touch on current applications and highlight the research and advances in low and high frequency ultrasound at CSIRO (The Commonwealth Scientific and Industrial Research Organisation, Australia), e.g., airborne ultrasound for defoaming and enhanced drying, low frequency ultrasound for altering casein micelle characteristics in dairy processing, high frequency ultrasound (megasonics) for non-solvent separation in oil extraction processes and separation of milkfat, as well as texture modification of processed vegetables.

Keywords: ultrasound, ultrasonics, megasonics, dairy processing, separation, palm oil, texture modification

1. INTRODUCTION

Due to the versatility and flexibility of ultrasound technology over a wide frequency range, spanning over more than two orders of magnitude from ~20 kHz to >2 MHz, and different effects of the acoustic waves attributed at certain frequencies, the technology gives rise to a plethora of promising applications.

In the food industry, ultrasonics has been mainly applied in diagnostics, e.g., non-destructive testing for flaw detection in material science, and processes such as cutting (e.g., frozen or soft foods through ultrasonic vibration on the edge of a cutting tool), homogenisation (e.g., sauces and mayonnaise through turbulent mixing induced by cavitation), extraction (e.g., enhanced yields of flavourings and nutraceuticals from plants caused by the breakdown of

cell walls), degassing (e.g., beverages before canning or bottling), anti-fouling (of e.g., heat exchangers and membranes) among others (Tiwari and Mason, 2012). Ultrasonic applications have widened as a result of fairly recent developments of systems able to generate ultrasound in air at reasonable power levels as well as systems able to generate ultrasound at higher frequencies (> 400 kHz) various power levels (in the order of > 100 W). Latest developments include the use of airborne ultrasound for defoaming and drying applications (e.g., Rodriguez, Riera, Gallego-Juarez, Acosta, Pinto, Martinez, Blanco, 2010; Sabarez, Gallego-Juarez, Riera, 2012), ultrasound at low frequencies to alter the casein micelles in dairy products (e.g., Liu, Juliano, Williams, Niere, Augustin, 2012), ultrasound at higher frequencies for texture modification of processed vegetables (by inducing biochemical stress responses; e.g., Day, Xu, Oiseth, Mawson, 2012), megasonics for enhanced palm oil separation (e.g., Juliano, Swiergon, Mawson, Knoerzer, Augustin, 2013) and megasonics for enhanced milk fat separation (e.g., Juliano, Temmel, Rout, Swiergon, Mawson, Knoerzer, 2013).

1.1 Fundamentals of ultrasonic and megasonic processing

In general, ultrasound refers to acoustic pressure waves with frequencies of 20 kHz or higher (Rastogi, 2011). Most effects of ultrasound at low and high frequencies are directly related to the cavitation occurring in the treated liquid, i.e., the growth of vapour bubbles, which violently collapse in low frequency applications (20 to 100 kHz), generating locally high pressures (in excess of 500 bar) and temperatures (up to 5000°C) (Tiwari and Mason, 2012), resulting in high shear forces (Figure 1), which can enhance cleaning, homogenisation or reduce fouling, among other effects. Cavitation also is more stable (less violent collapse of smaller bubbles) when applying high frequency ultrasound, while inducing more microstreaming.

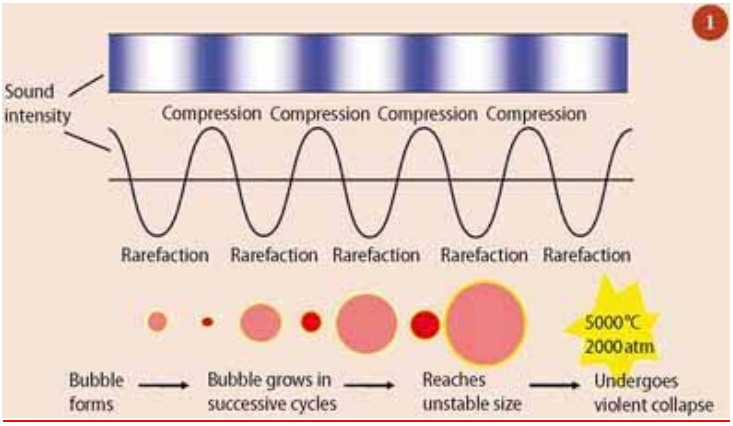

Figure 1: Visual representation of the growth and collapse of an ultrasound induced cavitation bubble (Mason, 2009).

Other effects in high frequency ultrasound applications include inducing biochemical stress responses in living tissue (e.g., fruits and vegetables), which can promote the synthesis of lignin reinforcing intercellular adhesion and the modification of the pectin structure enabling greater calcium ion bonding and cell wall stiffening (Day, Xu, Oiseth, Mawson, 2012). High frequency plate transducers also enable the formation of standing waves in purpose fit reactor designs (Juliano, Temmel, Rout, Swiergon, Mawson, Knoerzer, 2013). These standing waves can form between an ultrasound source and a reflector placed at a distance multiple of a half-wavelength in parallel orientation to the source. Particles or droplets suspended in a continuous phase experience an acoustic force, which, depending on the material properties, move either towards the nodes of the antinodes of the standing wave field (Figure 2).

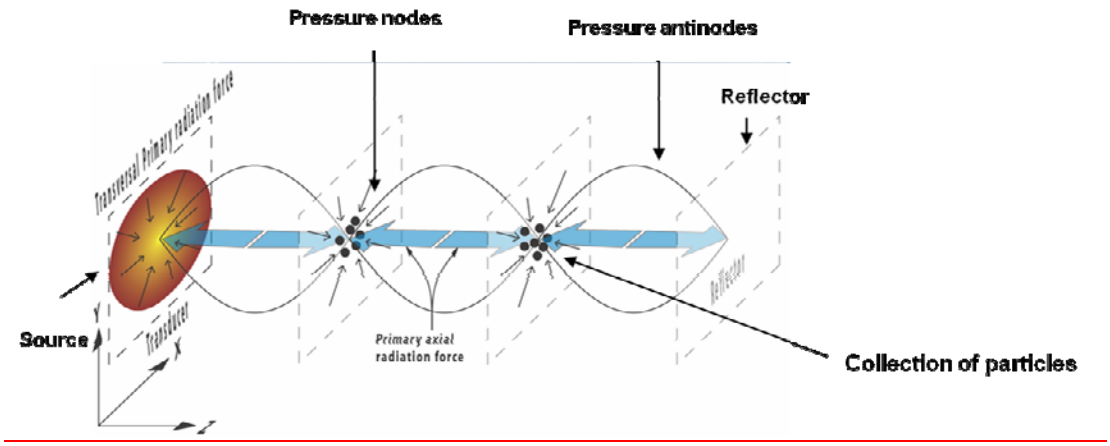

Figure 2: Visual representation of the formation of standing waves in a reflecting ultrasound system and collection of particles in the pressure nodes (Laurell, 2010).

2. REVIEW OF RECENT ADVANCES OF ULTRASONIC AND MEGASONIC PROCESSING AT CSIRO

2.1 Low frequency ultrasound

Over the last few decades, researchers around the world have investigated the use of ultrasound at low frequencies and high powers for various food processing aspects, including the aforementioned applications such as emulsification, dispersion, antifouling, but also the application for other purposes such as microbial and enzyme inactivation. Yet there are a few

applications of low frequency ultrasound that have not been investigated in great detail, such as airborne ultrasound for defoaming and utilisation of the technology for altering casein micelle properties in dairy products. The following section will give a brief overview on the studies performed at CSIRO for these purposes.

2.1.1 Airborne ultrasound for defoaming

Foam generation in tanks (e.g., in biotechnology applications such as fermenters, and tank applications in other processing industries) and on beverage bottling lines, through aeration and agitation of liquids, can lead to significant losses of product. Current methods to reduce foaming include cooling the product or using antifoaming agents. Both methods are not convenient because liquid cooling demands high energy input and the antifoaming agents contaminate the product (Rodriguez et al., 2010), can have a negative effect on the product quality, and impact on the environment. Reducing the product losses by defoaming through low energy airborne ultrasound application has a positive impact on water and energy consumption and, therefore, environmental sustainability. While the concept of the technology has been proven (Rodriguez et al., 2010), the underlying mechanism remains unclear. It was hypothesised that high frequency vibration of the bubble, and potentially induced cavitation in the bubble matrix, overcoming the surface tension of the liquid film, causes the bubble collapse. Research at CSIRO has evolved around the development of sonotrodes, capable of transmitting the mechanical energy generated in the transducer at relatively high powers into the air, where the sound waves lead to efficient defoaming capacity on soft drink bottling lines (Collings and Gwan, 2007).

2.1.2 Airborne ultrasound for enhanced drying

Drying is a very important process in the food and other manufacturing industries, but also high in energy consumption. In fact, drying is probably the largest energy consuming unit operation in the food industry. Furthermore, the use of air at relatively high temperatures negatively affects the quality and structure of the dried product. Recent studies on airborne ultrasound in drying applications have shown that product drying time can be reduced by over 50% (Sabarez, Gallego-Juarez, Riera, 2012). This can, therefore, be used not only to shorten the drying time but also to dry at reduced temperatures, both of which directly impact on the energy consumption . Furthermore, the reduced thermal load on the product leads to better

retention of quality attributes, such as colour and flavour, as well as the retention of health promoting heat sensitive components. The exact mechanism is unclear; some speculate that the acoustic pressure waves compress and decompress the material and, therefore, reduce the time-limiting stage in drying, where water needs to be transported through diffusion from the core of the product to the surface. The theory of formation of channels follows along this theory, where the occurring increase in tortuosity and, therefore, restrictions to the water transport internally, is reduced. An alternative theory is that the diffusion boundary layer forming across the surface of a dried product is disturbed by the pressure waves, which leads to easier water removal from the product and a faster secondary drying stage rate (Sabarez, Gallego-Juarez, Riera, 2012).

2.1.3 Low frequency ultrasound for altering casein micelle properties

Low frequency ultrasound induced cavitation can also offer opportunities to restructure milk proteins to further impact on milk processability. Liu, Juliano, Williams, Niere, Augustin (2012) have investigated the effects of ultrasound processing on the physicochemical properties of casein micelles in reconstituted skim milks and have shown that ultrasound at 20 kHz can disrupt casein micelles and reform micelle-like particles with smaller size and comparable ζ-potential to native micelles. In addition, the volume of the casein micelles that are soluble in the serum increased significantly after ultrasound treatment. As a result, the sonicated milk was able to shorten the cheese renneting time compared to untreated milks. Other ultrasound interventions integrated in dairy processing lines can potentially provide modified physicochemical properties of milk proteins and achieve desired functional properties that impact in food processes. For example, ultrasound can reduce viscosity of concentrates enabling drying at higher total solids content, subsequently increasing throughput and saving energy input into the dryer.

2.2 High frequency ultrasound (megasonics)

Megasonics, operating at frequencies at or above 400 kHz, has been commercially applied only for cleaning of fine structures, e.g., in the semiconductor industry. This is where the development of these systems at larger scale and relatively high powers was pushed over the last decade. Only with the availability of larger scale systems was it seen feasible to conduct studies related to food processing. The researchers at CSIRO have since spent considerable effort into developing a basic understanding of the mechanisms involved in utilising high

frequency ultrasound for various applications, such as enhanced separation, texture modification of processed foods among others

2.2.1 Megasonic milk fat separation

Recent research has shown that high frequency ultrasound (400 kHz to 3 MHz), can enhance milkfat separation in small scale systems able to treat only a few milliliters of sample (e.g., Grenvall, Augustsson, Folkenberg, Laurell, 2009). Juliano, Temmel, Rout, Swiergon, Mawson, Knoerzer (2013) have investigated the effect of ultrasonic standing waves on milkfat creaming in a 6 L reactor and the influence of different frequencies and transducer configurations in direct contact with the fluid. They selected a recombined coarse milk emulsion with fat globules stained with oil-red-O dye for the separation trials. Runs were performed with one or two transducers placed in vertical (parallel or perpendicular) and horizontal positions (at the reactor base) at 400 kHz, 1 MHz and/or 2 MHz. Creaming behaviour was assessed by measuring the thickness of the separated cream layer. Other methods supporting this assessment included the measurement of fat content, backscattering, particle size distribution, and microscopy of samples taken at the bottom and top of the reactor. They found most efficient creaming after treatment at 400 kHz in single and double vertical transducer configurations. Fat globule size increase was observed when creaming occurred. They concluded that there is potential for enhanced separation of milkfat in larger scale systems from selected transducer configurations in contact with a dairy emulsion, or emulsion splitting in general.

2.2.2 Enhanced palm oil separation

In the palm oil milling operation, depending on the efficiency of the mill, a substantial part of the contained oil can be lost in the effluent stream. Therefore, an intervention at any processing stage that increases recoverable oil and reduces oil in the discharged palm oil mill effluent is expected to improve palm milling performance. Juliano, Swiergon, Mawson, Knoerzer, Augustin (2013) have examined the effects of applying ultrasound on the oil recovery from the ex-screw press feed and the underflow sludge from a palm oil vertical clarification tank to determine the usefulness of an intervention based on ultrasound. Megasonics was applied at frequencies of 400 kHz to 1.6 MHz in a standing wave field and the effects on two process streams (containing oil, non-oil solids and water) in palm oil milling were examined. Ultrasonication of the ex-screw press feed obtained upon crushing of

the sterilised palm fruit and of the underflow sludge from the vertical clarification tank enhanced oil separation on gravity settling. Megasonics also enhanced the total oil recoverable, which consisted of the sum of the oil separated under gravity and the decantable oil separated upon centrifugation of the remaining fraction. It was concluded that ultrasound-assisted separation of oil from process streams was attributed to the acoustic forces exerted on the suspended particles in the feed , similar to the effects in milkfat separation, which causes the oil to migrate to the antinodes and non-oil solids (comprising vegetal matter and residual oil) to the nodes. This work demonstrated the potential of applying high frequency ultrasound to improve the separation of oil in the clarification tanks and reduce oil that is lost in the non-oil fraction from the separators (i.e., sludge underflow). This application represents a step-change innovation in palm oil milling operations to reduce oil loss during milling.

2.2.3 Texture modification in processed vegetables

Recent studies (Curulli, Klingler, Mawson, Suwanchewakorn, 2007) have shown that ultrasound at a selected frequency, energy and time profile can be used to modify the surface structure of plant tissue which has a cellular structure with substantial starch content (e.g. potatoes). Day, Xu, Oiseth, Mawson (2012) took this as a baseline to investigate the use of ultrasound pre-processing treatment, compared to blanching, to enhance mechanical properties of non-starchy cell wall materials using carrot as an example. They measured the mechanical properties of carrot tissue by compression and tensile testing after the pre-processing treatment prior to and after retorting and found that ultrasound treated samples at 400 kHz provided a higher mechanical strength to the cell wall structure than blanching for the same time period. They hypothesised that the mechanism involved appears to be related to the stress responses present in all living plant matter and concluded that the ultrasound treatment has great potential to improve the textural properties, i.e., firmness and crunchiness, in canned vegetables.

3. CONCLUSION

Ultrasound technology has proven to be a versatile technology for a number of different applications, with the potential to increase process speed with minimum energy requirements or allow for effects that are not accessible by any other means. Applications can have benefits from a quality perspective (e.g., better flavour, texture and nutrient retention of the processed

food material) but also from an environmental and economical sustainability perspective (e.g., increased throughput, higher extraction yields, lower energy consumption).

While low frequency ultrasound has been investigated extensively in food processing applications, there are still new opportunities that are at earlier stages of research and development (e.g., airborne ultrasound). However, particularly in the high frequency spectrum, the use of ultrasound for enhancing food processes is still in its infancy and many of the theories that would explain the observed effects have not been proven yet. Furthermore, with the development of higher performance systems and extension of the frequency range to frequencies in excess of 3 MHz, will give rise to new applications not investigated to date.

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NUMERICAL SIMULATION OF INNOVATIVE FOOD PROCESSING TECHNOLOGIES FOR EQUIPMENT DESIGN AND PROCESS OPTIMISATION

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ABSTRACT:

Innovative food processing technologies, such as high pressure (low and high temperature), pulsed electric field and ultrasonic/megasonic processing can be applied to manufacture better quality, yet safe, food products and potentially assist in reducing the carbon and water footprint in food processing. Thus, these technologies may play an important role towards satisfying consumer demand for safe and higher quality products and sustainable manufacture.

The design, application and optimisation of suitable equipment and the selection of process conditions for these technologies require further knowledge development. Computational Fluid Dynamics (CFD) is already established as a tool for characterising, improving and optimising traditional food processing technologies; innovative technologies, however, provide additional complexity and challenges because of interacting Multiphysics phenomena.

In this work, we will highlight selected Multiphysics models developed for the characterisation of various processing aspects and optimisation of innovative technologies.

Keywords: modelling, simulation, high pressure processing, HPP, pulsed electric fields, PEF, ultrasonics, megasonics

1. INTRODUCTION

The food industry is an increasingly competitive and dynamic arena with consumers being more aware of what they eat and, more importantly, what they want to eat. Important food

quality attributes such as taste, texture, appearance and nutritional content are strongly dependent on the way foods are processed.

In recent years, a number of innovative food processing technologies, also referred to as "emerging" or "novel" technologies have been proposed, investigated, developed and, in some cases, implemented with the aim to improve or replace, conventional processing technologies. These technologies take advantage of other physics phenomena such as static or dynamic high hydrostatic pressure, or electric and electromagnetic fields, and provide the opportunity for the development of new foods but also for improving the quality of established food products through gentle processing. The physical phenomena utilised by these technologies can potentially reduce energy and water consumption and, therefore, can play an important role towards environmental sustainability of food processing and global food security.

Apart from the underlying thermo- and fluid-dynamic principles of conventional processing, these innovative technologies incorporate additional Multiphysics dimensions, for example, pressure waves, electric and electromagnetic fields, among others. To date, they still lack an adequate, complete understanding of the basic principles of intervening in temperature and flow evolution in product and equipment during processing. Their proper application, development and optimisation of suitable equipment and process conditions still require a significant amount of further knowledge. Computational Fluid Dynamics (CFD) is an established tool for characterising, improving and optimising traditional food processing technologies. Innovative technologies, however, provide additional complexity and challenges for modellers because of the concurrent interacting Multiphysics phenomena (Knoerzer, Juliano, Roupas, Versteeg, 2011).

2. RECENT ADVANCES IN SIMULATING INNOVATIVE FOOD PROCESSING TECHNOLOGIES

Numerical modelling studies have been reported across the range of innovative food processing technologies, such as microwave and radiofrequency processing, ultraviolet light processing, high pressure (thermal) processing, pulsed electric field processing, ultrasonics and megasonics processing, extrusion cooking, electron beam and cool plasma processing (listed in order of technological readiness).

A common problem of innovative food processing technologies is the non-uniformity of the treatment, which can be caused by gradients of process variables such as temperature, electric field strength or sound pressure fields in the processing chambers. A non-uniform distribution of a certain process variable leads to non-uniformities in the resulting outcomes of the process (e.g., microbial inactivation).

While trial-and-error optimisation is always an option to improve equipment and process design, it is the least preferred way, as it is very cost-, labour-, and time-intensive and a good performance may be missed altogether, as not all possibilities can be tested following this approach. On the other hand, numerical modelling using CFD can be used exactly for this purpose at reduced costs and time of equipment use. This way, advantages and disadvantages of the respective technology can be identified and either utilised or minimised.

The following sections will highlight latest advances in modelling high pressure thermal, pulsed electric field and ultrasonics/megasonics processing.

2.1 High Pressure Thermal Processing

High pressure thermal processing is a technology that is not only effective in inactivating vegetative organisms, but also microbial spores, due to the elevated temperatures involved. Because the process can heat faster than thermal-only processing through rapid compression heating and decompression cooling, quality attributes, such as colour, nutrients, flavour and texture can be better retained.

A number of studies have been reported on the utilisation of Multiphysics modelling for equipment and process characterisation in terms of in process temperature and flow field distributions (e.g., Knoerzer, Juliano, Gladman, Versteeg, Fryer, 2007; Knoerzer and Chapman, 2011), prediction of microbial (spore) inactivation (e.g., Juliano, Knoerzer, Fryer, Versteeg, 2009) and equipment optimisation (e.g., Knoerzer, Buckow, Chapman, Juliano, Versteeg, 2010) (Figure 1).

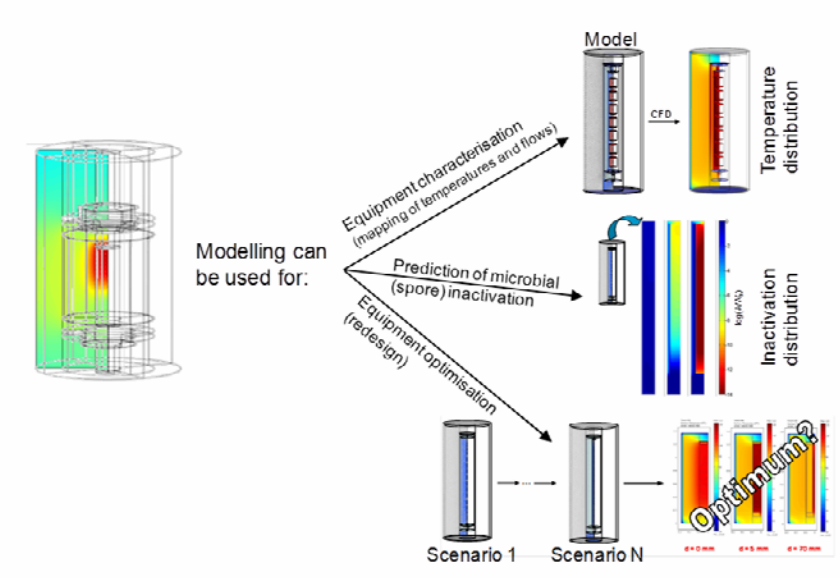

Figure 1: Uses of numerical models describing high pressure thermal processing.

Knoerzer, Juliano, Gladman, Versteeg and Fryer (2007) reported on the use of a numerical model to describe temperature and flow distribution in a 35 L pilot scale high pressure sterilisation system (Avure Technologies Inc., Seattle, WA, USA) and evaluated the differences of the process variables for a number of different product carriers made of metal and insulating plastic material. As expected, only the insulated carrier provided process conditions feasible for sufficient microbial spore inactivation. Juliano, Knoerzer, Fryer and Versteeg (2009) applied similar models to evaluate the differences in the extent and distribution of predicted inactivation of *Clostridium botulinum* spores in food packages. The CFD models were coupled to selected predictive microbial inactivation models, namely, the commonly known log-linear model, an n-th order model and a Weibull distribution model. The different inactivation models predicted very different levels of spore inactivation for the same pressure and temperature conditions. For example, the log-linear model predicted inactivation of *C. botulinum* spores in the order of 14 $\log_{10}$ after 3 min processing at 600 MPa and 121°C whereas the Weibull model indicated spore inactivation of only 9 $\log_{10}$ for the same process.

Knoerzer, Buckow, Chapman, Juliano, Versteeg (2010) then used a modified version of the model to evaluate the importance of the insulating product carrier and optimised the carrier's wall thickness to increase product load capacity. The carrier supplied by the manufacturer was designed with a wall thickness such that , sufficient heat retention was achieved during processing. The study showed that the wall thickness can be reduced from 28 mm to

approximately 4 mm without compromising temperature performance, leading to an increase of carrier load capacity by more than 100%.

2.2 Pulsed Electric Field Processing

Pulsed Electric Field Processing is a technology that can be applied for cold or low temperature pasteurisation of liquid products. It is able to inactivate vegetative microorganisms through the application of electric fields in the order of several ten thousand volts per centimetre for a very short time, leading to cell poration and cell death. Overall treatment times are in the order of microseconds. Other potential applications of this technology are for enhanced extraction processes or softening of fruit and vegetable tissue, for example for improving cutting performance and reducing cutting losses. Also, this technology can improve the quality attributes of foods compared to conventional thermal processing, such as flavour, colour, and nutrients among others. Being a continuous process, high throughputs are possible.

Published studies on numerical modelling of pulsed electric field processing include the utilisation of such models for equipment characterisation with respect to electric field, temperature and flow distribution (Buckow, Schroeder, Berres, Baumann, Knoerzer, 2010), the prediction of microbial or enzyme inactivation (Buckow, Semrau, Sui, Wan, Knoerzer, 2012; Knoerzer, Arnold, Buckow, 2011) and equipment optimisation to ensure uniform and effective processing (Knoerzer, Baumann, Buckow, 2012) (Figure 2).

Buckow, Schroeder, Berres, Baumann and Knoerzer (2010) reported on the development of a model for a pilot scale pulsed electric field system (Diversified Technologies Inc., Bedford, MA, USA) and an extensive validation of the model predictions through temperature measurements within the constrained space of the treatment chamber's active zone. They were able to utilise this model to characterise and evaluate the performance of the system as supplied by the manufacturer.

Buckow, Baumann, Schroeder and Knoerzer (2011) applied this model further to derive simplified equations to estimate accurate electric field strengths and specific energy inputs from treatment variables such as voltage, pulse frequency and duration among others. Also, the effects of changing treatment chamber geometry and configuration on these process variables were evaluated.

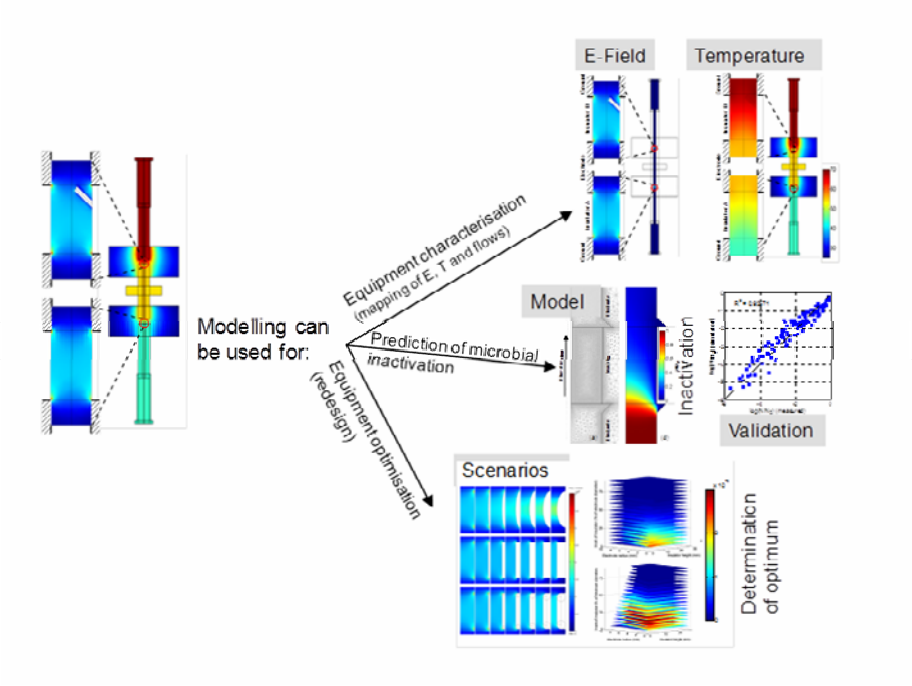

Figure 2: Uses of numerical models describing pulsed electric processing.

Buckow, Semrau, Sui, Wan and Knoerzer (2012) developed a model for a laboratory scale pulsed electric field system and evaluated the effect of the electric field on enzyme degradation by coupling the predicted process variables to predictive enzyme degradation models. The study indicated that the major effect on enzyme inactivation comes from the elevated process temperatures, but there was also some additional inactivation caused by the electric field.

Finally, Knoerzer, Baumann and Buckow (2012) developed an algorithm that was capable of automatically changing the treatment chamber configuration and dimensions in the Multiphysics models and to identify, out of more than 100,000 scenarios, the one that showed the highest degree of electric field uniformity, together with sufficient throughput, lowest pressure drop, among other evaluation characteristics. This geometrical configuration was found to be very different to that supplied by the manufacturer.

2.3 Ultrasonics and megasonics processing

Ultrasonics and megasonics processing span over a wide range of acoustic frequencies, starting as low as 18 kHz, up to several MHz. Applications are as diverse as the frequency spectrum is wide. At the lower frequency end where effects are caused mainly by instable cavitation, traditional applications such as emulsification, cleaning, anti-fouling, and more

novel applications used for improved drying and beverage defoaming in airborne ultrasound can be listed. When using higher frequencies the effects can be either mechanical through both standing pressure waves and microstreaming, and/or sonochemical (radical driven) or biochemical (stress response in living tissue). Novel high frequency applications include the separation of particles in standing wave systems and texture improvement of processed fruits and vegetables, through produce-internal stress responses.

Published studies on numerical modelling of ultrasonics and megasonics processing include the utilisation of Multiphysics models for equipment characterisation with respect to acoustic pressure, temperature and flow distribution (Trujillo and Knoerzer, 2009 and 2011), equipment optimisation (Trujillo and Knoerzer, 2009) and predicting particle separation in megasonics standing wave applications (Trujillo, Eberhardt, Moeller, Dual and Knoerzer, 2013) (Figure 3).

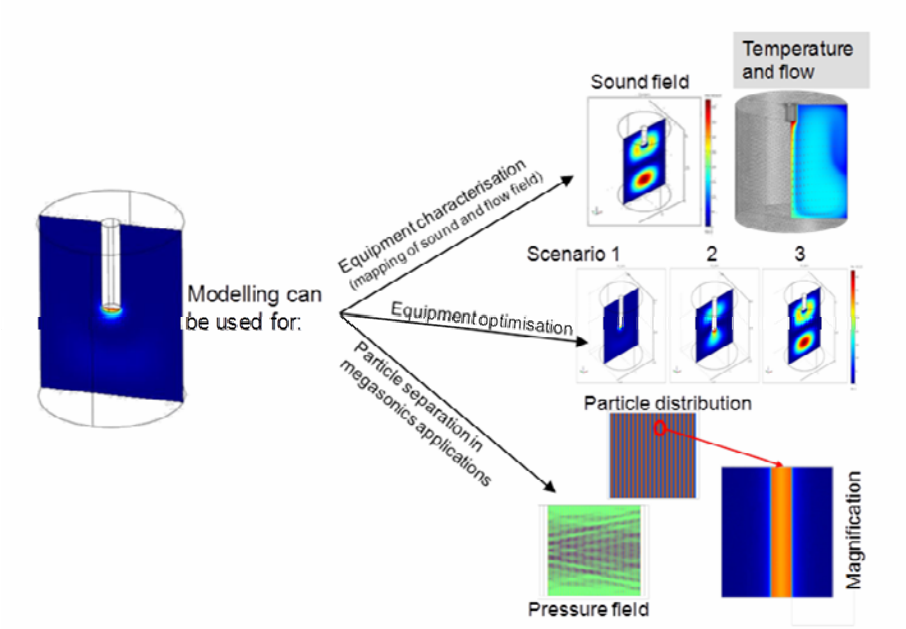

Figure 3: Uses of numerical models describing low and high frequency ultrasound processing.

Trujillo and Knoerzer (2011) reported on the development of a Multiphysics model capable of simulating the formation of a sound pressure jet produced by a sonotrode placed in water in a low frequency high power ultrasound application. The acoustic power was dissipated within close proximity to the horn and the acoustic energy was completely converted into kinetic and thermal energy leading to a jet being formed and directed away from the sonotrode while the temperature was increasing in the bulk of the treated fluid. The model was validated by

utilising published data (Kumar, Kumaresan, Pandit, Joshi, 2011) where fluid movement was measured by Laser Doppler Anemometry.

Trujillo and Knoerzer (2009) presented on the utilisation of an acoustics model, where they solved the linear wave equation to show how different configurations in a low frequency ultrasound system impact on the distribution of acoustic pressures in an ultrasound reactor. They were able to show that the depth of insertion of the sonotrode into the liquid had a pronounced effect on the treatment performance, where insertion of the sonotrode to about half the height of the reactor led to all power dissipated just underneath the horn, which can lead to significant horn erosion and poor product treatment, while insertion by two thirds of the overall height gave better distribution in the system, yet the maximum acoustic pressure was still at the horn tip. When inserting the sonotrode by only one third of the reactor height, they were able to show that the maximum ultrasound intensity was now shifted away from the sonotrode into the main bulk of the treated product, which can lead to less horn erosion and better product treatment.

Last, Trujillo, Eberhardt, Moeller, Dual and Knoerzer (2013) published the development of a Multiphysics model of a high frequency ultrasound application for separation of particles out of a continuous water phase. The model included solving for the acoustic pressure distribution, the movement of the particle phase to the nodes of the ultrasonic standing wave and frequency ramping, leading to active separation of the particles away from the transducer plate towards the reflector. They were able to validate the model through digitised images of the actual experiment, which they compared with the predicted particle band formation and transient band movement.

3. CONCLUSION

It is widely acknowledged that innovative technologies are the means to meet a need and capture an opportunity, particularly around the manufacture of attractive, new, high quality products with fresh-like quality attributes, ensured safety and long shelf-life. The main incentive for applying these new technologies should focus around inducing disruptive innovation in the food manufacturing industry rather than providing merely incremental improvements of existing processes.

Multiphysics modelling will be able to assist in further developing these technologies, while improving and optimising current equipment for effective and efficient implementation in the food manufacturing industry. Without such modelling capabilities, relying on the traditional

approach of trial-and-error, development will be slow and in some instances a sufficient performance justifying utilisation in industry may never happen.

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DEVELOPMENT OF A SYSTEM TO DETERMINE THE EFFECT OF HIGH PRESSURE ON MICROBIAL SPORE INACTIVATION IN HIGH PRESSURE THERMAL PROCESSING

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ABSTRACT:

High pressure processing at ambient temperature is effective for inactivation of vegetative organisms; however, microbial spores are resistant to pressure alone and can only be inactivated if elevated temperatures are applied. The combination of pressure and heat to inactivate microbial spores is commonly referred to as High Pressure Thermal Processing. Some published studies report synergistic effects of combined pressure and temperature, whereas others report equality in inactivation performance, i.e., the effect of spore inactivation under pressure to be thermal only. It remains to be proven whether synergistic effects exist, and if so, to what extent. This is challenging mainly because temperature profiles in high pressure thermal processing are very different to those obtained in thermal-only processing.

To obtain near-identical temperature profiles, two metallic containers, able to carry sample media under pressure, were purpose-designed so that only one of the samples in the devices undergoes high pressure changes, while the second one was kept close to atmospheric pressure throughout the process. Temperature data was successfully matched in both devices giving very similar F values at various ramping rates. These unique devices will enable investigating the effect of high pressure application on the inactivation behaviour of bacterial spores upon exposure to elevated temperatures based on near-identical temperature profiles.

Keywords: high pressure processing, high pressure thermal processing, microbial spore inactivation, synergy, pressure, temperature

1. INTRODUCTION

High pressure in combination with heat is claimed to be more effective for microbial spore inactivation than conventional thermal processing. It has the potential to deliver novel chill or shelf stable products with retained sensory properties by using either reduced temperatures or shorter processing times compared to heat processing alone.

To determine whether the pressure application shows synergy, equivalence or even protection of the bacterial spores, earlier work compared spore inactivation in a retort (conventional thermal processing) and a high pressure thermal process based on the same *F* values, i.e., equivalent thermal load (Bull, Olivier, van Diepenbeek, Kormelink, and Chapman, 2009). However, the very different temperature profiles in these two processes may affect spore inactivation behaviour.

To be able to investigate the pressure effect on spore inactivation, near identical temperature profiles of the spore samples are essential. This work presents two systems able to provide near-identical temperature profiles to demonstrate microbial kill.

2. MATERIALS AND METHODS

2.1 Experimental Setup

High pressure experiments were conducted in a Stansted ISO-LAB FPG11501 High-Pressure 3.6 L unit (Stansted Fluid Power Ltd., Stansted, Essex, UK; Figure 1(a)).

Two cylindrical devices, which can resist high hydrostatic pressures normally used in a high pressure unit (i.e., in the order of 600 MPa), were designed and manufactured from high tensile strength aircraft aluminium, allowing near-identical temperature profiles to be applied to spore samples sealed inside each device. A “pressure on” device transmits pressure to the sample through a channel sealed off with a moveable piston, while the ”pressure off” device shields the sample from pressure, i.e., maintains the sample close to atmospheric pressure (Figure 1(b)). The containers were filled with spore solutions prior to pressurisation.

Temperature profiles in the “pressure on” device were measured with T-type thermocouples inserted through the channel in the pressure-transmitting sample holder and the “pressure off” device contained a Thermochron iButton® DS1922T temperature logger (Maxim Integrated

Products, Inc., Sunnyvale, CA, USA), which was logging temperatures during the high pressure experiment.

Figure 1: (a) Stansted High Pressure unit utilised in this study; (b) "Pressure on" (left) and "Pressure off" (right) devices manufactured from high tensile strength aircraft aluminium for the investigation of the high pressure effect on thermal spore inactivation.

2.2 Process Conditions

The effect of the pressurisation rate during the compression and decompression stage of the high pressure process was evaluated. Temperature increase due to the compression heating created in the "pressure on" device was avoided by ramping the pressure slow enough to allow the compression heat to dissipate into the aluminium so that closely matched temperature profiles between the two devices can be achieved. Pressure ramp rates from 100 MPa/min up to 600 MPa/min were investigated at initial sample temperatures between 70 and 90°C.

2.3 Performance Evaluation

In order to evaluate the performance and closeness of temperature profiles in both systems, the measured temperature data from experiments at the selected process conditions were plotted over time and qualitatively compared.

Furthermore, a coefficient of determination (R^2) was calculated from the data sets extracted from the containers with and without pressure transmission channels according to equation 1.

This coefficient is a measure of the goodness of fit of the respective pair of temperature profiles.

$$R^2 = 1 - \frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2} \qquad (1)$$

where f_i are the temperature values from the sample holders with channel (open), y_i the temperature values from the sample holders without channel (closed) and $\bar{y}$ the mean of the values from the temperature values of the closed sample holders. Swapping the temperature data from open and closed sample holders did not change the value of R^2.

The systems will eventually be used to determine differences in microbial spore inactivation and, therefore, to evaluate the potential synergistic effect of pressure and temperature, therefore, thermal death times (F values) were calculated according to equation 2 and compared. This approach facilitates the comparison of spore inactivation due to the thermal component of the entire process, and allows comparison of spore inactivation independent from process conditions, e.g. maximum temperatures and temperature hold times, which have to be adjusted for each new organism investigated according to their resistance.

For comparison of the thermal death times in the temperature evaluation studies a target temperature of 110°C and a z-value of 10°C were selected. This z-value is representative for a number of organisms, including the spores of proteolytic *Clostridium botulinum*.

$$F = \int_0^t 10^{\frac{T(t) - T_{ref}}{Z_T}} dt \qquad (2)$$

where F is the thermal death time, i.e., time the product would be held at a T_{ref} of 110ºC to achieve the same microbial inactivation of the process, Z_T is the temperature increment that changes the rate of the (inactivation) process by a factor of 10, in this case Z_T = 10°C.

3. RESULTS

Temperature was successfully measured in both devices. Figure 2 shows the temperature profiles in both devices at pressure ramp rates (compression and decompression) of (a) 600 MPa/min and (b) 300 MPa/min. Initial temperature was 87°C and 84°C, respectively; pressure hold time was set to 5 min.

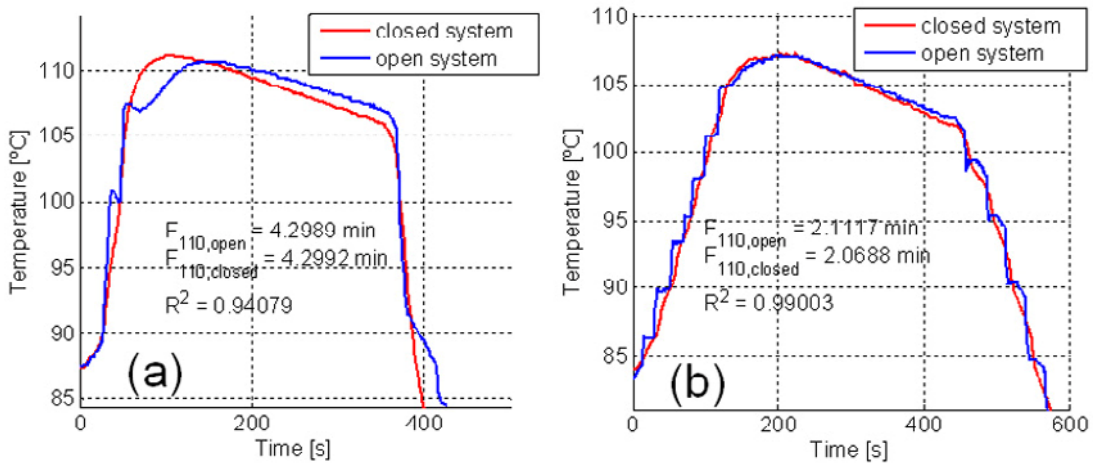

Figure 2: Temperature profiles recorded in both systems at a pressure rate of (a) 600 MPa/min and (b) 300 MPa/min.

As can be seen, although the *F* values at a target temperature of 110°C are almost identical for the experiment at a ramp rate of 600 MPa/min, the temperature profiles exhibit pronounced differences. Reducing the ramp rate to 300 MPa/min also reduced the differences in the recorded temperature profiles, while still delivering *F* values at target temperature of 110°C which are very close. Additionally, the coefficient of determination could be increased from 0.94 to 0.99, indicating a very close fit. Reducing the ramp rate further did not noticeably improve the temperature profiles (data not shown).

4. CONCLUSION AND OUTLOOK

Pressure "on" and "off" devices were able to provide near-identical temperature profiles in high pressure thermal processing experiments. Pressure ramp rates greater than 300 MPa/min provided more pronounced differences in the temperature profiles compared to those below 300 MPa/min. Future research will use these unique systems to determine, for the first time, whether, and to what extent, a synergistic effect of combined pressure and temperature

application exists for inactivation of relevant microbial spores, e.g., proteolytic and non-proteolytic *C. botulinum*, among spores of other pathogenic and spoilage bacteria.

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BIOTECHNOLOGICAL PROPERTIES OF YEASTS ISOLATED FROM FERMENTATION PROCESSES

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ABSTRACT:

In recent years there has been an increasing interest in characterizing the yeast population associated with fermentative processes in order to identify strains with interesting biotechnological applications. The objective of the present work was to investigate the extracellular enzymatic activity and antimicrobial potential of yeast strains isolated from olive fermentation. In this study 30 yeast isolates belonging to the genera *Cryptococcus*, *Candida*, *Pichia* and *Sporobolomyces* and 8 isolates of *Aureobasidium pullulans* were screened for the production of pectinases, amylases, xylanases, lipases, proteases and glycosidases. The antimicrobial activity of the isolates was also tested against yeast and foodborne bacteria. The majority of the *A. pullulans* isolates produced amylases and pectinases and was able to hydrolyze olive oil. None of the isolates tested were capable of degrading xylan or proteins but all produced catalases. Antibacterial activity against *Staphylococcus aureus* was detected in some isolates pertaining to the genera *Candida* and *Cryptococcus*. However, none of the isolates were effective against *E. coli*, *Salmonella* and *Cronobacter sakazakii*. This study revealed the potential of some isolates of the species *C. membranaefaciens* as inhibitors of some foodborne pathogens in the fermentation of olives.

COMPARISON OF DIFFERENT LINEAR AND NON-LINEAR TECHNIQUES IN THE GENDER DETERMINATION OF MACKEREL (*Scomber scombrus*) BY COLORIMETRY

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ABSTRACT

Mackerel is an infravalored fish captured by European fishing vessels. However, their female gonads have a high market value. Due to the absence of any external differences between mackerel males and females the unique solution for sex determination is looking atthe color of the fluid excreted when pressing the abdominal zone where the gonads are localized. To date,this classification is completely manual.This process entails some disadvantages, such as the increase of cost and the decrease of product quality, process productivity and efficiency. A manner to add value to the exploitation of this species can be achieved by trying to classify the samples automatically attending to their sex. The aim of this study was to find a combination of colorimetry measurements and multivariate data analysis techniques that could allow a further automatic classification with high level of accuracy.

The reflectance spectra of fresh and defrozen mackerel were collected and fourteen color parameters were analyzed in order to obtain differences between sexes.Then,several linear and non-linear classifiers such as DLDA (Diagonal Linear Discriminant Analysis), k-NN (k-Nearest Neighbor) and SVM (Support Vector Machines) were applied to this problem.

The experimental results suggest that non-linear classifiers such as SVM outperforms better than linear classifiers for both fresh and defrozen mackerel. SVM based on RBF (radial basis function) kernel improves the results of linear classifiers. Thus, this technique shows potential to be applied in an automatically classification process

Keywords pattern recognition, modeling, classification, color measurements, sensor neural networks

1. INTRODUCTION

Mackerel (*Scomber scombrus L. 1758*) is pelagic species of medium sizeplentiful in the Northeast Atlantic waters. In Basque Country, the mackerelhas certain economic importance in the traditional fishery [19]. The idea ofdistinguishing and selecting fish species is justified by the high value of female roescompared with the price of the fish. There are several methods developed todifferentiate the sex of different species. In this study, a minimally invasivemethod that can be implemented in line and on-line has been chosen.This method is based on the color difference between the gonads of maleand female fish consists in a sensor that allows, after an appropriate signalprocessing, characterizing the spectrum obtained after the incidence ofelectromagnetic radiation into the gonads.

A large variety of machine learning techniques have been proposed suchas Support Vector Machines (SVM), k-Nearest Neighbors or DiagonalLinear Discriminant Analysis (DLDA). However, the algorithmsconsidered in the literature rely frequently on the use of the Euclidean distancethat fails often to reflect accurately the proximities among the sample profiles [16, 17]. The classifiers mentioned above have been extended to workwith non-Euclidean dissimilarities [20]. In spite of this, the resulting algorithms misclassify a different set of patterns and fail to significantly reduce the errors. This can be explained because each dissimilarity reflects differentfeatures of the data and they induce different type of errors.

In this paper, we build the diversity of classifiers considering three different kinds of models such as SVM, k-NN and DLDA. The diversity isincreased considering a set of complementary dissimilarities for each model.In order to incorporatenon-Euclidean dissimilarities the base classifiers are modified in anappropriate way.The method proposed has been applied to the mackerel genderdetermination with remarkable results.

2. MATERIAL AND METHODS

2.1 Data acquisition

Mackerel (*Scomber scombrus*) was obtained directly from Basque fishing. The fish size tested was within the range 0.35-0.50 kilograms per fish unit. Samples were captured during their best period regarding to the maturity state of roes (from February to April). The tests were made both in fresh (n=1006) and defrozen mackerel (n=1439). The fish was defrosted at 5ºC

and both the fresh and defrozen samples were kept at 5ºCuntil analyzed. The gonads, which differ between females and males in terms of their color, were obtained by extraction from the whole fish.

Color measurements were performed on manually extracted gonads from mackerel females and males. The reflectance spectra were collected and twelve color variables (L, a, b, c, h, X, Y, Z, dL, da, db, and dE) were analyzed to characterize differences between both sexes.

The equipment used to collect the color data is composed by a spectrometer (AvaSpec-102-USB2-SPU2 Avantes, Netherland), a light source (AvaLight -HAL-S, Avantes, The Netherlands) and a fiber optic probe (FCR-7UV200-5-1.5x55 SR, Avantes, The Netherlands) composed of 7 optical fibers of 200um core (6 light-fibers and1 read fiber). The reflected spectra, which is representative of the color, acquisition and processing were integrated in Avasoft7USB2 (Avantes, The Netherlands) which includes the data processing software(AvaSoft-FULL, AvaSoft-COL and AvaSoft-PROC, Avantes, The Netherlands).

2.2 Dissimilarities for mackerel data analysis

An important step in the design of a classifier is the choice of a properdissimilarity that reflects the proximities among the objects. However, thechoice of a good dissimilarity is not an easy task. Each measure reflects different features of the data and the classifiers induced by the dissimilarities misclassify frequently a different set of patterns. Therefore, no dissimilarity outperforms the others.In this section, we comment briefly onthe main differences among several dissimilaritiesproposed to evaluate the proximity between spectrum samples.

Let $x= \{x_1,\ldots,x_d\}$ be the vectorial representationof a sample where x_i is the spectrum obtained for the wavelength i:

- The Euclidean distance evaluates if the spectrums obtained differ significantly across different samples.

$$d_{\text{euclid}}(\vec{x},\vec{y}) = \sqrt{\sum_{i=1}^{d}(x_i - y_i)^2}\,, \qquad (1)$$

- The correlation measure evaluates if the spectrums change similarly in bothsamples. Correlation based measures tend to group together samples whichspectrums are linearly related.

$$d_{\mathrm{cor}}(\vec{x},\vec{y}) = 1 - \frac{\sum_{i=1}^{d}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{d}(x_i-\bar{x})^2}\sqrt{\sum_{j=1}^{d}(y_j-\bar{y})^2}}, \quad (2)$$

- TheSpearman rank dissimilarity is less sensitive to outliers because itcomputes a correlation between the ranks of the spectrums.

$$d_{\mathrm{spearman}}(\vec{x},\vec{y}) = 1 - \frac{\sum_{i=1}^{d}(x'_i-\bar{x}')(y'_i-\bar{y}')}{\sqrt{\sum_{i=1}^{d}(x'_i-\bar{x}')^2}\sqrt{\sum_{j=1}^{d}(y'_j-\bar{y}')^2}}, \quad (3)$$

where $x'_i = \mathrm{rank}(x_i)$ and $y'_j = \mathrm{rank}(y_j)$.

- The chi-squared distance is a measure proposed for the analysis of the contingencytables. This measure can be seen as a generalization of theEuclidean distancebut considering weights that control the relevance of each variable

$$d_{\chi^2}(i,j) = \sum_{k=1}^{d}\frac{1}{x_{.k}}\left(\frac{x_{ik}}{x_{i.}} - \frac{x_{jk}}{x_{j.}}\right)^2 \quad (4)$$

In order to calculate the distance, the rows and columns of table have to be represented by their profiles which being given by:

$$p_i = x_i / x_{i.} \;\; ; \; p_j = x_j / x_{j.}$$

where $x_{i.}$ and $x_{.j}$ can be given by

$$x_{i.} = \frac{1}{p}\sum_{l=1}^{p} Xil \quad ; \quad x_{.j} = \frac{1}{p}\sum_{l=1}^{p} Xlj$$

Due to the large number of wavelengths, the sample profiles are codedin high dimensional and noisy spaces. In this case, the dissimilarities mentionedabove are affected by the `curse of dimensionality'. Hence, most ofthe dissimilarities become almost constant and the differences among dissimilaritiesare lost [15]. To avoid this problem, it is recommended to reduce thenumber of features before computing the dissimilarities.

2.3 Diagonal Linear Discriminant Analysis (DLDA)

This model easily handles the case where the within-class frequencies are unequal and their performances have been examined on randomly generated test data. This method maximizes

the ratio of between-class variance to the within-class variance in any particular data set thereby ensuringmaximal separability.

Under this assumption, the Bayes optimal solution is to predict points as being from the second class if the log of the likelihood ratios is below athreshold T, so that:

$$(x - \mu_0)T \sum\text{-}1y=0 (x - \mu 0) + \ln | \sum y=0| - (x - \mu_1)^T \sum y=1\text{-}1 - (x - \mu_1) - \ln | \sum y=0| < T \quad (5)$$

LDA focus the classification problem assuming that the conditional probability density functions are normally distributed p(x|y= 0) and p(x|y =1) with mean and covariance(μ_0, $\sum y=0$) and (μ_1, $\sum y=1$). The algorithm assumes homoscedasticity (equal covariances).

2.4.Support Vector Machines (SVM)

The SVM looks for a hyperplane $f(x,w) = w^Tx+b$ that maximizes the margin $\gamma = 2 / ||w||2$. γ determines the generalization ability ofthe SVM. The 'slack' variables allow the classificationerrors. The SVM can be extended easily to the non-linear case replacing the scalar products by Mercer kernels [21]. Besides, it can work with non-euclidean dissimilarities through the kernel of dissimilarities[21] or embedding the data in an Euclidean space via the multidimensional scaling algorithm [6].

2.5k-Nearest Neighbor

The classifier k-Nearest Neighbor (k-NN) finds a group of k objects in the training set that are closest to the test object, and bases the assignment of alabel on the predominance of a particular class in this neighborhood. Thereare three key elements of this approach: a set of labeled objects, a distanceor similarity metric to compute distance between objects, and the value of k,the number of nearest neighbors.

To classify an unlabeled object, the distance of this object to the labeledobjects is computed, its k-nearest neighbors are identified, and the class labelsof these nearest neighbors are then used to determine the class label of theobject.

Given a training set D and a test object x = (x, y) where x is the trainingdata and y the target. The algorithm computes the distance between z andall the training objects (x, y) D to determine its nearest neighbor list, Dz. Once the nearestneighborlist is obtained, the test object is classified based on the majorityclass of its nearest neighbors.

2.6 Dissimilarity based Classifiers

Classical Support Vector Machines (SVM) [21] and Diagonal Linear Discriminant Analysis (DLDA) [8] are not able to work directly from a dissimilarity matrix. The classical SVM algorithm is extended to work from a dissimilarity matrix by defining a kernel of dissimilarities. Next, DLDA is adapted following a different approach by embedding the patterns in a Euclidean space.It has been suggested in the literature that selecting a smaller subset of representatives does not help to improve the resulting classifier [20].

3. EXPERIMENTAL RESULTS

The classifiers proposed are applied to the genderdetermination of mackerel (*Scomber scombrus*). Mackerel obtained directly from Basque fishing ports was used to test the ensemble.The sampleswere analyzed in their best maturity stages (from February to April).
Two datasets from mackerel have been considered. The first consistedoffresh mackerel samples (less than 24-48 hours after its landing). 1006samples were analyzed captured from 2007-2010. The colorparameters were studied to determine effects of sex to determine the mackerel gender.

The second dataset consisted of 1439 samples of defrozen mackerel.The dissimilaritieshave been computed without normalizing the variables because this operation may increase thecorrelation among them.

The algorithm chosen to train the Support Vector Machines is C-SVM. The C regularization parameter has been set up by ten-foldcrossvalidation [4].We have considered non-linear kernels. The kernels considered in this paper are the following ones:

- The polynomial kernel is defined as follows:

$$k(\vec{x}, \vec{y}) = (\langle \vec{x}, \vec{y} \rangle + c_0)^d \quad (6)$$

Where c_0 is a constant and d is the degree of the polynomial. Thepolynomial kernel has a finite VC (Vapnik-Chervonenkis) dimensionand the complexity depends on the exponent d. It isa global transformation that has no null value throughout the space. It issemidefinite positive provided that c_0 be >= 0.

- The RBF kernel is defined as follows:

$$k(\vec{x},\vec{y}) = \exp\{-\gamma\|\vec{x}-\vec{y}\|^2\} \quad (7)$$

Where ϒ determines the complexity of the non-linear transformation.The RBF kernel has a finite VC dimension and the complexity dependson the complexity of the parameter. It is a local non null kernel andpositive semidefinite. High values give place to strong non-linear transformations.

TABLE1: Empirical results for the best dissimilarity based classifier for fresh mackerel.

Technique	Error (%)	False negative (%)	False positive (%)
SVM RBF(Cor)	6.75	4.57	2.18
K-NN(Cor)	8.34	5.06	3.28
DLDA(Man)	9.04	4.97	4.07

TABLE2: Empirical results for the best dissimilarity based classifier for defrozen mackerel.

Technique	Error (%)	False negative (%)	False positive (%)
SVM RBF(Euc)	3.12	1.56	1.56
K-NN(Chi)	3.26	1.63	1.63
DLDA(Chi)	3.97	2.00	1.97

Table1 and Table 2compare linear and non-linear classifiers for the best dissimilarityfor frozen and defrozen mackerel and the following conclusions can be drawn:

- The dissimilarity that minimizes the error dependsstrongly on the classifier and on the dataset considered.
- No dissimilarity outperforms the others for the models and datasets considered.The choice of a proper dissimilarity is not an easy task for human experts.
- SVM algorithms based on nonlinear kernels improve the best KNN classifier for both fresh and defrozen mackerel.
- Regarding the DLDA, both k-NN and SVM outperforms this classifier considering the best dissimilarity.
- Due to the interest of the industry in the mackerel gender determination, we report both, false and positive errors. The importance of the error depends strongly on the problem at hand.

- The algorithm performs better for defrozen mackerel obtaining an accuracy approaching 100 %.

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A FRAMEWORK FOR MODELING FREEZE DRYING FOOD PROCESSES USING CFD

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ABSTRACT:

The development of a framework for modeling freeze drying processes in ANSYS Fluent using Computational Fluid Dynamics (CFD) principles is described. The simulated physical system consists of frozen cubes of solid food (such as potato) placed on a heated plate in a low pressure drying chamber. In the model, the food domain was represented as a porous medium with the porosity increasing with time as ice sublimes. The immediate vicinity of the cube was modeled as a gaseous domain with constant temperature and pressure at its boundaries. Because of the absence of a sublimation model in Fluent, the sublimation rate and the corresponding evolution of porosity were implemented through User-Defined Functions (UDFs). Sublimation was assumed to occur in cells where the vapor pressure at the cell temperature was greater than the partial pressure of the vapor provided that adjacent cells allowed the produced vapor to escape. No a priori assumptions on the shape and evolution of the sublimation surface were made. Through the solution of the model dynamically in time, the retreat of the sublimation surface was observed and the drying time was recorded. The framework is intended to be used as basis for the development of freeze-drying models for specific food systems and envisioned as a means to enhance our understanding of the mechanisms involved and their contribution in the progression of freeze drying.

Keywords: freeze-drying, CFD, food process modeling

1. INTRODUCTION

Freeze-drying or lyophilization is the process by which frozen material is dried through the sublimation of ice under low pressure. The procedure consists of the following three stages: freezing, sublimation (or primary drying) and desorption (or secondary drying). Initially, the material is cooled below freezing temperature, causing a physical separation of the contained

water as ice from the solids. Ice is then removed from the product by direct conversion to vapor at vacuum pressure and transfer of the water vapors through the pores of the dried product.

Freeze drying is the gentlest process for preserving food stuff yielding high quality dried products. Because of the absence of water in liquid phase, shrinkage is low and solutes do not migrate to the surface (Bellows and King, 1973). The dried product has a light porous structure, which facilitates rehydration (Krokida and Maroulis, 2000). The temperature to which the product is exposed is lower than in most other methods of drying, so heat damage is relatively low. There is also good retention of volatile flavor compounds (Flink and Gejl-Hanse, 1972). However, some damage to the structure may occur during freezing which can result in some structural collapse on rehydration and a poor texture in the rehydrated product. Additionally, freeze-drying is considered as the most expensive method of drying food materials due to the relatively high energy and capital costs of freeze-drying equipment and also because of the long times required for its completion.

A suboptimal freeze-drying process may compromise food stability, take longer and entail higher costs. In order to design an optimum freeze-drying process, there is a need to know the optimum operating conditions of the process, such as chamber pressure, shelf temperature, product temperature which should always be several degrees below the collapse temperature of the product etc. (Tang and Pikal, 2003). One of the recently applied methods of research for optimizing the design of freeze-drying processes is Computational Fluid Dynamics (CFD). CFD is a state-of-the-art numerical technique for solving fluid flow problems. CFD calculations use a computational grid to solve the governing equations describing fluid flow, i.e., the continuity equation and any additional conservation equations, such as momentum and energy balance, across each grid cell by means of an iterative procedure in order to predict and visualize the profiles of velocity, temperature, pressure, etc. There are several examples of implementing CFD in food processes such as mixing, drying and refrigeration. Such processes are used regularly to enhance quality, safety, and shelf life of foodstuffs and have been evolving over the years to become more efficient (Wang and Sun, 2003). Various applications of CFD in food processing are presented by Sun (2007).

CFD has also been used for the modeling of the freeze-drying process in an attempt to predict the effect of operating conditions, such as chamber pressure and plate temperature, on the duration of the primary drying phase (Velardi and Barresi, 2008). Li et *al.* (2007) developed a model for atmospheric freeze-drying process of 10-mm apple cubes that enables the

simulation of phase change and water vapor diffusion process within the porous media. They used the Uniformly Retreating Ice Front (URIF) model which was first presented by King (1971) and is based on the assumption of equilibrium at the ice front interface. Cheng et *al.* (2002) developed a mathematical model for a freeze drying process with or without back surface heating by a combination of URIF and the theory developed by Sheng and Peck (1975), which takes into account the desorption of the bound water and predicts the drying rates with higher precision. Nam and Song (2007) studied numerically the freeze drying characteristics of planar and slab-shaped food products considering the conjugate heat and mass transfer, sublimation of ice, and motion of the sublimation interface. The design of freeze-drying devices is an important topic in the analysis of the freeze-drying process. Research work has also been focused on the study of the effect of shelf flow channel upon heat transfer performance of dried substances as the shelf is heated in the vacuum freeze-dryer, in order to dry materials with minimum energy (Cheng et *al.*, 2009). Furthermore, the duct connecting the chamber and the condenser can be used as an in-process mass flow meter to measure sublimation rate in real time during primary drying without interrupting the process (Patel et *al.*, 2010). Thus, the development of CFD applications on the freeze-drying process can lead to more accurate monitoring, control and optimizing of this process.

In this paper, we present an attempt to create a framework for developing CFD models of freeze-drying in Fluent^{TM} 14.0 (ANSYS, Inc., Canonsburg, PA, USA) without making any assumptions (such as URIF) about the shape or the retreat of the sublimation interface and no a priori distinction between the frozen and dried layers.

2. MODEL DEVELOPMENT

2.1 Physical System

Freeze-drying experiments were conducted with the use of a Gamma 1-20 lab-scale lyophilizer (Martin Christ GmbH, Osterode, Germany). Potato cubes of 1 cm^3 in volume were placed on the top tray of the lyophilizer, they were frozen to a preselected temperature and then vacuum was applied. The vacuum pressure was set to correspond to the saturation vapor pressure of ice at the chamber temperature. Heating of the tray to a given temperature was also initiated when vacuum was applied. At regular intervals during the process, cubes were taken out of the chamber to visually inspect the progression of the sublimation interface and

determine the end time of primary drying. During the process, the product temperature cannot exceed the collapse temperature, which for potato cubes is 261 K (Fellows, 2000).

2.2 Model Setup and Assumptions

A 2-D CFD model of the process was developed in Fluent to predict the retreat pattern of the sublimation interface during primary drying and the evolution of operating conditions within the freeze-dried food. The objective was for the sublimation interface to appear and evolve through the solution of the model and not have it predefined as an integral entity of the model structure. To account for any effects of pressure and temperature variations in the chamber, the flow domain was expanded outside the food cube to include the cube's immediate vapor phase vicinity. It was assumed that the temperature and pressure at the domain boundaries were equal to the freeze dryer chamber settings. The solution domain and the corresponding grid are shown in Figure 1.

The domain representing the frozen food was modeled as a porous zone with the flow equations solved only in the porous part of the food domain. In the development of the porous model, an important issue proved to be the handling of ice. After several failed attempts to treat ice as a fluid component occupying the pores along with water vapor, it was concluded that the best approach was to consider ice as part of the solid matrix. This meant that porosity, γ, could not be considered constant but it had to vary in time and in space to account for the sublimation of ice.

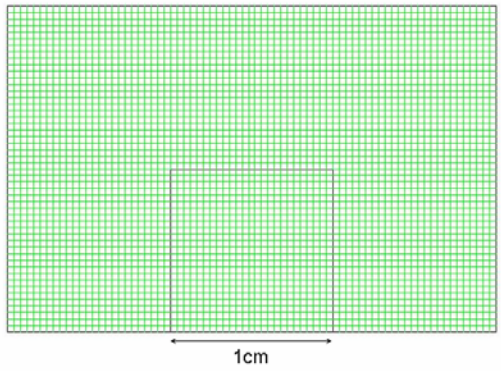

Figure 1. The modeled flow domain and computational grid.

Through a simple balance that equates the volume of sublimed ice with the increase in porosity, the following equation can be obtained to represent the change in porosity, $d\gamma$, due to sublimation:

$$V\frac{d\gamma}{dt}=\frac{S}{\rho_{ice}} \qquad [1]$$

(S is the sublimation rate, in kg/s, ρ_{ice} is the ice density and V is the cell volume.) Equation [1] can be integrated to yield the values of γ at every time point. In Fluent it was implemented through a User-Defined Function (UDF) and was solved in every cell of the domain and at every time step of the numerical solution process to yield the local value of porosity.

The difference between the saturation vapor pressure of ice and the partial pressure of water vapor is the driving force for sublimation (Tang and Pikal, 2004). The sublimation rate S of ice was thus modeled as:

$$S=\frac{P^{sat}(T)-P^{w}}{R} \qquad [2]$$

where, P^{sat}(T) is the saturation vapor pressure at temperature T, P^{w} is the partial pressure of water vapor, and R an adjustable parameter. Equation [2] is a simplification of the true equations governing sublimation rate with all resistances lumped for convenience in one parameter. Equation [2] was implemented again through a UDF and solved in every cell of the domain and at every time point using the local cell temperature and the local partial water pressure. The computed value of S was inserted as a source term in the continuity equation to account for the generation of water vapor in the fluid porous region. To activate sublimation in a given cell the following conditions have to hold:

- saturation vapor pressure at the cell temperature must be greater than the vapor partial pressure,
- in the immediate neighborhood of the cell there must exist at least one dried cell.

The last condition stems from the fact that for ice to sublime, there must be a path for the generated vapor to diffuse out and this is not possible if a cell is well within the still frozen volume. To account for transport resistances through the pores, terms representing viscous resistance were included in the momentum equations in the porous zone.

From early modeling results it became obvious that initial inert gases present in the chamber are removed rather quickly through the application of vacuum and the chamber is practically filled with water vapors from that point on; this conclusion is also in agreement with similar observations by Tang and Pikal (2004) and Nam and Song (2007). Therefore, the fluid (gas)

phase in the porous zone and the cube surroundings was considered to be comprised of water vapor only.

Following the previously described experimental setup, it was assumed that heat was provided only through the plate at the bottom of the cube (no heat radiation effects were considered since the samples were placed on the top tray.) The contribution of the sublimation process in the energy balance equation was modeled through a negative source term also implemented by a UDF. With respect to fluid properties, the ideal gas law was considered for the fluid phase. The properties (density, specific heat and heat conductivity) of the solid phase were calculated as weighted averages of potato and ice properties and implemented through UDFs. In each cell, the volume fraction of solid potato was assumed to be constant at 0.15 while that of ice was dependent on the local porosity and was set to 0.85-γ.

2.3 Model Equations

The physical system described above was modeled by Fluent using the partial differential equations representing mass, momentum and energy balances. The mass and energy balance equations are presented below to emphasize the presence of the relevant source terms representing the vapor generated and the energy removed due to ice sublimation.

Mass Conservation (Continuity) Equation

$$\frac{\partial(\gamma\rho)}{\partial t} + \nabla \cdot (\gamma\rho\vec{u}) = \gamma S_m$$

where, S_m is the user-defined mass added source representing vapor generation due to sublimation.

Energy equation

$$\frac{\partial}{\partial t}\left(\gamma\rho_f E_f + (1-\gamma)\rho_s E_s\right) + \nabla \cdot \left(\vec{u}\left(\rho_f E_f + P\right)\right) = \nabla \cdot \left(k_{eff} \nabla T\right) + S_f^h$$

where, E_f and E_s are the total fluid and solid medium energy respectively, S^h_f is the (negative) energy source term corresponding to energy loss due to sublimation, and k_{eff} is the effective conductivity which is computed as the volume average of the fluid conductivity and the solid conductivity:

$$k_{eff} = \gamma k_f + (1-\gamma)k_s$$

The starting point of the simulation was the time point when vacuum was set in the chamber and heating was applied to the cubes (i.e. the beginning of the primary drying phase). The initial temperature and pressure within the entire solution domain were set to those of the freeze-dryer chamber.

3. RESULTS

The simulation results for a freezing temperature of -24°C (corresponding to a saturation vapor pressure of approximately 71 Pa) and a hot plate temperature of 20°C are presented here. Figure 2 shows the evolution of the sublimation interface through the porosity plots at various time points. Areas outside the sublimation curve represent dried cells (with porosity 0.85) while the area inside the curve is the still frozen potato (with almost zero porosity).

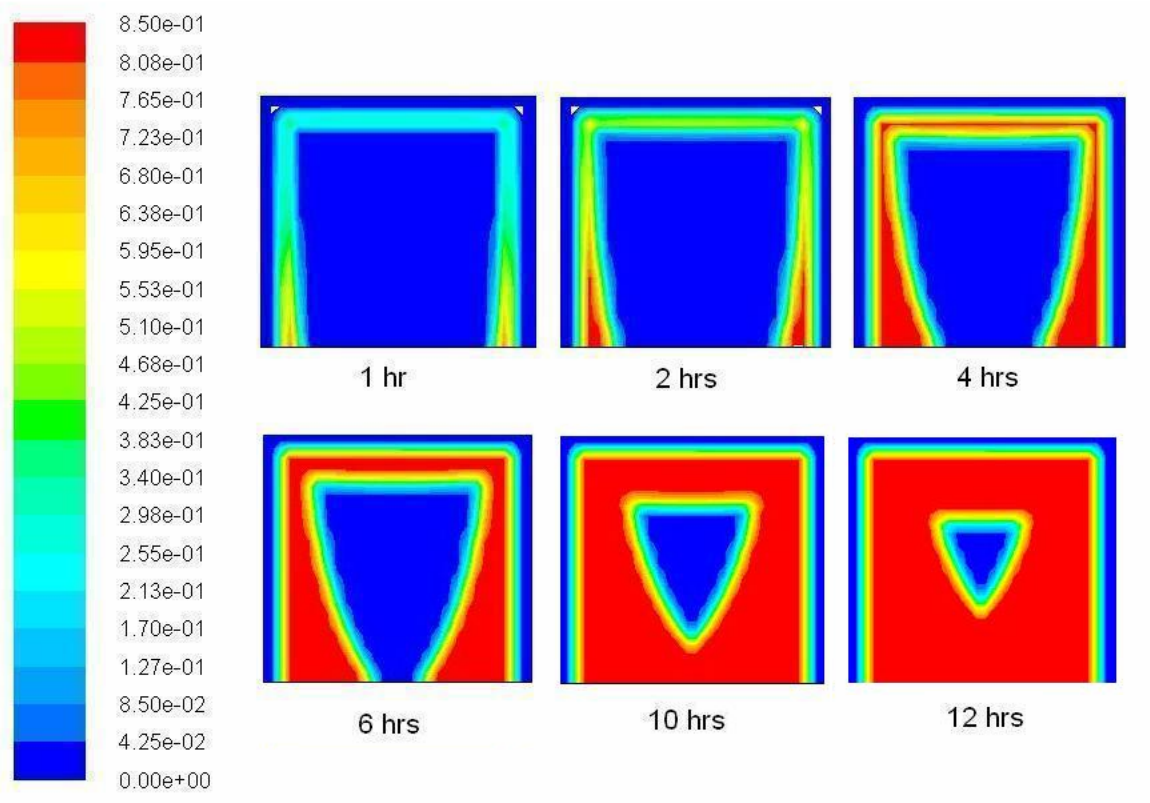

Figure 2. The evolution of porosity (and the sublimation interface) at different time points.

Unlike previously reported results where the sublimation interface retreats almost uniformly around the cube, the results shown in Figure 2 show that sublimation proceeds faster at the bottom of the cube as compared to the top. This is due to the assumption that heat was exclusively transferred through the bottom surface.

Although comparison of simulated with experimental results is beyond the limited scope of this paper, it can be noted that the simulation time for completion of the process (approximately 13 hrs) is very close to the times (11.8-12.7 hours) obtained experimentally.

Figure 3 shows the velocity vector field and the pressure profile in the flow domain after 4 hours into the process. The diffusion of vapor within the food cube and the chamber can be clearly seen.

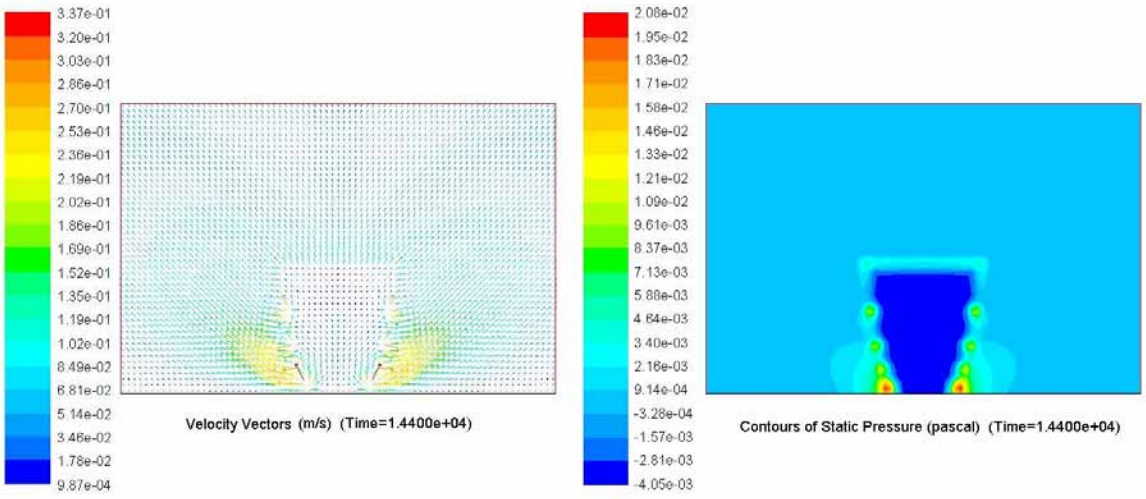

Figure 3. The velocity vector field and the pressure profile after 4 hours.

4. CONCLUSIONS AND FUTURE WORK

The generation of a CFD modeling framework of freeze-drying was presented. An attempt was made to make as few as possible a priori assumptions about the rate of the progression of the phenomenon and have as few as possible adjustable parameters. Initial results look promising and indicate that the framework could be used as a basis for developing freeze-drying models for specific food systems with different properties and geometries. After the model is validated with detailed experimental data, it is hoped that it can become the basis for better understanding of how different mechanisms and conditions involved in the process affect the rate at which freeze-drying evolves.

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METAL CONTENTS IN HONEY AND MUSHROOMS FROM SERPENTINE SOILS IN THE MORAIS SITE, PORTUGAL.

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ABSTRACT:

Morais site, located in the northeast of Portugal, is known by its geology because it is one of the most representative areas of ultramafic rocks. This kind of soils has particular chemical characteristics, such as high levels of some heavy metals, namely chromium (Cr) and nickel (Ni). However, in Morais site several non-wood forest products (NWFP) are collected by the local community, and, so, it is of great importance to determine the contents of these metals in such food products. In this connection, in the present work three honeys of Morais site (additionally three commercial honeys – control), and nineteen mushroom species collected on four locals were analyzed in relation to calcium (Ca), magnesium (Mg), manganese (Mn), Cr and Ni contents.

The honeys of Morais site showed higher levels of Ni in relation to the control ones. For the other metals, no differences were detected between these two types of honeys. Even though Morais honeys showed higher Ni levels than the control ones, they do not put in danger human health.

Regarding mushrooms, the *Trametes* sp. (non-edible) was the one that showed always the highest metal concentrations. In relation to the edible species, the *Suillus* sp. was the one that had the highest levels of Ca, Mn, Cr and Ni. On the other hand, the *Agaricuscampestris* showed the highest Mg concentration. Nevertheless, the metal levels found in mushrooms collected on the Morais site were identical to those reported for other sites.

Keywords: Honeys; Mushrooms; Metals; Serpentine soils.

1. INTRODUCTION

Morais site is located in the northeast of Portugal and integrates theNatura 2000 conservationnetwork(PTCON0023), occupying an area of 12878 ha. This site has high geological and botanical interest because it is one of the few places in the world where it is possible to see the formation process of the Earth, being called the "navel of the world". This area has in its core the Morais Ophiolite Complex that is one of the most representative areas of ultramafic rocks, and the largest continuous serpentine unitin Portugal. Studies performed in serpentine soils have reporteddeficiencies in certain nutrients (P, N, K, Ca), imbalances in some elements (low Ca/Mg ratios) and high concentrations of Cr,NiandMn (Fernández et al 1999; Miranda et al, 2009).

In the Morais site several non-wood forest products (NWFP) are collected, consumed and, sometimes traded by the local community. These products contribute to the sustainable management of the world's forests, to preservetheir biodiversity, and to improve income generation and food security. However, as soils of Morais may have high contents on heavy metals, it is of great importance to analyze their contents in such food products. In the present study, to infer on the effect of soil chemistry on food security we analyzed the contents ofCa, Mg, Mn, Cr and Ni in three honeys from theMorais site and nineteen mushroom species collected in four locations within the serpentine unit.

2. MATERIALS AND METHODS

2.1 Sampling

The mushrooms were collected in late October and early November 2010in four locations in the Morais site. Plant cover was dominated by holm oak (*Quercusrotundifolia*). Undergrowth was comprised mostly of common gum cistus (*Cistusladanifer*). It was given particular attention to edible species indicatedby the local community.A total of nineteen mushroom species were collected, fiveof whichedible:*Agaricus* sp., *Bovista* sp., *Fistulina* sp., *Trametes* sp., *Hygrophorus* sp., *Lepiota* sp., *Lactarius* sp., *Russula* sp., *Suillus* sp., and *Xerocomus* sp. Three honey containers were purchased to beekeepers ofMorais Site. At the same time three commercial honeys were purchased to serve as control.

2.2 Chemical Analysis

Upon arrival at the laboratory, the mushrooms were washed with ultrapure water andthe excess of water was removed with absorbent paper. Afterwards, the samples were dried at 50-60 °C and subsequently ground. One gram of each sample was digested with concentrated nitric acid in amicrowave digestion system (MARS Xpress, CEM), applying the following program: 1600 W (100%), with a 15 minutes temperature ramp and holding at 200 ºC for 15 minutes.

The honey samples were kept in the dark at ambient temperature. Ca, Mg, Mn, Ni, and Cr levels were determined by FAAS asdescribed byFrías et al. (2008).

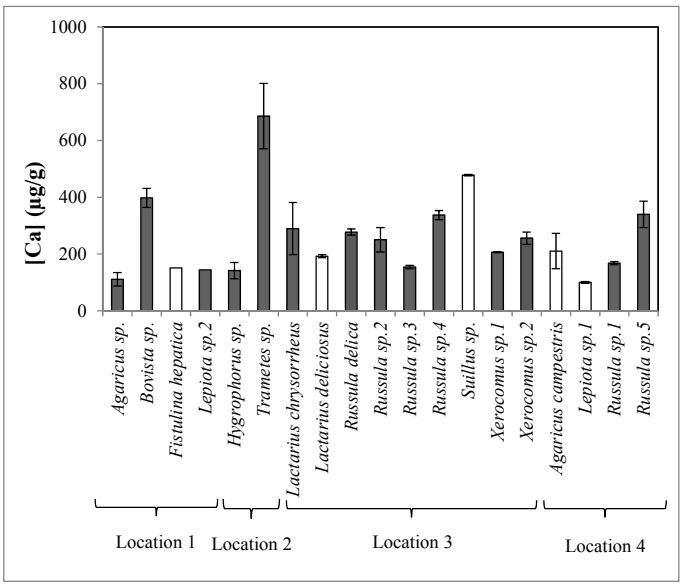
Internal matrix modifier, lanthanum nitrate hexahydrate, was added to the samples in which Ca and Mg were determined. The reliability of the method used inthe mushroomanalysis was tested with the NCS DC 73349certified reference material (Bush branches and leaves). The agreement between the analytical results for the reference material and the certified values wassatisfactory (recovery 77–110%).

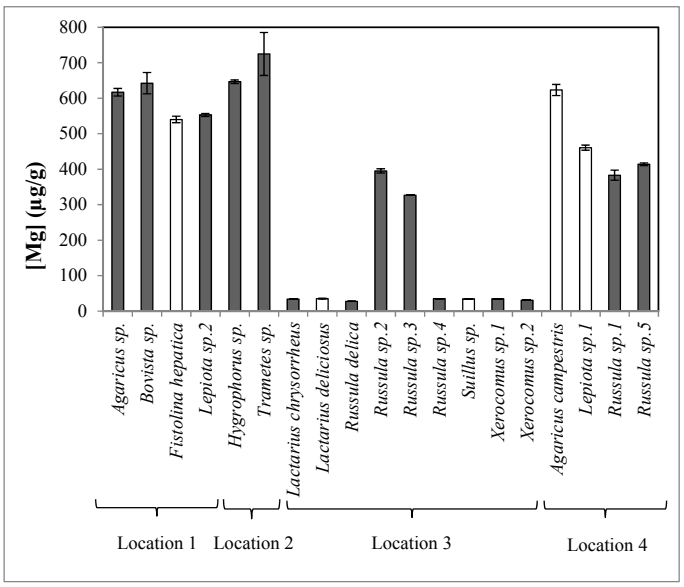
3. RESULTS AND DISCUSSION

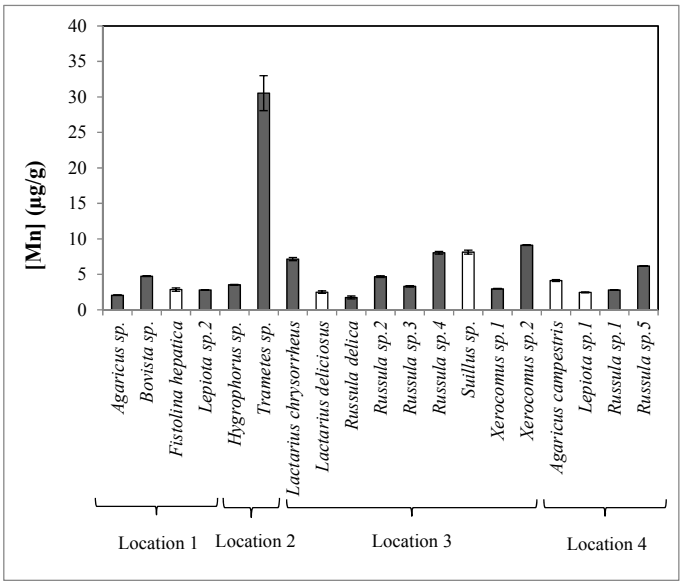
3.1 Mushrooms

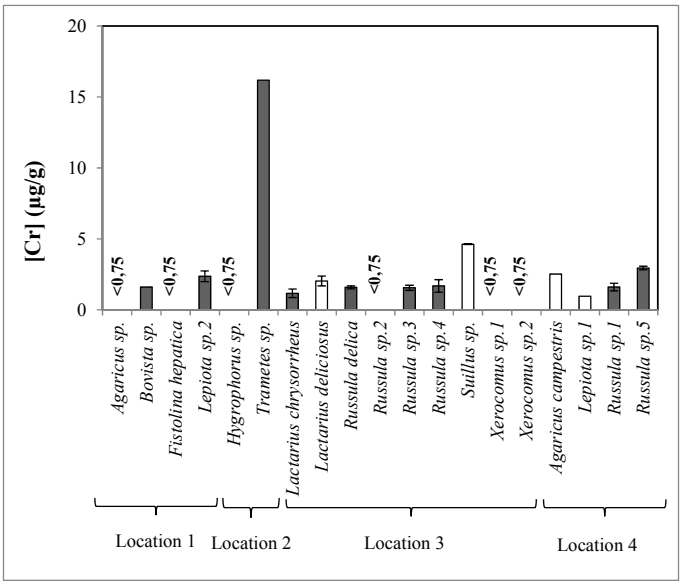
The Ca, Mg, Mn, Cr and Nicontents determined for mushrooms species are presented in Figure 1.Differences were detected amongmushroom species and collection sites. In general, it was observed that *Trametes* sp. was the speciesthat consistently presented the highest concentrations of all metals. This may be due to the fact that this speciesgrowsin decaying woodthat might have accumulated a higher amount of metals over time, passing for the mushrooms. Additionally, *Trametes* sp.are perennial allowing accumulation of metals in tissues.

In relation to the edible species, *Suillus* sp. presented the highest levels of Ca, Mn, Cr and Ni. On the other hand, *Agaricuscampestris* showed the highest Mg concentration, followed by *Fistulina hepatica* and *Lepiota* sp..*Lactariusdeliciosus* presented a lower Mg level than the speciesmentioned previously but a similar content to *Suillus* sp.; however, both mushrooms differed in other metal concentrations.


[Ca] (µg/g)
1000
800
600
400
200
0
Agaricus sp.
Bovista sp.
Fistulina hepatica
Lepiota sp.2
Hygrophorus sp.
Trametes sp.
Lactarius chrysorrheus
Lactarius deliciosus
Russula delica
Russula sp.2
Russula sp.3
Russula sp.4
Suillus sp.
Xerocomus sp.1
Xerocomus sp.2
Agaricus campestris
Lepiota sp.1
Russula sp.1
Russula sp.5
Location 1
Location 2
Location 3
Location 4


[Mg] (µg/g)
800
700
600
500
400
300
200
100
0
Agaricus sp.
Bovista sp.
Fistolina hepatica
Lepiota sp.2
Hygrophorus sp.
Trametes sp.
Lactarius chrysorrheus
Lactarius deliciosus
Russula delica
Russula sp.2
Russula sp.3
Russula sp.4
Suillus sp.
Xerocomus sp.1
Xerocomus sp.2
Agaricus campestris
Lepiota sp.1
Russula sp.1
Russula sp.5
Location 1
Location 2
Location 3
Location 4


[Mn] (µg/g)
40
35
30
25
20
15
10
5
0
Agaricus sp.
Bovista sp.
Fistolina hepatica
Lepiota sp.2
Hygrophorus sp.
Trametes sp.
Lactarius chrysorrheus
Lactarius deliciosus
Russula delica
Russula sp.2
Russula sp.3
Russula sp.4
Suillus sp.
Xerocomus sp.1
Xerocomus sp.2
Agaricus campestris
Lepiota sp.1
Russula sp.1
Russula sp.5
Location 1
Location 2
Location 3
Location 4


[Cr] (µg/g)
20
15
10
5
0
<0,75
<0,75
<0,75
<0,75
<0,75
<0,75
Agaricus sp.
Bovista sp.
Fistolina hepatica
Lepiota sp.2
Hygrophorus sp.
Trametes sp.
Lactarius chrysorrheus
Lactarius deliciosus
Russula delica
Russula sp.2
Russula sp.3
Russula sp.4
Suillus sp.
Xerocomus sp.1
Xerocomus sp.2
Agaricus campestris
Lepiota sp.1
Russula sp.1
Russula sp.5
Location 1
Location 2
Location 3
Location 4

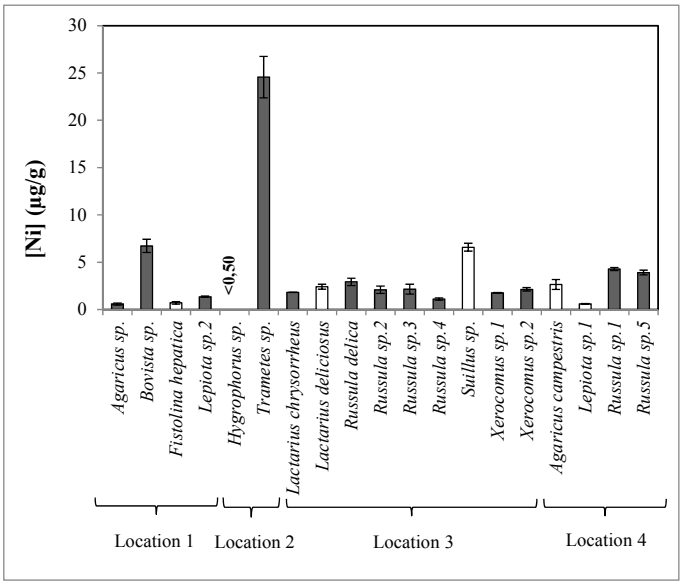

[Ni] (µg/g)
30
25
20
15
10
5
0
<0,50
Agaricus sp.
Bovista sp.
Fistolina hepatica
Lepiota sp.2
Hygrophorus sp.
Trametes sp.
Lactarius chrysorrheus
Lactarius deliciosus
Russula delica
Russula sp.2
Russula sp.3
Russula sp.4
Suillus sp.
Xerocomus sp.1
Xerocomus sp.2
Agaricus campestris
Lepiota sp.1
Russula sp.1
Russula sp.5
Location 1
Location 2
Location 3
Location 4

Figure 1 - Ca, Mg, Mn, Cr and Ni contents (μg/g) inmushroomsanalyzed from the Morais site (white bars – edible species; grey bars –non-edible species).

In terms of locations, the lowest Mg contentswere determined in edible mushrooms fromlocation 3. No differences were detected in other metal levels. Similar results were obtained for non-edible species. The metal levels found in edible mushroom species collected in the Morais site were identical or lower to those reported by other authors such as Siobud-Dorocant et al. (2011) for *Agaricus*sp., *Lepiota*ps. and *Fistulina hepatica*collected in several locations in Paris (France) orIsologlu et al. (2001) and Dermibas (2001) for *Lactarius*sp. in north western Turkey and in the East Black Sea region, respectively.

3.2 Honey

Ca, Mg, Mnand Ni contents determined for the honey samplesstudied in this work, are presented in Figure 2. We found that chromium concentration was lower than 0.060 μg Cr/g in all cases.

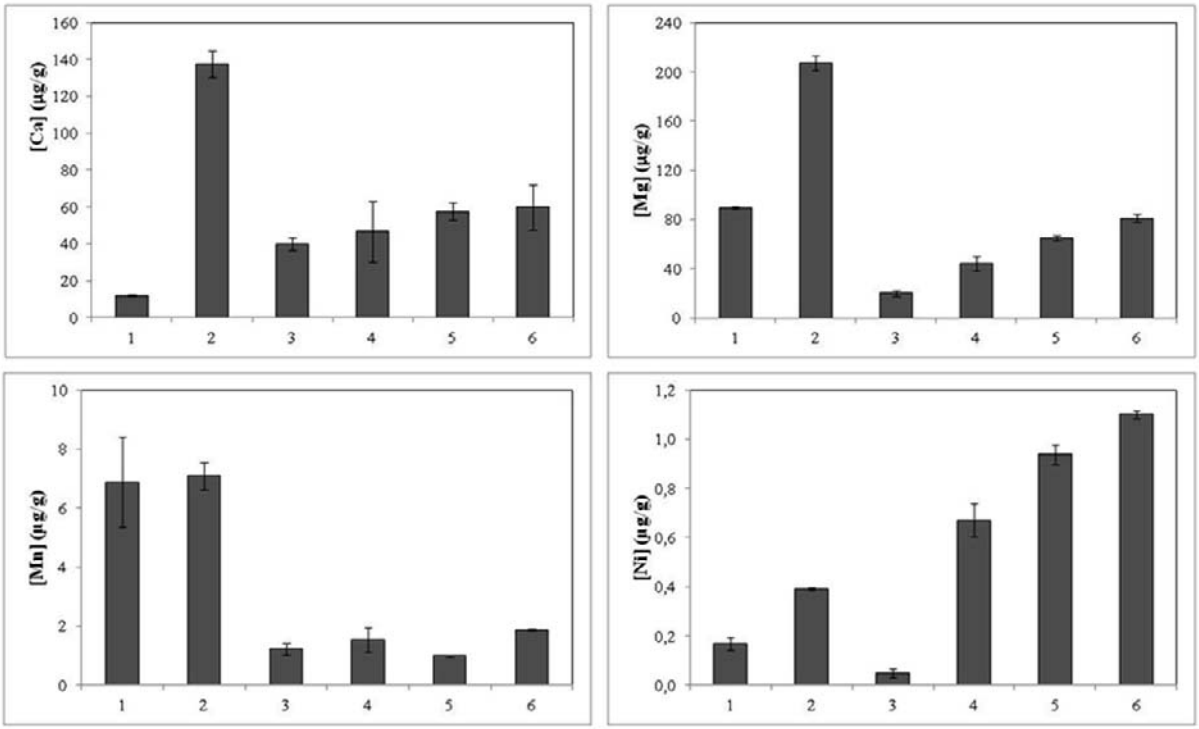


Figura 2 - Ca, Mg, Mn and Ni contents (μg/g) in honeys from the Morais site: 1 – Commercial A; 2-Commercial B; 3- Commercial C; 4 – Morais site A; 5 – Morais site B; 6 – Morais site C.

Honeys collected in the Morais site (4, 5 and 6)showed similar Ca and Mncontents. Calcium is required for bone formation and development, and manganese is important for the good performance of structural and enzymatic functions. The Mg and Ni levels increased from honey 4to honey 6, indicating variability on the metal contents on honeys produced in the same region.Honey collected in the Morais site always showed higher Ni concentrations than commercial honeys. This is probably due to the high Ni concentrations found in soil and vegetationat the Morais site. When present in high concentrations, Ni is toxic; however, it is required in small quantities for the production of red blood cells.

When comparing ourresults with other reported in the literature, we observed that honey collected in Turkey and analyzed by Yilmaz and Yavuz (1999) had Ca and Mn concentrations of51 and 1 μg/g, respectively, of the same order of magnitude ofCommercial Honey no. 3 (Ca = 40 μg/g, Mn = 1.2 μg/g) and all honeys collected inthe Morais site (Ca = 46.6-60.0 μg/g, Mn = 0.99-1.9 μg/g)(Honeys no. 4, 5 and 6).Ca and Ni contents reported by Omode and Ademukola (2008) for Nigerian honeys (Ca = 144-270 μg/g, Ni = 5.0-13.0 μg/g) were higher than thosedetermined in our study (Ca = 12.1-137.5 μg/g, Ni = 0.06-1.1 μg/g). Taking into account theinternational recommendation for Tolerable Upper Intake Level (UL) for Ni of 1 mg Ni/day(National Research Council, 2001), we estimatedthat for the honeywith the highest Ni contentof Moraissite (1.1 μg/g) it would be necessary to consume about 900 g of honey per day to exceed the UL. Thus, these results indicate that the three honeys collectedin the Morais site are not hazardous tohuman health.

4. CONCLUSIONS

Trametes sp. (non-edible) was the mushroom speciesthat showed consistentlythe highest metal concentrations. In relation to the edible species, *Suillus* sp. was the one that presentedthe highest levels of Ca, Mn, Cr and Ni. On the other hand, *Agaricuscampestris* showed the highest Mg concentration. The metal levels found in mushrooms collected in the Morais site were, however, identical to those reported elsewhere.

The honeys collected inthe Morais site showed higher levels of Ni in relation to commercial honeys (control). For the other metals, no differences were detected between these two types of honeys. Even though Morais honeys showed higher Ni levels than the control ones, they are safe for human consumption.

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B GROUP VITAMINS CONCENTRATION IN YOGURT WITH FLAKES FROM BIOLOGICALLY ACTIVATED HULL-LESS BARLEY GRAIN AND MALT EXTRACT

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ABSTRACT:

The influence of added flakes from biologically activated hull-less barley grain in concentration of 5% and malt extract in concentrations of 2%, 4% and 6% was studied during milk fermentation with yogurt culture YF-L811 on B group vitamins: thiamin, riboflavin, pyridoxine and cobalamin concentration.

The added flakes from biologically activated hull-less barley grain (BA-HL-BG) and malt extract (ME) significantly increased the content of vitamins B_2 and B_6 ($p<0.05$) in yogurt samples comparing with control. In addition the content of vitamins B_1, B_2 and B_6 in yogurt sample with flakes from BA-HL-BG and ME in concentration of 6% significantly differed from other yogurt samples ($p<0.05$). The adding of flakes from BA-HL-BG and ME resulted in significant losses of vitamin B_{12} ($p<0.05$). The decrease of vitamin B_{12} content depended on concentration of added ME, i.e., the content of vitamin B_{12} was significantly lower in yogurt sample with flakes from BA-HL-BG and ME in concentration of 6% ($p<0.05$) as in yogurt samples with flakes from BA-HL-BG and ME in concentrations of 2% and 4%.

Keywords: B group vitamins, barley grain, malt extract, yogurt

1. INTRODUCTION

Milk is naturally and highly nutritive part of a balanced daily diet (Saxelin et al, 2007), which provides water-soluble vitamins such as: thiamin, riboflavin, pyridoxine and cobalamin in variable quantities (Miller et al, 2000). The nutrient composition of fermented milk is similar to that in milk, but concentrations of vitamins are generally lower, with the possible exception of folic acid (Gurr, 1987). Therefore it was necessary to add bioactive compounds for increasing the nutritional value of fermented milk products. One of the possibilities is to

add hull-less barley grain and malt extract; they are excellent source of many valuable nutrients, such as dietary fibers, vitamin B complex and minerals (Škrbić and Cvejanov, 2011; Lebiedzińska and Szefer, 2006). B group vitamins have many different fundamental biological properties, such as, protection against cancer, heart disease, and birth defects (Lucock, 2004); they have important role in cell metabolism in humans (Turpin et al, 2010). Increase in consumer awareness has indicated that typical Western diet falls short of the recommended daily allowance of some nutrients, such as vitamins (Papastoyiannidis et al, 2006). Therefore it is important to produce the natural food-stuff with increased vitamins content. The good alternative could be fermented milk with barley grain. The nutritional value of food supplemented with barley depends on the level of supplementation as well on the type of used barley (Škrbić et al, 2009). Hull-less barley grain has a better nutritional value than hulled (Soares et al, 2007). The barley contains thiamin 0.356±0.012 mg 100 g^{-1}; riboflavin – 0.136±0.031 mg 100 g^{-1} and pyridoxine – 0.262±0.012 mg 100 g^{-1} (Lebiedzinska and Szefer, 2006). During the activation time the amount of vitamin B_1 is decreased by 17.39%, whereas the amount of vitamin B_2 increased by 88.9% in hull-less barley grain (Rakcejeva and Skudra, 2007). Therefore biologically activated hull-less barley grain (BA-HL-BG) in fermented milk product could be significant source of B_2 in diet. Adult requirements of riboflavin are 1.1 – 1.3 mg/day (WHO/FAO, 2001).

Barley and malt are now gaining renewed interests as ingredients for the production of functional foods (Qingming et al, 2010). The task of research was to investigate the effect of flakes from biologically activated hull-less barley grain and malt extract on B group vitamins: thiamin, riboflavin, pyridoxine and cobalamin concentration in fermented milk.

2. MATERIALS AND METHODS

Pasteurized milk with fat content 2.5% and the yogurt culture YF-L811, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Chr.Hansen, Denmark), were used for experiments. The culture was stored at -18 ^{o}C.

Flakes from biologically activated hull-less barley grain (BA-HL-BG) (Latvia) in concentration of 5% and malt extract (ME) (Igezeem, Latvia) in different concentrations of 2%, 4% and 6% were added to milk. Milk samples with flakes from BA-HL-BG and ME were inoculated with yogurt culture and fermented at 43 ^{o}C for 4 h. The control was prepared without the flakes from BA-HL-BG and ME for comparing results.

The content of B_1 and B_2 vitamins was determined by AOAC Official Method 986.27 and 970.65, B_6 vitamin – using J. Odincovas method (Vulfa, 1977) and B_{12} vitamin – using *Escherichia coli 113-3* (Valdmanis, 1959).

The differences in the concentrations of vitamins B_1, B_2, B_6 and B_{12} were analyzed using the analysis of variance (ANOVA). T-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences. Experiments were carried out in triplicate.

3. RESULTS AND DISCUSSION

Lactic acid bacteria are able to produce B group vitamins (Turpin et al., 2010), which depend on species and strain specificity. Crittenden et al (2003) have indicated that *S.thermophilus* strains are able to synthesize vitamins during milk fermentation, while lactobacilli are known for their inability to produce vitamins. On the other hand the stability of B vitamins depends on each vitamin and on external factors, such as presence of oxygen, light and acids, as well thermal treatment, whereas riboflavin is the most thermo able (Rivas et al., 2007). The ability of yogurt culture YF-L811 to synthesize vitamins B_1, B_2, B_6 and B_{12}, as well as the content of synthesized vitamins influenced by added flakes from BA-HL-BG and concentration of added ME were studied.

The concentration of vitamins B_1, B_2 and B_6 in yogurt samples with flakes from BA-HL-BG depending on the added ME concentration is presented in Figure 1.

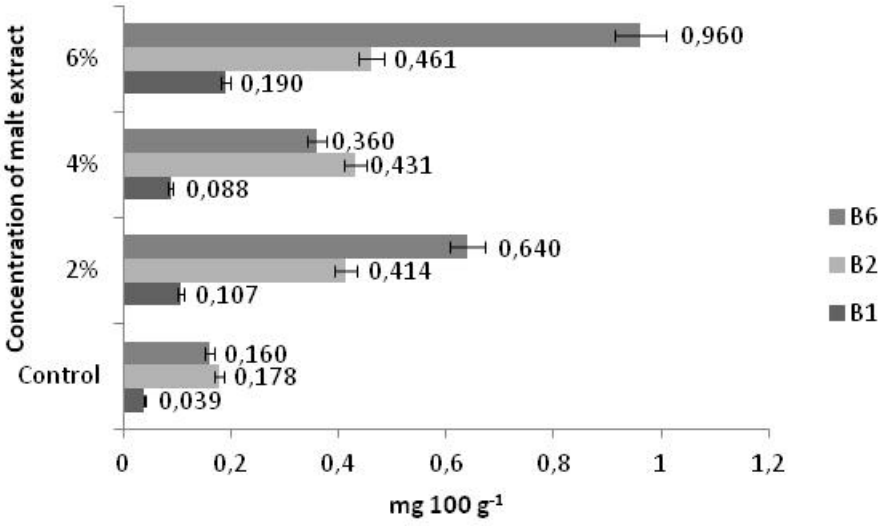

Figure 1 The influence of ME concentrations on the content of vitamins B_1, B_2 and B_6 in yogurt samples with flakes from BA-HL-BG

Results showed that by adding flakes from BA-HL-BG and ME it was possible considerably to increase the content of vitamins B_1, B_2 and B_6 in yogurt samples. Significant importance was also gained by increased concentration of ME. The highest concentration of vitamins B_1, B_2 and B_6 was determined in yogurt sample with flakes from BA-HL-BG and ME in concentration of 6%, respectively 0.190 mg 100 g^{-1}, 0.461 mg 100 g^{-1} and 0.960 mg 100 g^{-1}.

The results of ANOVA showed that content of vitamins B_1, B_2 and B_6 in the yogurt sample with flakes from BA-HL-BG and ME in concentration of 6% significant differed from other samples ($p<0.05$). In addition the content of B_2 and B_6 vitamins were significantly increased by adding flakes from BA-HL-BG and ME in all concentrations ($p<0.05$) comparing with control. Also, significant differences of B_6 vitamin content were determined between fermented milk samples with flakes from BA-HL-BG and ME in concentration of 2% and 4% ($p<0.05$).

Comparing the obtained results with in literature mentioned content of B_1, B_2 and B_6 vitamins in cow milk, respectively 0.04 mg 100 g^{-1}, 0.17 mg 100 g^{-1} and 0.04 mg 100 g^{-1} (Raynal-Ljutovac et al, 2008), there were shown the contradiction with Gurr (1987) conclusion that concentration of vitamins is generally lower in fermented milk. The results of research showed that the content of vitamins B_1 and B_2 in the control sample was similar to milk furthermore the content of vitamin B_6 was significantly higher. It should be explained that the ability of *S.thermophilus*, which made a part of the used yogurt culture, was used to synthesize vitamins. It confirmed the results of Crittenden et al (2003) research.

Analyzing the content of vitamin B_{12} in yoghurt samples with flakes from BA-HL-BG and ME (Figure 2), contrary tendencies can be observed.

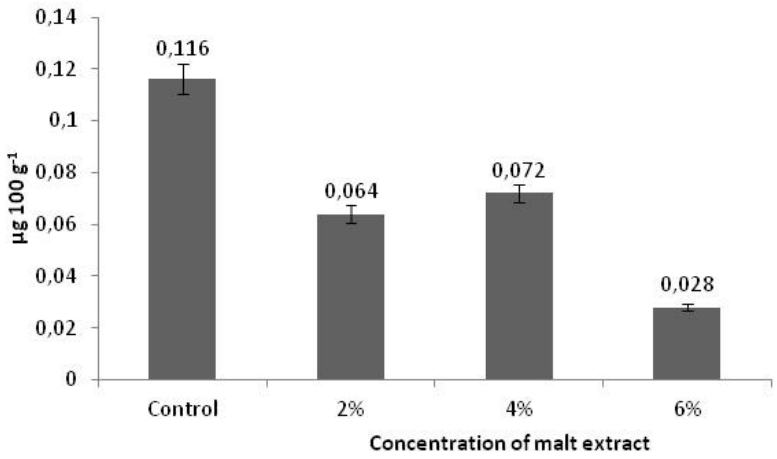

Figure 2 The influence of ME concentrations on the content of vitamin B_{12} in yogurt samples with flakes from BA-HL-BG

The fermentation of milk with flakes from BA-HL-BG and ME resulted in vitamin B_{12} losses. In addition the decrease of vitamin B_{12} depended on the concentration of added ME, i.e., the content of vitamin B_{12} was significantly lower in yogurt sample with flakes from BA-HL-BG and ME in concentration of 6% ($p<0.05$) as in yogurt samples with flakes from BA-HL-BG and ME in concentration of 2% and 4%. There were determined significant differences between control and yogurt samples with flakes from BA-HL-BG and ME ($p<0.05$). Comparing the obtained results with in the literature mentioned the concentration of vitamin B_{12} (0.35 µg 100 g^{-1}) in milk (Raynal-Ljutovac et al, 2008), the decrease of vitamin B_{12} content was apparent, as control the losses of vitamin B_{12} were circa 66%, it could be explained that during milk fermentation vitamin B_{12} synthesis was essentially less in comparison with its usage for providing the life processes of lactic acid bacteria cells. In addition Arkbåge et al (2003) have indicated that the losses of vitamin B_{12} in yogurt made 25% after fermentation and 33% after storage, taken together, yogurt contained 40-60% of vitamin B_{12} originally present in the milk, when consumed at ”use by date”. It could be concluded that the addition of flakes from BA-HL-BG and ME in yogurt promoted the decrease of vitamin B_{12} content in yogurt samples.

CONCLUSIONS

The added flakes from biologically activated hull-less barley grain (BA-HL-BG) and malt extract (ME) significantly increased the content of vitamins B_2 and B_6 ($p<0.05$) in yogurt samples comparing with control. In addition the content of vitamins B_1, B_2 and B_6 in yogurt sample with flakes from BA-HL-BG and ME in concentration of 6% significantly differed from other yogurt samples ($p<0.05$). The adding of flakes from BA-HL-BG and ME resulted in significant losses of vitamin B_{12} ($p<0.05$). The decrease of vitamin B_{12} content depended on the concentration of added ME, i.e., the content of vitamin B_{12} was significantly lower in yogurt sample with flakes from BA-HL-BG and ME in concentration of 6% ($p<0.05$) as in the yogurt samples with flakes from BA-HL-BG and ME of 2% and 4%.

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EVALUATING THE EFFECTS OF OLIVE OIL PROCESSING ON THE LIPOPHILIC OLIVE BIOACTIVES: SQUALENE, STEROLS AND TOCOPHEROLS

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ABSTRACT:
In addition to phenolics, olive oil contains a number of bioactive compounds including squalene (up to 0.7%), tocopherols (up to 300 ppm) and sterols (1000-2300 ppm). Growing evidence on the bioactivity of these compounds in vitro and in vivo including anticancer, anticholesterol and antioxidant activities have put increased focus on research on their health effects. During olive oil processing, these compounds may undergo chemical transformations and are distributed between the oil and waste streams to varying extents. The effect of processing conditions on these compounds should be considered during process design and optimization to maximize their retention in the final product and to facilitate their value added utilization by improving the quality of the waste streams. In this study, a systematic review of the academic was carried out to determine the factors affecting the sterol, tocopherol and squalene profiles of olive oil and waste streams. Information on the distribution of these compounds in the olive fruit (skin, pulp, seed) and their chemical and physical properties and mass transfer behavior were compiled and used to assess the effects of olive oil process parameters (such as malaxation time and temperature) and proposed process modifications to increase the yield and quality of olive oil (such as destoning, nitrogen flush, extraction method, enzyme addition, ultrasound and pulsed electric field application) on the bioactive profile. The implications of the findings for process design for the retention of bioactives in olive oil and for waste utilization were evaluated.

Keywords: Olive oil processing, squalene, sterols, tocopherols

INTRODUCTION

The health benefits of olive oil (OO), a prominent element of the Mediterranean diet, such as anticancer, anticholesterol and antioxidant activities, are mainly ascribed to the presence of high content of monounsaturated fatty acid and bioactives (phenolic compounds, tocopherols, squalene, sterols) (Newmark, 2006; Watson & Preedy, 2010).

Virgin olive oil (VOO) processing is a physical extraction process including crushing, malaxation and centrifugation steps. The bioactive composition and content of VOO are

affected by extraction methods and parameters (Koutsaftakis, Kotsifaki, & Stefanoudaki, 1999). Physical separation of VOO is done either using the classical press system or the modern continuous two-phase or three-phase centrifugation system. In addition to OO, pressing or the three-phase system also generates solid waste (olive cake or "orujo", containing olive pulp and stones) and aqueous liquor, which comes from the vegetation water and the soft tissues of the olive fruits, and water added during processing (so-called "alpechin" or "olive-mill waste water"). The use of a modern two-phase processing technique to which no water is added generates oil and a new by-product that is a combination of liquid and solid waste, called "alperujo" or "two-phase olive mill waste" (2POMW) (Dimitrios, 2012).

The objectives of this review were to summarize information on the properties, stability and mass transfer behavior of the major lipophilic OO bioactives (e.g. squalene, tocopherols and sterols), their distribution in the olive fruit and the effect of processing methods and parameters on the bioactive content and composition of olive oil, which is required for the optimization of VOO processing to realize effective transfer of the bioactives from olive fruit to oil.

LIPOPHILIC BIOACTIVES

Squalene is a polyunsaturated hydrocarbon of the triterpene type which is liquid at room temperature. Due to its strong hydrophobic nature and its unsaturated structure, squalene is not very stable and gets easily oxidized (Spanova & Daum, 2011). Moreover, it protects lipids against oxidation (Dessì et al., 2002). Decomposition of squalene in olive oil was reported as in the range of 26–47% after 6 months storage in the dark and at room temperature (Manzi, et al., 1998). Squalene, at up to 0.1-0.8% (w/w), is uniquely high in VOO as compared to other fats and oils (Lou-Bonafonte, et al., 2012).

Phytosterols, which are triterpenoid isoprenoids, are crystalline solid at room temperature. They are soluble in vegetable oils and fats, non-polar solvents such as hexane, iso-octane and 2-propanol and insoluble in water (Cantrill & Kawamura, 2008). Sterols are present in olive oil between 1000 and 2300 ppm (Ghanbari et. Al., 2012), the most abundant being β-sitosterol. Phytosterols and their fatty acid esters are quite stable compounds and undergo only limited degradation during oil processing. Only harsh conditions, such as high temperatures (>100°C) in the presence of oxygen may increase the oxidation rate (Zhang et

al., 2006; Cantrill & Kawamura, 2008; Rudzińska, Przybylski, & Wąsowicz, 2009; Thanh et al., 2006a).

Tocopherols (α, β, δ, γ) and tocotrienols, which belong to the vitamin E family, are particularly important antioxidants in vegetable oils. Tocopherols consist of a chroman ring and a long saturated phytyl chain. Tocopherols are viscous oils at room temperature and are slowly oxidized by atmospheric oxygen (Güçlü-Üstündağ & Temelli, 2004). Tocopherols are sensitive to light, heat, alkali, and metals; therefore, they are easily oxidized to tocoquinones, which no longer have antioxidant properties. α-tocopherol is insoluble in water and soluble in oils, fats and fat solvents (Merck Index, 2006). In VOO, 90 % of tocopherols is α-tocopherol with up to 300 mg/kg concentration while others (β-, γ-, and δ-) are present in low amounts, up to 25 ppm (Ghanbari et al., 2012; Boskou, Blekas, & Tsimidou, 2006). Total tocotrienols has been reported in trace amounts (<0.1 mg/100 g) in extra virgin olive oil (EVOO) (Schwartz, Ollilainen, Piironen, & Lampi, 2008).

3. DISTRIBUTION OF LIPOPHILIC BIOACTIVES IN OLIVE, OLIVE OIL AND WASTE STREAMS

3.1. Composition of the olive fruit

The average composition of an olive fruit (*Olea europaea L.*) used for oil extraction is oil (22%), water (50%) and total solids (28%) including carbohydrate (19.1%), cellulose (5.8%), proteins (1.6%), inorganic substances (min. 1.5%), minor constituents such as phenolic compounds (1-3%), other lipohilic bioactives (<1%) (sterols, squalene, tocopherols, volatiles, and pigments such as chlorophyll and carotenoids (Niaounakis, Michael, Halvadakis, 2006**;** Ghanbari et al., 2012). The olive drupe consists of 70-90%-pulp, 2-5.5%-seed and 1.5-7.7%-skin by weight.

Oil and water are distributed through anatomic parts of olive fruit (skin, pulp, seed) to different extents such that for 100 g of olive fruit, oil and water contents are 16-23 g, 2-4 g and 0.11-2 g, respectively (Niaounakis, Michael, Halvadakis, 2006; G. Bianchi & Vlahov, 1994; Conde, Delrot, & Gerós, 2008). As most of the oil is present in the pulp, composition of oil in the pulp is the determining factor for the quality of extracted oil.

Analysis of data on bioactive content of anatomic parts of the olive fruit, which is rather scarce, shows that while β-sitosterol and total sterol concentrations were highest in the seed,

squalene was mainly concentrated in pulp oil and tocopherol concentration in the seed was higher than that in the pulp (Table 1).

TABLE 1. Lipophilic bioactive profiles of whole olive fruit and its anatomic parts (mg/kg oil)

	Whole olive fruit	**Skin** (Epicarp)	**Pulp** (Mesocarp)	**Seed** (Kernel)
Squalene	nr	nd	1000[3]	tr[3]
	4359[2]	nr	nr	nr
β-sitosterol	2705[1]	1648[1]	2331[1]	4407[1]
	1496[4]	nr	1481[4]	3556[4]
Total Sterol	nr	1840[5]	1906[5]	2945[5]
	nr	nr	1832[6]	4128[6]
	1043[7]	nr	nr	4939[7]
α-tocopherol	nr	193[5]	122[5]	216[5]
	55[2]	nr	nr	nr

* The underlined values are calculated according to specified references and oil content (22%).
**tr:trace amount, nd: not detected, nr: no data
[1](Guillaume, Ravetti, Ray, & Johnson, 2011a), [2](Manzi, Panüli, Esti, & Pizzoferrato, 1998), [3](Bianchi & Vlahov, 1994), [4](Ranalli, Pollastri, Contento, Di Loreto, Lannucci, et al., 2002), [5](Cortesi, Rovellini, & Fiorino, 1999), [6](Ranalli & Contento, 2010), [7](Mulinacci et al., 2005)

3.2. Bioactive content of olive oil and waste streams

Most olive phenolics are amphiphilic in nature with a higher solubility in the water than in the oil phase. Consequently, a large amount of them is lost (approximately 98%) with the wastewater (53%, w/w) and pomace (45%, w/w) (Rodis, Karathanos, & Mantzavinou, 2002; Klen & Vodopivec, 2012). Accordingly, the lipophilic bioactives are expected to be mostly retained in the oil phase rather than in the water phase, and will be present in the VOO and pomace streams.

The recovery of VOO during olive oil processing is around 80-98% with the remaining oil being lost in the waste streams mainly in the pomace (Ranalli & Martinelli, 1995; Vlyssides, Loizides, & Karlis, 2004; Pattara, Cappelletti, & Cichelli, 2010; AGRITEM, 2011). Highest oil loss was observed in the pomace of press system (Vlyssides, Loizides, & Karlis, 2004). Oil contents of pomace of 2-phase and 3-phase systems were in the range of 2.4-4.5 % (Ranalli & Martinelli, 1995; Vlyssides, Loizides, & Karlis, 2004; Pattara, Cappelletti, & Cichelli, 2010a; AGRITEM, 2011). Oil left in the pomace (olive-pomace oil (POO)) can be further recovered using solvents or other physical treatments (IOC, COI/T.15/NC No 3/Rev. 6 Nov.2011). Figure 1 provides the flow chart of olive oil extraction with a mass balance (using data from Vlyssides et al., (2004)) showing the water and oil content of the waste streams.

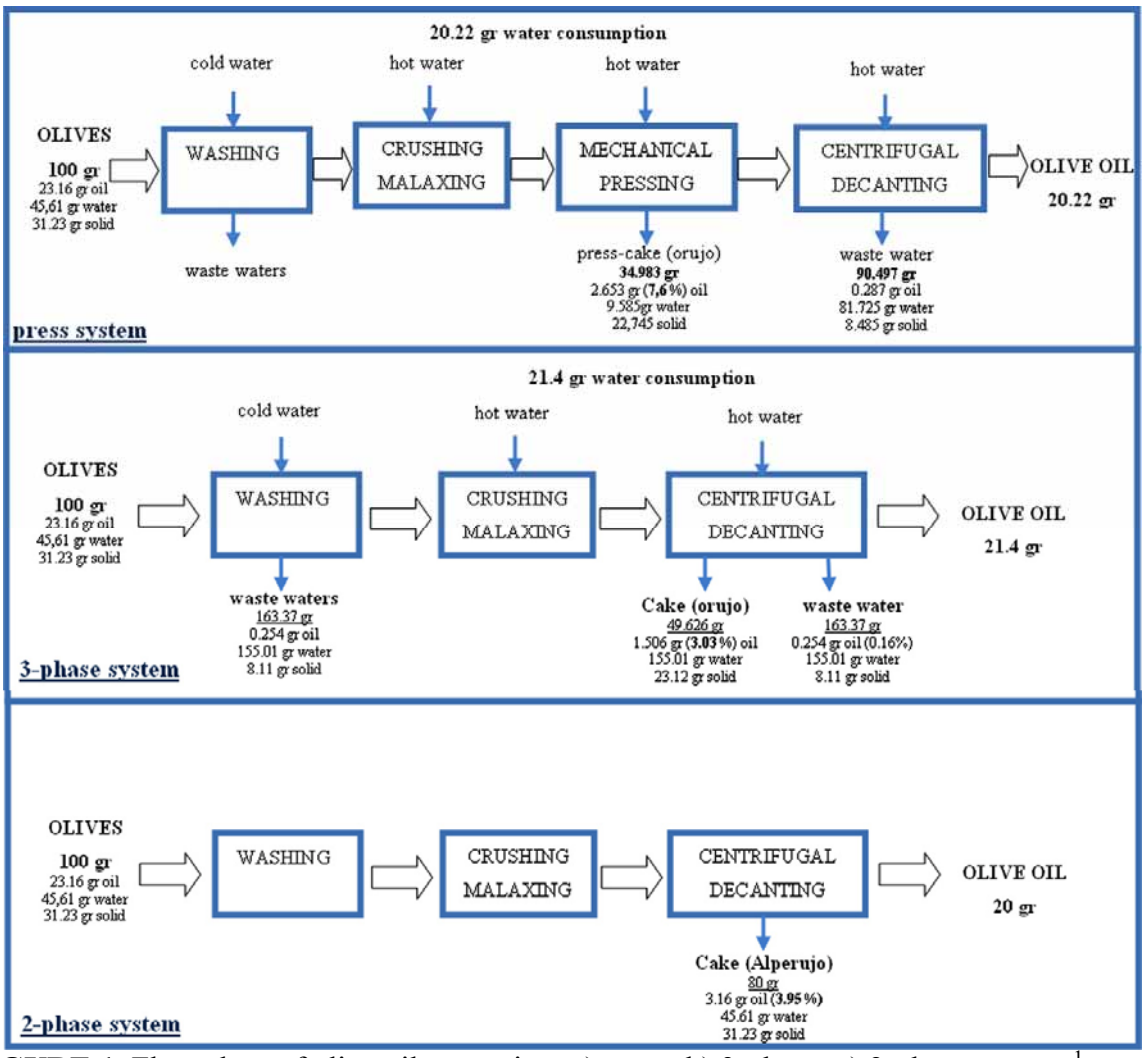

FIGURE 1. Flow chart of olive oil extraction: a) press, b) 3-phase, c) 2-phase system.[1]
[1] Calculations based on data from Vlyssides et al. (2004)

Table 2 shows loss of lipophilic bioactives (%) calculated using literature data. □-sitosterol, □-tocopherol and squalene loss during VOO processing was calculated as 88%; 30%; 15%, respectively. The higher sterol concentration in POO (up to 2600 mg/kg) than in VOO (up to 1600 mg/kg) (Covas et al., 2006) may be attributed to the uneven distribution of these bioactives in olive fruit parts (Table 1). Oil in pomace has higher amount of bioactive compounds from the seed due to the lower yield of seed oil.

TABLE 2. Loss of lipophilic bioactives during olive oil processing

	Olive Fruit	Virgin Olive oil	System	% loss
Mass balance (according to Figure 3)	1kg	Press; 3-phase 0.20 kg; 0.21 kg		
Squalene (mg/kg)	959[1]	4083[1]	press	15%
β-sitosterol (mg/kg)	2705[2]	1550[2]	3-phase	88%
α-tocopherol (mg/kg)	55[1]	184[1]	press	30%

[1](Manzi et al., 1998), [2](Guillaume et al., 2011a)

THE EFFECT OF PROCESSING ON OLIVE OIL LIPOPHILIC BIOACTIVES

Processing conditions that affect olive oil extraction yield are the main determining factors for the recovery of olive bioactives during processing in addition to chemical and enzymatic transformations.

Common applications

4.1.1 Crushing

The objective of crushing (fruit milling) is to break the olive tissues in order to liberate the oil drops contained in the mesocarp cells using a crusher. Chemical species contained within the anatomic parts of olive fruit (skin, pulp, stone and seed) are mixed and distributed according to their affinity and concentration during crushing (Herrera, 2007).

The effects of crusher type (stone-mill, disc and hammer), sieve/hole size, and rotation speed on the oil yield and content of volatile and phenolic compounds were widely investigated (Caponio et. al., 2003; Caponio & Gomes, 2001; Inarejos-García, Fregapane, & Salvador, 2010; Veillet et. al., 2009).

The crusher type affects the oil yield through its effect on the extraction mechanism. While in hammer crushers oil diffuses across the opened cells which have been clearly cut, exudation of oil from broken and damaged cell walls occurs due to a strong mechanical effect in stone mills resulting in lower oil yields (Veillet et al., 2009). Higher strength hammer crushing (achieved by smaller grid holes and a higher rotation speed) resulted in higher oil yield (30%), and □-tocopherol, phenolic content in the olive paste and in the oil due to better cell cuts (Inarejos-García, Fregapane, & Salvador, 2010; Giovacchino et al., 2002). As expected, the crushing parameters that resulted in higher oil yield resulted in higher recovery of lipophilic bioactives such □-tocopherol (Inarejos-García, Fregapane, & Salvador, 2010).

4.1.2. Malaxation (Kneading)

Malaxation, which involves continuous kneading of the olive paste at a carefully monitored temperature, prepares the paste for separation of the oil. It is very relevant to the quality, yield and chemical composition of the final product as it affects the partitioning of olive components between oil and water phases and activity of enzymes, which are released during the crushing step owing to the disruption of the cell tissues (Clodoveo, 2012). The efficiency of malaxation depends on malaxation time, temperature and oxygen concentration,

and addition of coadjuvants for better rheological characteristics, such as water, talc, (Cert, Leo, Moreda, & Pe, 1996), salt and enzymes (section 3.2.) (Clodoveo, 2012).

An increase in malaxation time (Mt) or malaxation temperature (MT) increases VOO yield (Giovacchino et al., 2002; Clodoveo, 2012) as the small oil droplets merge into large drops due to a reduction in the oil viscosity with increasing temperature (Ranalli, Contento, Schiavone, & Simone, 2001). Kalua et al., (2007) investigated the effect of MT (15, 30, 45, 60 ^{0}C) and Mt (30, 60, 90, 120 min) on yield and observed that the lowest yield (45 ^{0}C, 30 min) and the highest yield (30 ^{0}C, 90 min) were 33.8% and 44.3%, respectively. Malaxing at 15 °C and 60 °C displayed a distinct difference in quality and composition of minor components (Kalua et al., 2007).

The concentrations of □-sitosterol in olive oil (% total sterols) obtained at MT of 30 ^{0}C and 45 ^{0}C were similar (75.6% and 75.9%, respectively) (Koutsaftakis, Kotsifaki, & Stefanoudaki, 1999b). In another study (Guillaume, Ravetti, Ray, & Johnson, 2011b), MT (18, 28, 38 ^{0}C) and Mt (15, 30, 60 min) did not have much of an effect on □-sitosterol content of VOO. Similarly, squalene contents of three extra virgin olive oil varieties were not affected by MT (20, 25, 30, 35 ^{0}C) (A. Ranalli, Malfatti, Lucera, Contento, & Sotiriou, 2005). □-tocopherol content in three different VOO varieties increased approximately 27% with MT in the range of 20-35°C (A. Ranalli, Contento, et al., 2001).

4.1.3. Centrifugation

Liquid and solid/semisolid phase separation takes place during centrifugation. Two types of decantation systems, in addition to classical press system, are used to separate the oil from olive paste: two-phase and three-phase systems.

Classical press system reportedly produced oils with higher amount of □-sitosterol (76, 75.7 and 75.5 % total sterols; classic, 2- and 3- phase) and lower campesterol/stigmasterol ratio (4.5, 5 and 5.1) than continuous systems (Koutsaftakis et al., 1999a). In spite of statistical differences, these values are too close to declare as a general trend. Similar values of □-sitosterol % were reported for 2- and 3- phase systems in another study (Ranalli, Angerosa, Sperimentale, & Pe, 1996).

In two-phase systems no water is added resulting in a higher retention of polar phenolic antioxidants. Although extraction method affected the level and nature of other quality characteristics (aroma, total phenolic content), no observable differences □-tocopherol levels were found (Psomiadou, Tsimidou, & Boskou, 2000). These results are in agreement with

those reported for Italian oils (A. Ranalli, Angerosa, Sperimentale, & Pe, 1996). Other studies reported higher (Fregapane, Salvador, Aranda, & Go, 2003) and lower □tocopherol contents for 2 phase systems (Stefanoudaki, Koutsaftakis, & Harwood, 2011).

4.2. Process modifications and new developments

4.2.1. De-stoning

In the last few years, much attention has been paid to the de-stoned olive processing technique to improve quality of VOO (Ranalli et al., 2008; Yorulmaz et. al., 2011; Servili et al., 2007). De-stoning decreased the oil yields by 1-1,5% due to the absence of the draining effect of pit pieces during malaxation (Ranalli et al., 2008; Mulinacci et al., 2005).

De-stoned oils (DO) had higher contents of phenolics and pleasant volatile compounds (Ranalli et al., 2008; Del Caro et. al., 2006; Amirante, et. al. 2006). The increase of phenolic concentration of oils with destoning, which was attributed to the absence of kernel tissues in the olive paste which contain polyphenoloxidase (PPO) and peroxidase (POD) enzymes, was different for each phenolic compound (Yorulmaz, et al., 2011)**.**

Although effects of de-stoning on tocopherol content were reported in several studies, no trend could be established. De-stoning resulted in a 4-20% increase (Ranalli et al., 2008; Del Caro, 2006), a 6-12% reduction (Lavelli & Bondesan, 2005) (Yorulmaz et al., 2011) and no significant difference in the concentration of total tocopherols (Gambacorta et al., 2010). Destoning did not have an effect on squalene concentration of olive oil (Ranalli et al., 2008).

While no significant effect of destoning on sterol, mainly □-sitosterol, concentration of olive oil was observed in some studies (Yorulmaz et al., 2011; Mulinacci et al., 2005; Ranalli et al., 2007), there are reports stating the sterol lowering effect of de-stoning, owing to the removal of olive seeds which are rich in phytosterols (Table 1). But this reduction was limited to 8% due to the lower amount of oil in the seed (Ranalli, et. al. 2010). As the olive seed constitutes a small proportion of the whole fruit (□2-3% compared to 78-85% for pulp) containing only 3% of the oil in the olive fruit, in general its removal does not significantly influence the composition of the olive oil (Ranalli et al., 2002).

4.2.2. Nitrogen application

In the presence of oxygen, bioactive compounds are subjected to oxidation (Yorulmaz et al., 2011; Vierhuis et al., 2001; Giovacchino, Mucciarella, Costantini, Ferrante, & Surricchio,

2002). Although some enzymes present in the olive fruit are deactivated during crushing, enzymes (such as LOX and PPO), retain some detectable activity in olive paste and in VOO (De Faveri et al., 2008).

The use of an nitrogen flush during malaxation increased the phenolic concentration and lead to higher resistance to oxidation during storage extending shelf life of VOO (Vierhuis et al., 2001; Yorulmaz et al., 2011; Giovacchino et al., 2002). Malaxation under inert gases, nitrogen and argon, increased the tocopherol concentration of olive oil by 10% due to protective effect of nitrogen against oxidation (Yorulmaz et al., 2011). However, malaxation under nitrogen decreased the carotenoid and chlorophyll concentrations, and had no effect on sterol concentration of VOO (Yorulmaz, et al., 2011). Thus nitrogen application mainly affects the antioxidants in VOO, which are susceptible to oxidation such as tocopherols and phenolic compounds, whereas sterols, which are relatively stable are not affected.

4.2.3. Ultrasound and electric field assisted extraction

Non-thermal processes involving ultrasound and pulsed electric field (PEF) technologies are being investigated to increase the yield and quality of olive oil (Pieralisi, 2012; Abenoza et al., 2012; Jiménez et al., 2007).

During malaxation, ultrasound provides a quick-heating of olive paste (ambient to 30^0C), improves process yields and quality without use of additives (Jiménez et al., 2007). In a study by Jimenez et al. (2007), malaxation using indirect (with an ultrasound-cleaning bath) and direct sonication (by an ultrasound probe horn) both increased oil yield, with direct sonication resulting in higher yields for high moisture olives (>50%), and indirect sonication for low moisture olive fruits (<50%). No clear effect on tocopherol content was established, however a decrease in the total phenolic content was observed (Jiménez et al., 2007).

Pulsed electric field is an effective method for extraction of the oil, present in free or colloidal form, due to irreversible permeabilization of cell membranes in tissues without a significant temperature increase (Abenoza et al., 2012). In a study on the effect of pulsed electric field at different intensities (0–2 kV/cm), a higher oil yield, which increased with the electric field intensity, and a slight increase in α-tocopherol content (from 248 to 252 mg/kg) together with a decrease in the total phenolic content (from 149 to 112 mg gallic acid equivalent/kg) was observed (Abenoza et al., 2012).

4.2.4. Enzyme assisted extraction

Enzyme preparations are used as extraction aids to improve oil yield and quality (Chiacchierini, Mele, Restuccia, & Vinci, 2007a). Enzyme solutions are usually added during malaxation process mostly in liquid form, and as they are water soluble, they are completely removed in the vegetation water without leaving any residue (A. Ranalli & Mattia, 1997).

Enzyme formulations, such as those involving pectinase and cellulolytic hemicellulolytic enzymes, improves the rheological characteristics of the paste, degrade the walls of the oil-bearing cells and break up the liquid/solid emulsions and the liquid/liquid emulsions before malaxation, improving the separation of phases (oil, water, solid) resulting in higher extraction yields (Ranalli & Mattia, 1997; Chiacchierini, Mele, Restuccia, & Vinci, 2007).

A cytolase enzyme (pectolytic) added before malaxation in a two-phase system increased the oil yield by 5-7%, phenolic compounds by 15%, □-tocopherol by 1-14%, and had a variable effect on β-sitosterol and total sterol content based on the olive variety (Ranalli & Mattia, 1997). In another study (Septiembre, Iconomou, Arapoglou, & Israilides, 2010), two enzyme formulations, Olivex (rich in pectinolytic, hemicellulolytic and cellulolytic enzymes) and Glucanex (β-glucanase), used in a three-phase system improved the yield but Glucanex resulted in a higher increase (15%). Both enzymes did not influence the sterol and □-sitosterol content. Similar results were obtained for Olivex in another study (Ranalli & Serraiocco, 1996). The use exogenous “Cytolase 0” enzyme complex in de-stoned olives resulted in 1-2% increase of oil yield, an increase in tocopherol content (19%), but no influence on squalene content (Ranalli, Marchegiani, Pardi, Contento, Girardi, et al., 2008; Ranalli, De Mattia, & Ferrante, 1998; Ranalli, De Mattia, & Ferrante, 1997).

Enzyme assisted extraction thus had an impact mainly on oil yield and concentration of antioxidants (phenolics and tocopherols) rather than squalene and sterols.

5. CONCLUSIONS

In this study, information on the properties, stability and mass transfer behavior of lipohilic OO bioactives, their distribution in the olive fruit and their concentration in the oil and waste streams as affected by processing conditions have been reviewed and integrated to study their recovery. Our findings indicate that oil yield is the main factor determining the recovery of these compounds as they are mainly retained in the oil phase. Therefore their recovery can mainly be improved by process modifications aimed at yield improvements.

Bioactive content of OO can be modified by processes that result in selective extraction of oil present in different anatomical parts due to the uneven distribution of olive bioactives in the olive fruit. New processes aimed at improvements in olive oil quality and yield affected the bioactives to different extents. For example, although de-stoning did not have much of an effect on lipid bioactives, nitrogen application during malaxation and enzyme treatment increased the recovery of □-tocopherol.

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WEB-BASED SIMULATION AS AN EDUCATIONAL TOOL IN FOOD ENGINEERING

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ABSTRACT:
This paper presents preliminary results from a research project aiming at the development of simulation-based web modules on mass and energy balances, flow mechanics and heat transfer to be used as e-learning tools in Food Engineering courses. In the presented case studies, MATLAB-based simulations of steady-state heat conduction/convection in planar and cylindrical surfaces and a Fluent-based model of transient heat transfer in a food can are used to interactively expose the dependence of heat rate on the geometry and the properties of the materials involved. Through the simulation applications, users can experiment with the values of heat conductivity and the characteristic lengths of the geometry while the underlying models are solved to compute the values of the overall heat transfer coefficient, the heat transfer rate and produce the temperature profiles. The results are displayed as numerical values as well as in charts and videos where the trends of the output variables with respect to the changes in the input variables can be easily observed and appreciated. It is expected that the use of process simulation technology can enhance the learning experience by offering an interactive platform for quick and effortless experimentation with physical phenomena. Web-based simulation makes this experience available to everyone from anywhere in the world.

Keywords: Web-based simulation, food engineering, education, MATLAB, Fluent

1. INTRODUCTION

Deduction is the process by which logical conclusions are extracted from valid propositions. Teaching in science and engineering traditionally follows the deduction paradigm: the behavior of progressively more complex physical systems is described through mathematical equations deduced from simpler fundamental principles and laws. Using, for example,

Fourier's law of heat conduction and the energy balance, the equations describing the steady-state and transient heat transfer through a solid medium can be derived and this is how the topic is presented to the engineering students. However, knowing how a mathematical model of a physical process emerges is very different from understanding the underlying cause-effect and quantitative relationships between the variables that it encompasses. This understanding can be induced by observation of the relevant phenomena under varying conditions.

Induction is the process of extracting global conclusions from local observations and, consequently, the ability to predict the behavior at unfamiliar situations. In education, induction is the process of learning by examples and can complement the traditional deductive approach as a way for students to comprehend the cause-effect relationships and non-obvious interdependencies between the variables that govern the behavior of a physical system. Performing physical experiments is one way of inductive learning. However, there are many limitations on the number and complexity of experiments that can be performed in the lab and the degree of involvement of each student in the experiment. Experiments allow a close contact with the physical system, whose behavior is studied, but they do not allow direct visualization of the variable dependencies, i.e. how the value of one variable is affected by a change in another.

Simulation technology offers an alternative to experimentation which may be devoid of any physical contact with the actual system, but with many advantages on the simplicity and speed at which experiments can be performed. A computer program embodying a simulated physical system allows fast and inexpensive repetitions of the same experiment under variable conditions, offers a personalized experience to the student, and provides feedback in many different forms. As claimed by Leung (2003), the use of simulation in education serves the purpose of shifting the learning paradigm from direct instruction to a more open-ended and learner-centered process to permit sustained exploration by the learners.

There are many software tools that allow the development of engineering simulations. There exist tools dedicated to specific applications and others which are generic. MATLAB is a general-purpose mathematical package that allows the simulation of any engineering system and, thus, has numerous applications (Ibrahim, 2011). On the other hand, ANSYS Fluent is a dedicated tool equipped with computational models for modeling fluid dynamics phenomena including those present in food processes (Sun, 2010).

Web-based simulation is the integration of the Web with the field of simulation (Byrne et al., 2010). Its greatest advantage is accessibility: anyone with access to the internet can experiment with web-based simulations without having to install and learn to use software packages and without having to worry about licensing issues. The role of the web browser is central in web-based simulation; it offers the graphical user interface (GUI) through which the user interacts with the server-based simulation engine and it may also contain and execute the simulation engine itself. Issues of security, stability, connection speed and GUI limitations (compared to stand-alone executables) may limit the usability of web-based simulation; however, the ease of access that it offers is by itself the single most important and adequate reason for considering it as a tool to deploy applications. Web-based simulation has been used in a variety of cases such as applications that require huge quantities of data (e.g. meteorology), collaborative projects from multiple sites, applications that require user input (like in manufacturing) and, finally, applications in education and training for distant students (Kuljis and Paul, 2001).

Within the context of web-based simulation for education, we have embarked on a project to develop simple simulations that will allow students in food engineering (or, process engineering, in general) to explore and experiment with fundamental phenomena in flow mechanics and heat transfer. The hope is that through an open, learner-centered environment better understanding of the phenomena and grasping of the causal-effect interdependencies can be achieved. This paper presents some early developments in the field of heat transfer.

2. HEAT TRANSFER

Heat transfer is present in almost all food processes and, therefore, is an important subject in food engineering. Food technologists should master the mechanisms underlying heat transfer and comprehend the relative significance of all the factors affecting its distribution and rate. In any typical food process, modeling the heat transfer mechanisms through the energy balance is a quite complex process involving the construction and solution of 3-D partial differential equations. As usually, teaching of a complex subject involves its decomposition into simpler, idealized sub-entities that can be modeled and studied independently. So, for example, students in heat transfer are first exposed to the basic mechanisms (conduction, convection, radiation) in isolation from each other, under simple geometric configurations

(planar, cylindrical, spherical) that are amenable to single-dimensional modeling, and under the assumption of homogeneity in material properties.

One of the very first subjects in the study of heat transfer is the steady-state conduction/convection through a (possibly multi-layer) solid surface separating two fluid phases. As defined in any basic heat transfer text book (e.g. Pitts and Sissom, 1998), the heat transfer rate, q, is driven by the temperature difference, ΔT, between the two fluids, and is proportional to the heat transfer area, A, and the overall heat transfer coefficient U:

$$q = UA\Delta T \qquad [1]$$

The overall heat transfer coefficient embeds all the resistances in heat transfer including the conductive solid layers characterized by the layer's thermal conductivity, k, and the fluid phases characterized by the convective heat transfer coefficient, h. The form of the overall heat transfer coefficient defining equation depends on the geometry. For planar layers:

$$U = \frac{1}{\frac{1}{h_i} + \sum_i \frac{L_i}{k_i} + \frac{1}{h_o}} \qquad [2]$$

where, L_i is the thickness of the convective layer i. In cylindrical configurations, U is given by:

$$U = \frac{1}{\frac{r_n}{r_1 h_i} + r_n \sum_{j=2}^{n} \frac{1}{k_j} \ln\left(\frac{r_j}{r_{j-1}}\right) + \frac{1}{h_o}} \qquad [3]$$

where, r_i is the radius of layer i. Equation [3] assumes that the heat transfer area is calculated at the outer surface with radius r_n.

Students are not only expected to be able to use the above relations to compute the overall heat transfer coefficient and the heat rate but also understand the relative effect of the parameters involved and, more importantly, how the temperature profile is affected by changes in their values. It is a typical misconception among the students that an insulating material (with a small value of heat conductivity, k) would have a smaller temperature

difference between its two surface walls than other non-insulating layers. This misconception arises from the fact that students learn to relate heat transfer rates with temperature differences (ΔT), so, an insulating material must cause a small heat rate and, therefore, small ΔT. They do not realize, however, that in a given steady-state case, the heat transferred is the same everywhere and that the presence of the insulating material causes a relatively large temperature difference between its two sides while a heat conductor maintains an almost constant temperature profile within its volume.

In cylindrical coordinates, there are two competing factors that determine the value of heat rate as the thickness of the insulating layer increases. The increase in thickness of a layer may provide extra insulation but it also causes an increase in the heat transfer area which, in turn, means larger heat transfer rate. Under certain circumstances, the surface increase dominates and it is possible to have greater heat losses even when expanding the insulating layer.

Non-steady state heat transfer through a solid surface is governed by the following equation:

$$\nabla^2 T = \frac{1}{\alpha}\frac{\partial T}{\partial t} \qquad [4]$$

where, α, is the material's thermal diffusivity. To obtain the temperature profile, equation [4] should be solved in the solid's domain with proper boundary and initial conditions. For simple geometries and one-dimensional heat transfer, students learn to use approximate methods for small values of the Biot number (lumped analysis), or pre-compiled charts to compute the actual solution (Pitts and Sissom, 1998). Even then, however, any computation with these methods will yield some isolated temperature values at specific positions in the solid and in specific time points making it difficult for the student to visualize how the temperature profiles in the entire solid evolve over time, let alone how the profiles are affected by the material properties. A computer simulated model of the above process would have no problem providing and exposing that information. Therefore, there are still important lessons to be learned even in the simplest of the cases like the ones just presented.

3. MODELS AND USER-INTERFACES

MATLAB was used to formulate two application modules aimed at teaching the students the steady-state heat transfer process in planar and cylindrical geometries. Fluent was used to

generate temperature profiles in transient conditions in a cylindrically shaped object like a food can. In the case of the steady-state modules, MATLAB was used to both model the process and also formulate a GUI through which the student can interact with the model. No such functionality is available in Fluent, so the GUI was developed on the webpage itself that invokes pre-solved Fluent models and displays graphically the results through videos and charts. In all cases, the scope of the module was to expose the phenomenon to the users and

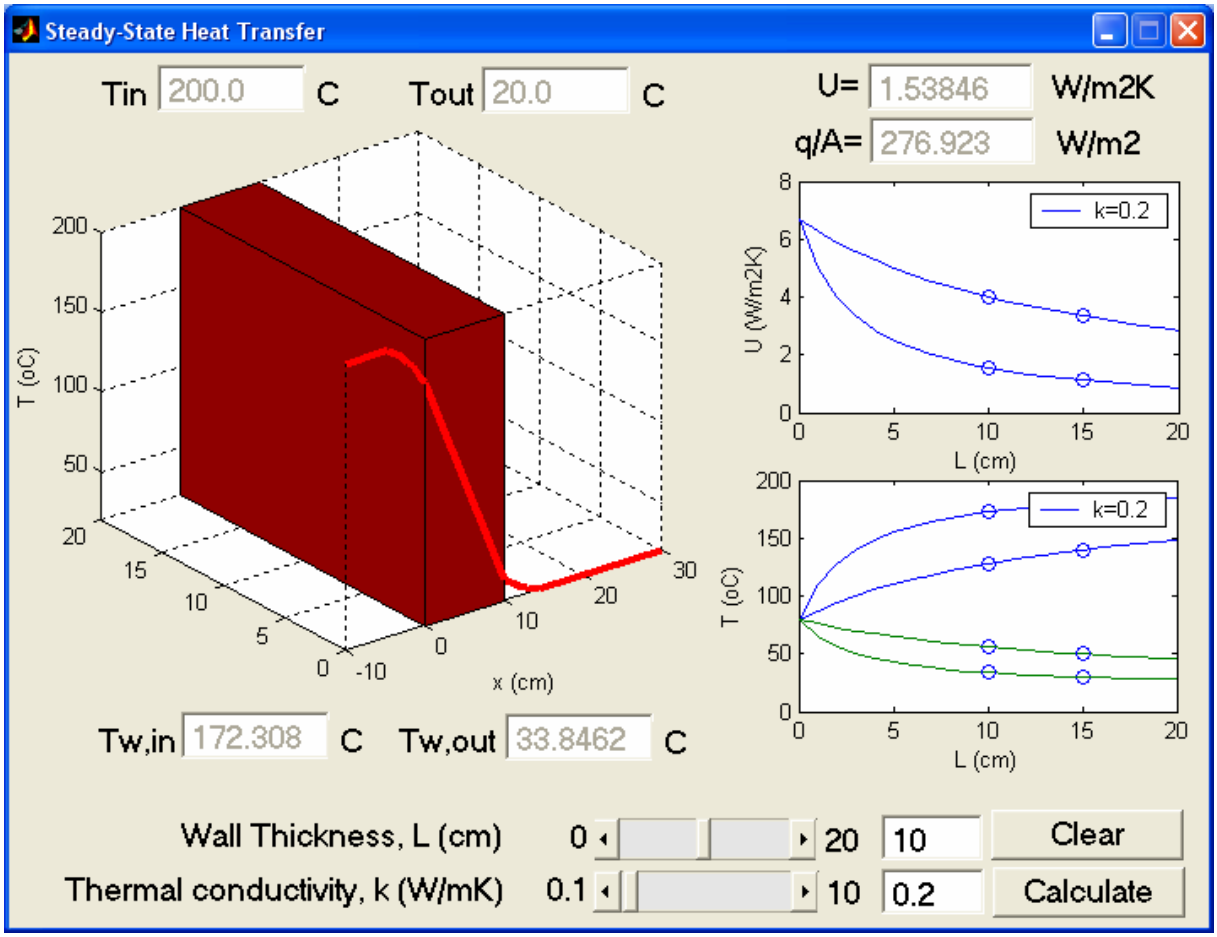

Figure 4. MATLAB GUI for modeling heat transfer in planar surfaces.

allow them to experiment freely with it without requiring any coding or knowledge on their part in MATLAB or Fluent.

Figures 1 and 2 present the GUIs for steady-state convective/conductive heat transfer for planar and cylindrical configurations respectively. A description of the GUI elements in both cases follows. The graph on the left hand side of the model window shows the physical wall through which heat is transferred with the temperature profile projected on the front surface. Edit controls and sliders at the bottom allow the student to change the values of the wall thickness and the thermal conductivity of the solid material. Charts on the right-hand side of

the window display the values of the heat transfer coefficient, the heat rate and/or the wall temperatures as functions of the wall thickness. Different lines in the graphs correspond to different values of heat conductivity. As soon as a parameter value is changed by the user, the figures are updated and new dots in the charts indicate the new values for the overall heat transfer coefficient and the temperature profile. In this way, the student can easily discern the effect of changing the values of parameters either independently or simultaneously.

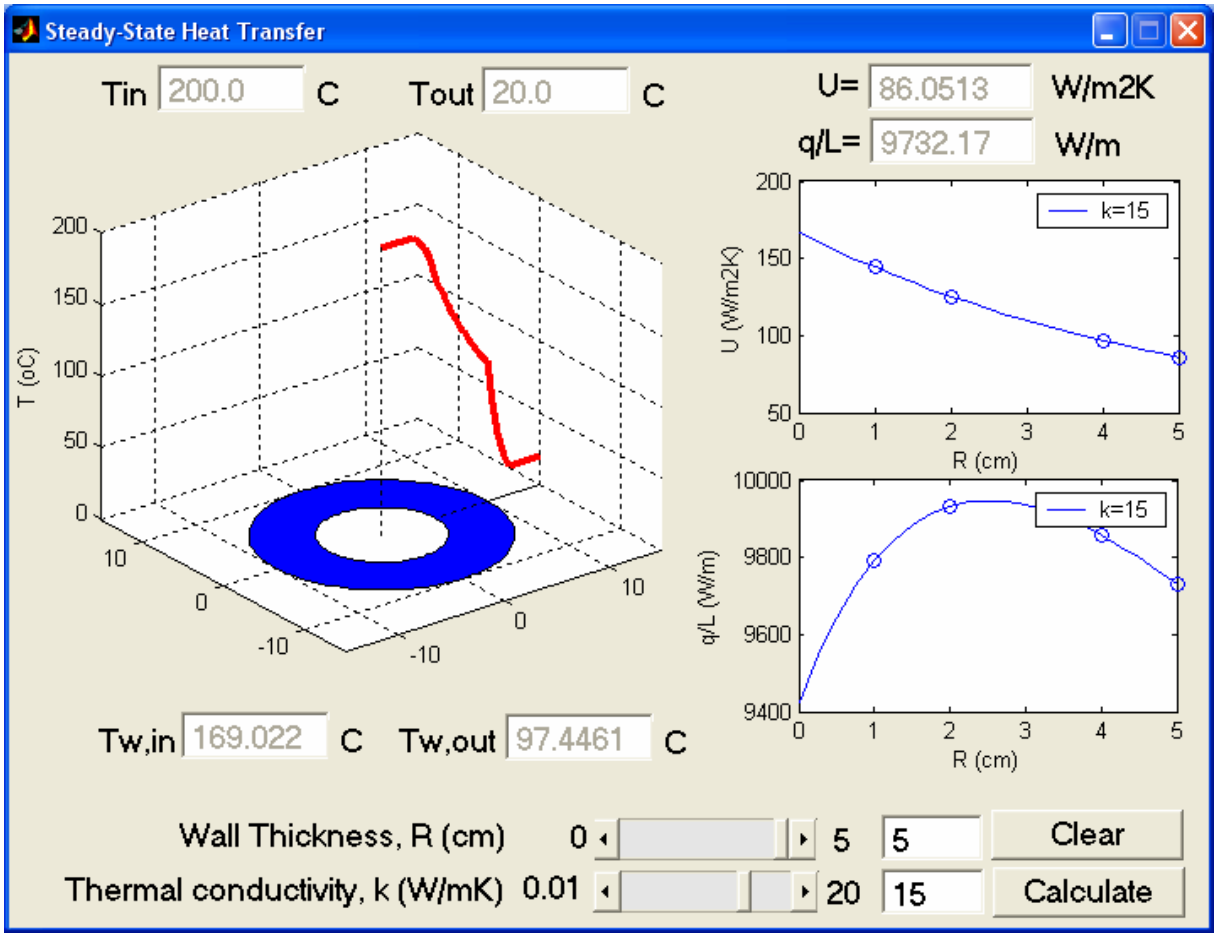

Figure 5. MATLAB GUI for modeling heat transfer in cylindrical surfaces.

As shown in Figure 1, the experiment makes apparent to the student that as the wall thickness increases, the value of the overall heat transfer coefficient decreases (as expected) but also the temperature difference between the two walls of the solid layer increases. As the wall becomes thinner or the solid's heat conductivity is increased, the burden of maintaining a given temperature gradient between the two fluids separated by the solid surface moves to the convective terms in the fluids side. In Figure 2, the competing effects of increasing the wall thickness and the inevitable increase in the surface area become apparent. Although the value

of the overall heat transfer coefficient decreases monotonically as wall thickness increases, the profile of heat rate per unit length shows a maximum demonstrating the fact that increased heat losses are possible even when adding more insulating material around a pipe.

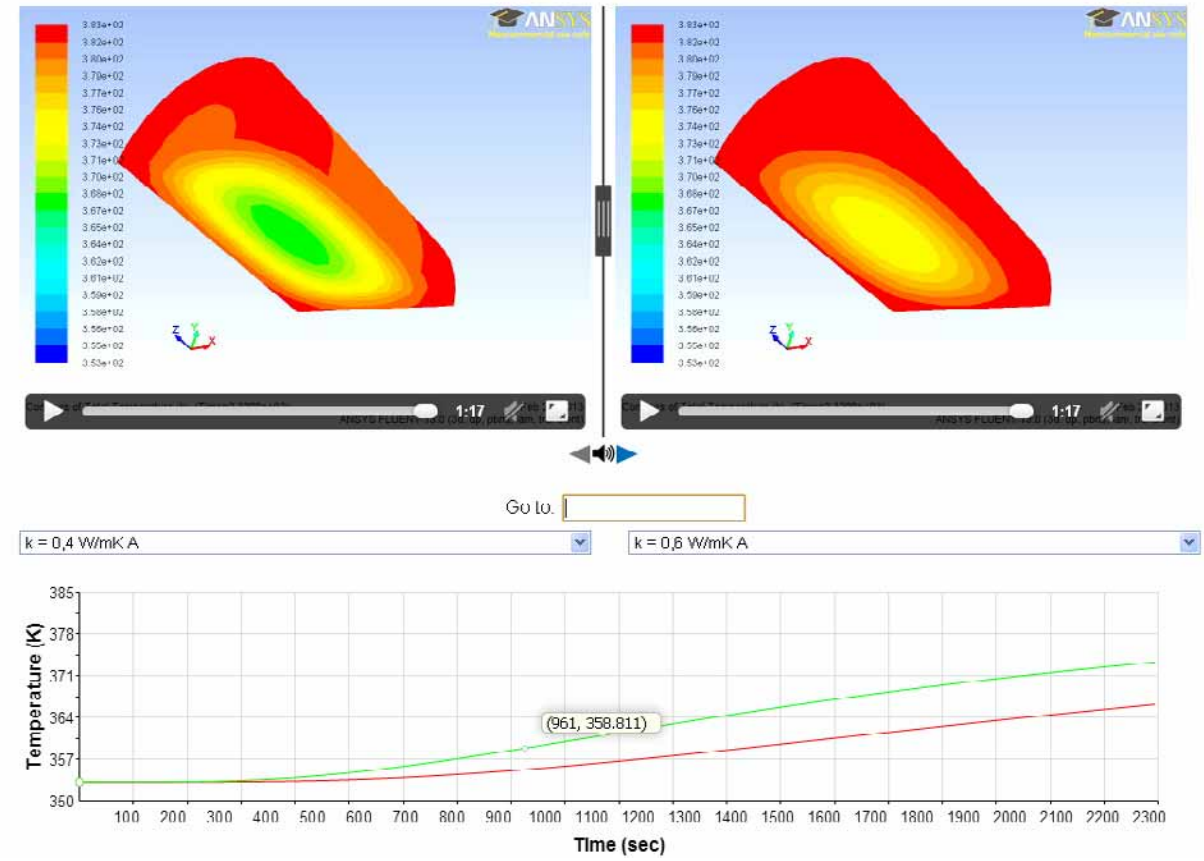

Figure 6. Web-based comparison of transient temperature profiles in heated cans.

Figure 3 shows an HTML5-based GUI through which videos and graphs of temperature profiles can be selected and shown in comparison for different parameter values (such as heat conductivity). The profiles correspond to a heated can containing a solid food with no natural convection present. The user can observe the evolution of the temperature profile for a single set of values or by comparing two different sets. In the latter case, the videos are synchronized to correspond to the same time instance for all displays. The chart displaying the temperature at the center of the can is also evolving as the video time progresses. The temperature value at a specific time instance is shown through a fly-over tooltip. The user can stop the video, move forward or backwards in time or jump to a specific time instance. In other words, the student can reproduce the phenomenon in any way possible while at the same time recording the values of key variables (temperature in this case) at selected time instances.

4. EDUCATIONAL SCOPE

The modules described in the previous section will be offered as web-based applications (and standalone applications in some instances) that users can interact with. In all cases, there exist input variables that the user can manipulate by changing their values (e.g. thermal conductivity) and output variables that are computed through the model simulating the process (e.g. the temperature profile).

These applications can be used by a teacher to provide visual context to the equations describing heat transfer phenomena but they can also be used outside the classroom for self-learning. In the latter case, there are two modes of interaction with the application:

- free interactive mode: students are completely free to 'play' with the model by changing any parameter values they want and observing the effect on the output variables,
- guided interactive mode: students are guided to use specific sets of parameter values, observe important effects that are known (to the module developer) to occur at those values and are prompted to offer explanation.

The free interactive mode allows students to observe and learn at their own pace in an open, learner-centered environment. Even if changes are random, it is still possible for some noteworthy behavior to occur that will prompt the user to search for an explanation. In the guided interactive mode, the student will be offered specific problems to simulate (textbook exercises or real-world situations) and will be asked through multiple-choice questions to predict the process behavior and explain it after the model is solved.

5. CONCLUSIONS AND FUTURE WORK

Preliminary results for the development of a simulation-based web environment for learning basic food processing phenomena were presented. Through its different modules, this learning framework is planned to cover phenomena in fluid mechanics (flow in pipes, around submerged objects etc.) for various fluid properties (e.g. rheological behavior, viscosity, density), heat transfer, mass and energy balances in single and integrated processes etc. It is expected that by offering to the students an open, interactive, learner-centered environment that exploits the benefits of the web and process simulation technologies, a deeper understanding of these phenomena can be achieved.

ACKNOWLEDGEMENTS

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VALUE ADDED UTILIZATION OF BLACK TEA FACTORY WASTE:ANTİOXİDANT ACTİVİTY AND PHENOLİC CONTENT

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ABSTRACT:

Research on value added utilization of food industry wastes for the development of novel food ingredients is driven by consumer demand for natural ingredients and sustainability concerns. Black tea waste is an attractive raw material for the development of novel food additives with antioxidant properties due to its bioactive components and large volume of consumption in tea drinking countries.Black tea factory waste streams containing tea leaves, tea dust, stalk and fibers to varying extents are physically removed during processing and have no commercial value. In this study, the antioxidant activity (using DPPH assay) and the phenolic content (using Folin-Ciocalteu assay and HPLC-DAD analysis) of 70% methanolic extracts of six black tea waste samples were determinedto assess the potential of their value added utilization. The six samples -two oven drying waste samples (OW1, OW2), a thin fibrous stream separated after the oven drying step (FW), two grading waste samples (GW1, GW2), and tea dust collected from filters of the processing line (TD)- and black tea were collected from the same processing line at a black tea factory in the Black Sea region of Turkey at the second harvest season of 2012. Antioxidant activity and phenolic content of samples ranged between 33.1 and 48.2 µmol TE/g and 3.3 and 6.3g GAE /100 g, respectively. FW, OW1 and GW1 had the highest antioxidant activity amongst the waste samples, which were comparable with that of black tea. Total phenolic content of OW1 and FW was also significantly higher than black tea ($P< 0.05$). These results demonstrate the potential of tea factory waste, particularly OW1, FW and GW1 fractions, as a source of bioactive ingredients with antioxidant properties.

Keywords: Black tea, waste, antioxidant activity, phenolic content

1. INTRODUCTION

Recovery of bioactive compounds from food industry wastes has gained importance due to environmental and economic considerations. There is also a growing interest in natural food ingredients obtained from food industry wastes. Tea and tea processing wastes are rich in bioactive compounds, especially catechins, which are important antioxidants. Tea, the most widely consumed drink in the world after water, is produced fromyoung tea leaves and buds of the *Camilla sinensis* plant.However,due to different picking practices, old tea leaves, tea stalks and stems may enter the black tea fermentation process. Since old tea leaves and tea stalks contain less amount of catechins compared to young tea leaves (Lin, Juan, Chen, Liang and Lin, 1996)they are partially fermented and do not have the typical dark brown-black tea color due to the loweramount of polymeric tea compoundsformed; theaflavins and thearubigins. This leads to lower quality tea liquor and an inhomogeneous structure in the final product. Therefore, fibrous material arising from old tea leaves, tea stalksand stems are physically removed during black tea processing. Hairy structures present in fresh tealeaves form tea dust and are separated together with other wastes during processing.Black tea factory wastes are generally burned at the factory site since they do not have any commercial value.

Black tea processing in general includes withering, curling, fermentation, oven drying and grading steps while actual process may differ for each country and factory resulting in different waste streams. The waste streams based on the actual process of a black tea factory in Turkey are as follows:oven waste 1 (OW1) mainly consists of light particles of tea, tea fiber and tea dust obtained by filters from the air exiting the drying ovens. Heavier tea fibers, which are notdragged by air movement in the oven, are separated by fiber collectors situated at the exit of the oven(oven waste 2, OW2). Grading of dried tea is performed by screening tea using a series of sieves, which contains fiber collectors on them to collect fibrous particlesgiving two waste fractions; (i) Fibrous waste (FW)consists of tea particles and fiber and stored for processing later to remove residual black tea particles, and (ii) Grading waste 1 (GW1) consistsof mainly tea fiber.After appropriate grades of black tea are separated, the remaining part on the sieves, which consists of unbroken tealeaves and tea stalks, is removed and may also be reprocessed (Grading waste 2, GW2). Tea dust (TD) is collected from filters placed in the processing area.

Research on the utilization of tea factory wastes mainly focused on caffeine extraction (Gürü and İçen, 2004; İçen and Gürü, 2009, 2010) and isolation (Shalmashi, Abedi, Golmohammad, Eikani, 2009). While there are individual studies reporting the antioxidant activity of black tea

waste (Farhoosh, Golmovahhed, Khodaparast, 2007; Thea, Lloret, Brumovsky, Schmalko, 2012), there is no systematic study on the different waste streams produced during black tea processing. In this study, different waste streams collected at a black tea factory were evaluated in terms of their antioxidant potential and phenolic content to show their utilization potential.

2. MATERIALS AND METHODS

2.1. ChemicalsandStandards

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol reagent (2N),(+)-catechin (C; ≥99.0%), gallicacidmonohydrate (≥98.0%), caffeine (≥99.0%), (-)-epicatechin (EC; ≥98%), (-)-epicatechingallate(ECG; ≥98%), (-)-epigallocatechin (EGC; ≥95%), (-)-epigallocatechingallate(EGCG; ≥95%),teaextractfromblackteacontainingtheaflavinmixture(≥80%),acetonitrile (HPLC grade), EDTA (ethylenediaminetetraacetic acid disodium saltdihydrate), and ascorbic acidwerepurchasedfromSigma-Aldrich. HPLC grade methanol from VWRandmilli-Qwater produced using a Millipore Water Purification System (Milli-Q A10) were used for the experiments.

2.2. Samples

Samples (six different black tea factory waste samples (two oven wastes -OW1 and OW2-, twograding wastes -GW1 and GW2-, fibrous waste -FW- andtea dust –TD)and black tea (BT-Grade 2))were obtained from the same processing line at a tea processing factory in the Black Sea region of Turkey at the second harvest season of 2012.Samples were kept at -80°C until they were used.Waste products containing high amount of bulky fibers (GW2 and FW)were ground by an IKA analytical mill for 10 seconds under nitrogen before extraction. Moisture content of the samples determined using oven drying method according to ISO1573:1980 (1980) was used to express results on a dry weight basis.

2.3. Extractpreparation

The extraction procedure was performed in triplicatesas described by ISO 14502-2:2005 (2006). Briefly, 0.2 g of sample was extracted twice with 5 ml of 70% methanol at 70°C for

10 min. After centrifugation at 3500 rpm/min for 10 min, the supernatant was decanted and final volume was made up to 10 ml with 70% methanol. Extracts were filtered using 0.45 µm regenerated cellulose filters and stored at -20°C until further analysis.

2.4. Antioxidantanalysis

Antioxidant capacity of the samples was determined using DPPH radical scavenging activity assay (Brand-Williams, Cuvelier, Berset, 1995) as modified by Fukumota andMazza(2000). Seven dilutions of extracts were prepared with 80% methanol.Trolox standards were prepared from 1 mg/mL stock solution in concentrations between 0.01 and 0.07 mg/mL in 80% methanol. 22 µl of diluted sample or Trolox standard and 200 µl of DPPH solution were mixed and absorbance readings were made at 30°C and 515 nm after 10 seconds shaking and a 30min incubation period using a microplate reader (Thermo Scientific, Multiskan Go, Finland).A plot of absorbance versus concentration was made for each sample and Trolox standard. DPPH free radical scavenging activity was calculated as µmolof Trolox equivalent (TE) per gram dry weightby dividing the slope of the linear portion of the sample (R^2=0.98-0.99) by that of theTrolox standard (R^2=0.99).

2.5. Determination of total phenoliccontent

Total phenolic contents of extracts were determined using the Folin-Ciocalteu assay (Ainsworth and Gillespie, 2007). Gallic acid standard solutions in the range of 0.02 and 0.2 mg/mL were prepared by diluting 1 mg/mLstock solution. 100 µLof diluted samples or gallic acid standard was mixed with 200 µL of 10% Folin-Ciocalteu reagent in a microcentrifuge tube and vortexed. Then, 800 µL of 700 mM Na_2CO_3 wasadded. After incubation at room temperature for 2 hours, 200 µL of mixture was transferred to a microplate(Thermo Scientific, Multiskan Go, Finland)and absorbance readings were taken at room temperatureat 765 nmin triplicates. The results were expressed as grams of gallic acid equivalents(GAE) per 100 g of dry weight.

2.6. HPLC analysis

Caffeine, catechins and theaflavin contents of samples were determined usingan HPLCsystem equipped with Accelaautosampler, Accela 600 pump, and Accela PDA dedector(Thermo Scientific) according to ISO 14502-2:2005 (ISO, 2006). Identification was done by comparing retention timesand UV spectra of unknown peaks with standard compounds.

Quantification of gallic acid and catechinswasdone using caffeine standard in conjunctionwith individual relative response factors (RRFs) with respect to caffeine given in ISO (2006). Total theaflavin content was expressed as percent area of total identified compounds.

2.7. Statistical analysis

Analysis of variance (ANOVA) and Tukey test was applied to determine significant differences ($P < 0.05$) among samples using the software program MINITAB (Release 16).

3. RESULTS AND DISCUSSION

Six different waste fractions (OW1, OW2, FW, GW1, GW2and TD)collected at a black tea processing factory were analyzed to determine their antioxidant capacity and phenolic content.Antioxidant activity and total phenolic content of samples are shown in **Figure 1** and **Figure 2**, respectively.

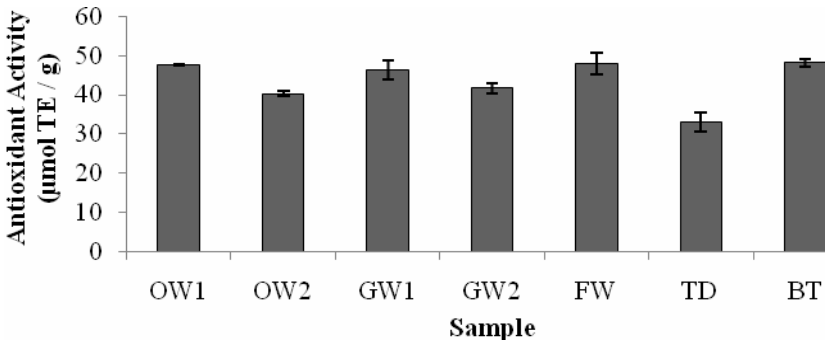

Figure 1. Antioxidantactivity of extracts of black tea factory waste.

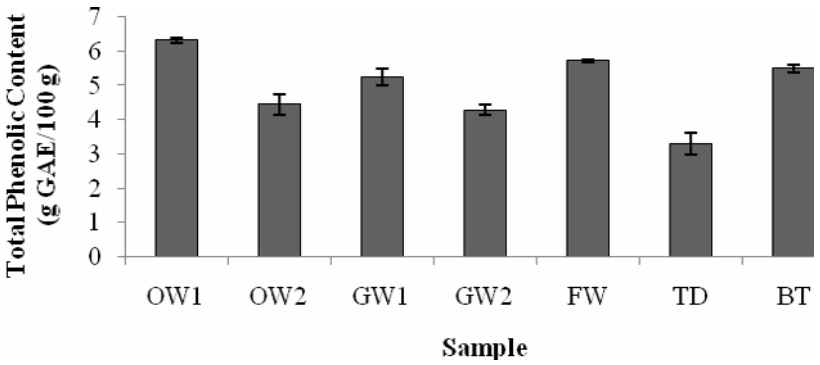

Figure 2. Total phenolic content of extracts of black tea factory waste.

Antioxidant activity of waste samples ranged between 33.1 and 48.2 μmolTE per grams dry weight. FW, OW1, GW1 had antioxidant activity comparable to that of black tea(ranging from 46 to 48 μmol TE/g).OW2 had significantly lower antioxidant activity compared to both black tea and OW1 ($P<0.05$). This result is expected as while OW1 containsboth small tea particles and tea fibers, OW2 contains mainly fibers. TD, which contains mainly the hairy structures present on the tea leaves, hadthe lowest antioxidant activity. This might be due to the uneven distribution of antioxidant compounds in tealeaves. There are a few studies on the antioxidant activity of black tea factory wastes, however direct comparison of the results is not possible due to the different antioxidant assays used. In the study of Farhoosh, Golmovahhed, Khodaparast (2007),antioxidant activity of black tea wastes obtained from a tea factory in northern Iranwasdeterminedby thiocyanate method, which measures antioxidant activity as the rate of reduction of lipid oxidation.Antioxidant activity of black tea waste, commercially available fiberous grading waste used for low grade tea production in Argentina, was found lower than that of black tea using DPPH antioxidant method by Thea, Lloret, BrumovskyandSchmalko(2012), however,as theirresultswereexpressed as ascorbicacidequivalents, a directcomparisonwithourresultswas not possible.

Gallic acid, caffeine, catechins (C, EGCG, ECG),and theaflavins were detected and quantified in the black tea and waste samples by HPLC(**Table 1)**.

TABLE 1. Content of (mg/g dry weight) main tea compounds**.**

Compounds	OW1	OW2	GW1	GW2	FW	TD	BT
Gallic acid	0.56±0.02^{b}	0.34±0.01^{d}	0.46±0.04^{c}	0.50±0.03^{c}	0.61±0.01^{b}	0.30±0.03^{d}	0.87±0.05^{a}
(-)-EGC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(+)-C	2.49±0.06^{a}	1.49±0.23^{c}	2.09 ± 0.15^{b}	1.73±0.14^{c}	2.58±0.03^{a}	1.16±0.13^{d}	2.45±0.09^{a}
Caffeine	16.59±0.53^{b}	10.46±0.65^{e}	13.97±0.93^{c}	12.48±0.43^{d}	16.63±0.16^{b}	9.13±0.84^{f}	18.81±0.68^{a}
(-)-EC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(-)-EGCG	1.26±0.02^{b}	0.97±0.06^{cd}	1.31±0.04^{b}	1.09±0.05^{c}	1.52±0.01^{a}	0.91±0.09^{d}	1.49±0.12^{a}
(-)-ECG	0.45±0.02^{c}	0.38±0.04^{d}	0.56±0.04^{ab}	0.46±0.06^{c}	0.62±0.03^{a}	0.34±0.02^{d}	0.51±0.04^{bc}
Total catechins	4.75±0.08^{b}	3.18±0.31^{d}	4.42±0.33^{b}	3.77±0.2^{c}	5.34±0.02^{a}	2.71±0.26^{e}	5.32±0.27^{a}
TFs (%Area)	22.15±0.23^{a}	19.02±0.29^{c}	18.96±0.23^{c}	17.86±0.43^{d}	20.72±0.39^{b}	18.16±0.29^{d}	19.96±0.81^{b}

[1]Means in rows followed by a different letter are significantly different ($P<0.05$), [2]n.d.=not detected.

BT, OW1 and FW had the highest caffeine content (16.6 to 18.8 mg/g). Caffeine content of black tea and a black tea grading waste mainly consisting of tea fibers was reported to be in the range of 10 to 36 mg/g in the study of Thea, Lloret, Brumovsky and Schmalko (2012) which is consistent with the finding of this study and shows the potential of tea fiber as a caffeine source. Total catechin contents of FW and BT (5.3 mg/g) were also highest followed by OW1 and GW1. The order of samples according to their total catechin content was similar with the results of antioxidant activity and total phenolic assays. All black tea factory wastes contained EGCG (Table 1); there was no significant difference between EGCG content of FW and BT ($P < 0.05$), which were the highest. Sample OW1 had the highest theaflavin content (total area %)whereas it had lower total catechin content than BT ($P<0.05$).OW1 also had the highest total phenolic content as stated above, which might result from its high theaflavin content. Comparison of our findings with literature could not be carried out as compositional analysis of black tea wastes by HPLC has not been reportedpreviously.

4. CONCLUSIONS

In conclusion, our results show that black tea factory wastes may be a good source of bioactivephenolic compounds. Recovery of these compounds from black tea factory wastes and their application in food industry warrants further investigation. This study makes an important contribution to the field as it provides valuable information on the characterization of the different black tea waste streams in terms of their phenolic content and bioactivity, which is required for process development but has been lacking in the literature. The differences between the composition and bioactivity of the waste streams collected at different sites should be considered while selecting raw materials for successful process design.

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VOLATILE COMPOUNDS IN FRESH AND PROCESSED OYSTER MUSHROOMS (*PLEUROTUS OSTREATUS)*

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ABSTRACT

Oyster mushroom' (*Pleurotus ostreatus*), a saprobic fungus, is known as a promising medicinal mushroom exhibiting haematological, antiviral, antitumor andhypocholesterolemic activities. Ambient temperature vacuum distillation is used to extract the volatile organic compounds (VOC) from fresh, boiled under reflux and dried mushrooms. The volatiles are also investigated during 10 days storage at 5 °C in order to detect spoilage and find the optimum conditions for usage and preservation of mushrooms. Different drying conditions (40, 50, 60 and 70 °C) are also investigated for the preservation and characterisation of VOC. The relative concentration (RC) of 1-octen-3-ol and 3-octanol decreased drastically as the mushrooms were boiled under reflux but remain the highest concentration among volatiles. The RC of 1-octen-3-one increased at D10. Benzaldehyde content did not fluctuate with the storage conditions. The amount of 2-ethyl,1-hexanol increased with time then was stabilized at D3. Results also showed that drying time and temperature affected the VOC profile with new compounds being formed while others are being lost.

Keywords: Oyster mushrooms, ambient temperature vacuum distillation, VOC, drying.

1. INTRODUCTION

Mushrooms are a valuable component of the diet whose culinary and commercial value is due to their organoleptic properties, being possible to distinguish edible mushrooms species on the basis of their characteristic odour or aroma (Zawirska-Wojtasiak*et al,* 2004, Pinho*et al,*2008). The oyster mushroom (*Pleurotus ostreatus*) is one of the most widely cultivated mushrooms (Bano andRajarathnam, 1986). It is regarded as a delicacy due to its characteristic texture and pleasant flavour.Having a high moisture content ~90 % , *P.ostreatus* are a good source of carbohydrates and proteins, low in fat generally the unsaturated are predominant over the

saturated fatty , a source of minerals (potassium, phosphorous, magnesium) and vitamins (folic acid, thiamine, riboflavin, vitamin C). They also contain ergosterols which can be converted to vitamin D under ultraviolet irradiation (Bano and Rajarathnam, 1988).The main compounds responsible for the fresh, stored and thermally processed mushrooms are a series of aliphatic alcohols and ketones known as C8 compounds: 1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octen-3-one, and 3-octanone.These compounds are formed by the oxidation of linoleic acid by the enzymes lypoxygenase and hydroperoxidelyase(Tressl *et al*., 1982 ;Chen and Wu, 1984; Mau *et al*., 1992; Assaf*et al*., 1997).

Pleurotus mushrooms are a delicate variety of mushrooms owing to their high moisture content.Therefore they require adequate postharvest storage in order to maintain their quality. Lowering the temperature reduces respiration and transpiration rate, delays senescence, prevents wilting and shrivelling thus extending their shelf life (Burton and Twyning, 1989; Beit- Halachmy and Mannheim, 1992).Drying is an effective technique for the preservationof mushrooms and other food commodities. As water is removed, there is a fractional loss of the VOC, increase in other compounds as well as changes in the enzymatic activity. During drying, Maillard reactions between sugars and amino acids may result in the formation of new compounds including VOC whose entity forms a specific aroma and flavour of the dry product (Misharina, 2009).

Conventional VOC sampling techniques for mushrooms includes solvent extraction and simultaneous distillation extraction (SDE) (Zawirska-Wojtasiak et al., 2004; Chen and Wu (1984, Cheung and Cheung 2005). However, these methods present some drawbacks, such as thermal decomposition and degradation of compounds during thermal extraction or distillation (Kimbaris*et al.,* 2006). Ambient temperature vacuum distillation (Ashmore *et al*, 2013) was proven to yield a complete and representative extract of the original and fresh mushroom aroma. Solid-phase micro-extraction (SPME) developed by Arthur and Pawliszyn (1990) and Pawliszyn (1995), is a simple, fast and solvent free sampling method. SPME has been widely used in environmental (Penalve*et al.,* 1995), biological (Mills and Walke, 2000), pharmaceutical (Ulrich, S., 2000), field analyses (Kozie*let al.,* 1999), and fragrance-and-aroma studies (Augusto *et al.,* 2003 and Soini*et al.,* 2006). In recent years, several applications of the analysis of mushroom VOC by HS-SPME (Tressl*et al.,* 1982; Wurzenberger and Grosch(1983); Cheung et al., 2009) have been published. It is safe and straightforward procedure that is amenable to automation and has been successfully applied to the analysis of a wide range of VOC in food and beverages (Polaskova*et al.,* 2008).

This study aimed at the investigation of the qualitative and quantitative composition of VOC in fresh mushrooms and their changes as results of the drying procedures and their intensity as well as indicator of spoilage as the raw mushrooms are stored over a period of 10 days at 4°C.

2. MATERIALS AND METHODS

2.1 Mushrooms

Fresh oyster mushrooms (*Pleurotus ostreatus*) were harvested from Li Sun exotic mushrooms (Mittagong, NSW, Australia) and were assigned day 0. The mushrooms were then packed and stored.A sample of 50 g was cleaned, then blended to a puree to fragment hyphae, gills and veils thus facilitating greater release of VOC.

2.2 Treatments

a)Fresh mushrooms (F) were cleaned, blended to a puree and fed into a 2 L round bottom flask with water for ambient temperature vacuum distillation.

b) Boiled under reflux mushrooms (B): Same as a) then cooked with water for 30 min, then VOC are extracted by ambient temperature vacuum distillation.

c) Dried mushrooms (D): The mushrooms were cleaned, sliced, dried then fed into a 2 L round bottom flask with water for ambient temperature vacuum distillation.

2.3 Ambient temperature vacuum distillation

Ambient temperature vacuum distillation (4 h at 30 Pa) was performed on the several mushroom samples according to the method published by Ashmore *et al.*, (2013)

2.4 Concentration using SPME

An SPME holder (Supelco, PA, USA) was used in performing the second stage of the experiment. SPME was performed on the combined cold traps to extract the VOC using a fused silica fibre coated with 50/30μmlayer of divinylbenzene/carboxen/polydimethylsiloxane (Supelco). The fibre was conditioned following the manufacturer's instructions prior to its use and was thermally cleaned between analyses by injecting it to the GC injection port at a 250 °C for 10 min. The sample vial was placed inside the bath at 25 °C and was allowed to condition for the equilibrium time (10 min) with no fibre exposition. The fibre was then introduced into the sample vial and was exposed to the headspace of the sample during the corresponding extraction time (1.5 h).

2.5 Identification and quantification of volatiles compounds using GC-MS

The SPME was injected in the splitless mode into the GC-MS and left for 0.5 min allowing the VOC to desorb. GC-MS analysis was performed using an Agilent 19091S-433 equipped with a DB-5ms column (30 m length * 0.25 mm i.d. * 0.25 nm film thickness). The carrier gas used was helium at a constant flow rate of 1.1 ml/min. The oven temperature was initially held at 30 °C for 5 min, and then raised to 250 °C at a rate of 3 °C/min held at 250 °C for 25 min. All the chromatogram peaks were identified by means of mass spectral library then confirmed by comparison with the retention time of the authentic reference (Wiley 275, Hewlett-Packard Company, Palo Alto, CA.).

2.6 Detection of spoilage

Fresh oysters mushrooms were stored at 5 °C until needed. The quality was assessed by extracting the VOC using ambient temperature vacuum distillation then followed by SPME and GC-MS. The analysis was carried on day 0 (collection date), day 1, day 2, day 3, day 4, day 7, and day 10.

2.7 Drying

The drying procedures were carried out using heat pump drier at 40, 50, 60 and 70 °C. The drier was run without the sample for 1 h to set the desired drying conditions before each drying experiment. Then 1 kg of mushroom sample was uniformly spread on a metal tray in a single layer. The tray was hung over the inside of the drying chamber and connected to the hook of an electronic balance with a sensitivity of 0.1 mg. The change in the sample weight was monitored at 5 min intervals for 10-15 h depending on the drying temperature. The weight data were collected using a PC –based data acquisition system. The drying experiments were performed in duplicate. The moisture content was determined according to AOCA method (2002).The fresh and dried weighed were used to calculate the moisture content which was expressed as g water/g dry solid.

2.8 Quantification

VOC were quantified using the method of standard addition. A mixture of standards (benzaldehyde, 3-octanone, and nonanal) of known concentrations was added progressively

and the relative concentration (RC) of the volatiles was determined using the standard addition curve.

3. Results and discussion

3.1 Detection of spoilage

Volatiles in mushrooms are a mixture of organic compounds belonging to a range of chemical classes and exhibiting a wide range of physical and chemical properties. The amount of the main C8 compounds varied with the storage time and the boiling under reflux.The concentration of 1-octen-3-ol and 3-octanol decreased drastically as the mushrooms were boiled under reflux but remain the highest RC among volatiles (Figure1). Under thermal processing, the amount of 1-octen-3-one decreased from 0.049 ppm to 0.006 ppm and 3-octanone decreased from 0.664 ppm to 0.234 ppm. Previously, Picardi and Issenberg (1973) isolated 1-octen-3-one only in cooked *A.bisporus* and concluded that 1-octen-3-one is either found in cooked mushrooms or it is present in the fresh mushroom but its level is well below the detection limit of the instrument. In this work, the latter compound was found in fresh and boiled under reflux samples which suggest that fresh *P.ostreatus* contain the compound previously detected in cooked mushrooms.

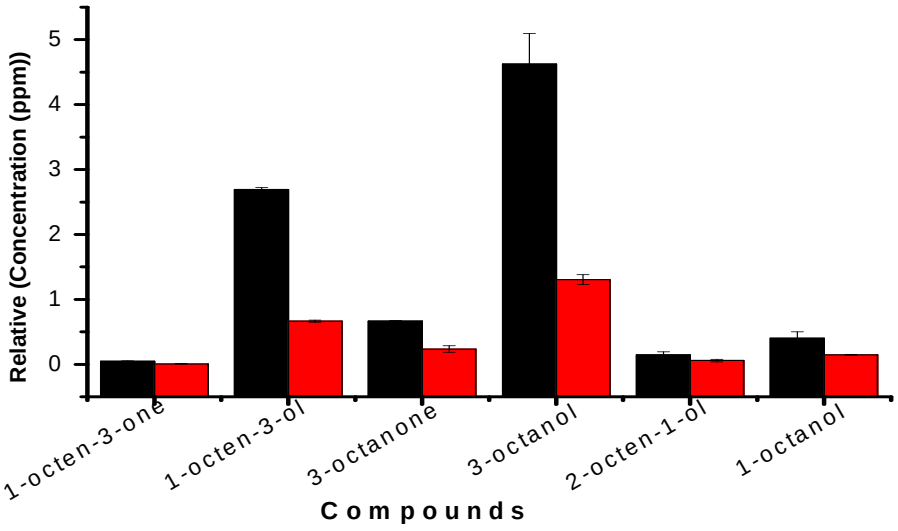

Figure1. The changing trends of main C8 compounds in fresh (D_0 inblack)and boiled under reflux (B, in red) oyster.

The RC of 1-octen-3-one varied with the storage time with the highest amount recorded at d_2 (0.082 ppm) and the lowest amount at d_4(0.0236 ppm) (Figure 2). No significant difference

was observed between the harvesting day (D_0, 0.049 ppm), D_1 (0.054 ppm), D_3 (0.049 ppm), and D_7 (0.054 ppm). However, the RC of 1-octen-3-one increased at D_{10} (0.072 ppm).

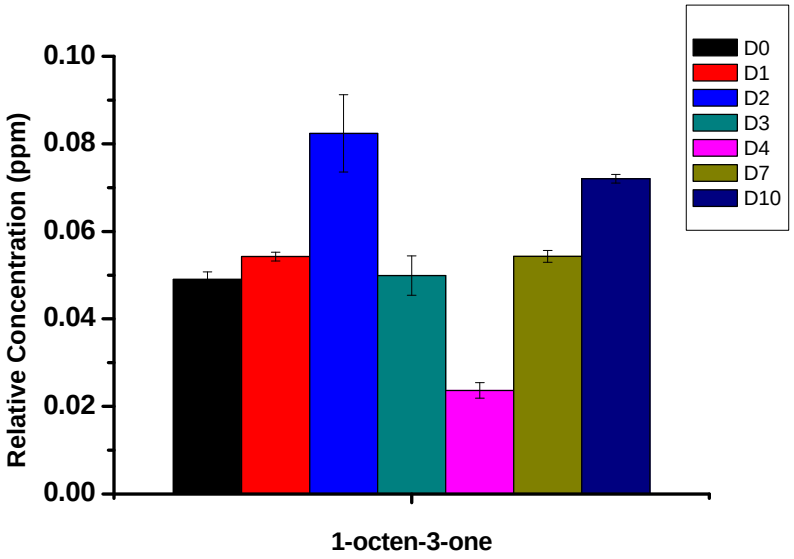

Figure2. Changes in the concentration of 1-octen-3-one as a function of storage time

The RC of benzaldehyde did not vary throughout the storage period (Figure 3) and was found to be 0.002 ppm +/- 0.0003. 2-ethyl,1-hexanol content fluctuated with time with the lowest concentration recorded at D0 (0.004 +/- 0.0002),then it increased at D3 (0.134 ppm +/- 0.008)to finally reach plateau from D3 to D10 (0.200 ppm +/- 0.028).As for 1-octen-3-ol, it had a considerably higher RC at D0 compared to the 2 former compounds (2.69 ppm +/- 0.050) reaching its maximum at D2 (3.07 ppm +/-0.008) then started to decline to reach a minimum of 0.200 ppm +/- 0.0001.

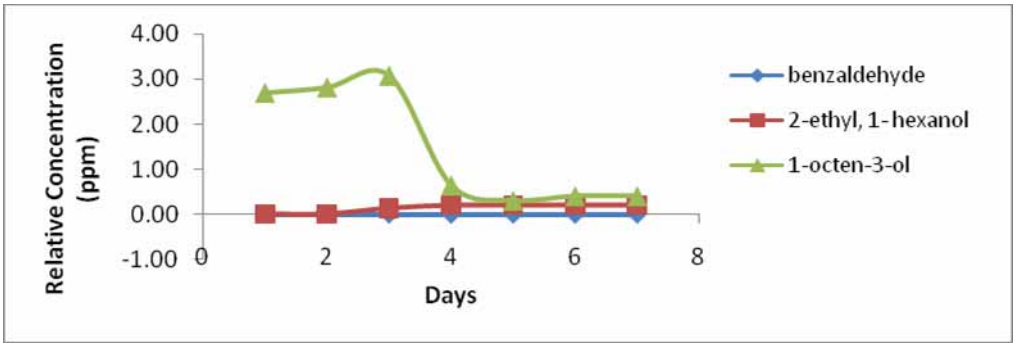

Figure3. Fluctuations in benzaldehyde (blue), 2-ethyl, 1-hexanone (red)and 1-octen-3-ol (green) concentrations during storage.

3.2 Drying

Four different drying temperatures were used to determine the effect of drying time and temperature and the quality of the mushrooms in terms of their VOC content.The moisture

ratio of the samples as a function of drying time was presented in Figure 4 for 40,50,60 and 70 °C drying air temperatures. All lines have two stages: the moisture ratio rapidly declined then slowly decreased with increasing drying time. It can be also seen that drying temperature has a tremendous effect on the total drying time. The rate of moisture loss was higher at 70 °C and the lowest at 40 °C.

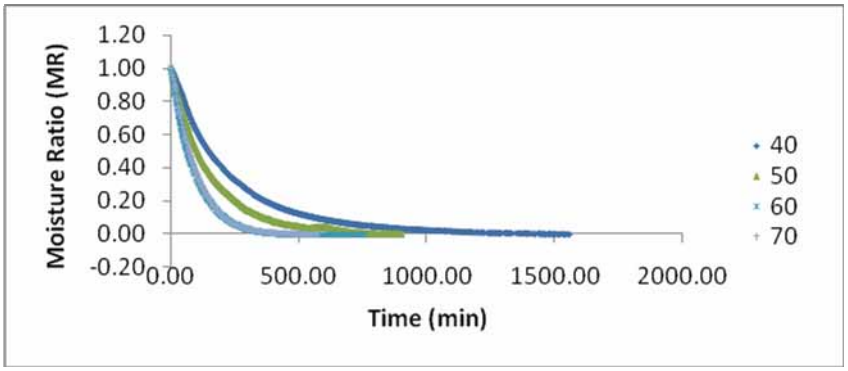

Figure4.Moisture ratio versus time at 40, 50, 60 and 70 °C.

In terms of VOC content, new compounds were formed, 1 compound disappeared while the RC of the some compounds fluctuated depending on the drying temperature and time (Table 1). 2,5 –hexadienal was only detected at 60 and 70 °C with a RC 0.004+/-0.001 and 0.013+/-0.0001 ppm respectively. 2,3,4-trimethylfuran and 2-undecanone were absent from samples at 40°C. The amount of hexanal was quadrupled when 70 °C was used compared to 40 °C (0.001+/-0.0003 versus 0.004+/-0.0001ppm). The highest amount of 1-hexanol, nonanol, decanal, and phenol,2,4-bis-(1,1-dimethyl) was noted at 50 °C but decreased with increasing temperature and increasing time unlike n-octanol whose RC decreased with increasing temperatures. The lowest RC of 1-octen-3-ol was observed at 60 °C (0.005+/-0.0003 ppm) and it doubled at 40, 50 and 70 °C.The amount of 3-octanol and 2-octen-1-ol was greatly affected at 60 and 70 °C where almost half of the compound is lost (3.007+/-0.147 ppm at 40 °C versus 1.370 +/-0.3050 ppm) and (0.023+/-0.0053 ppm at 40 °C versus 0.007+/-0.0012 ppm at 70 °C). 3-octanone and 1,3-benzendiol, 3-methyl, 1-hexanol, 2-ethyl were almost unaffected by the temperatures.1-octen-3-one could not be detected which can be explained by either its level is well below the detection limit of the instrument or it did not survive the drying conditions.

TABLE 1.Relative concentration (RC) of VOC during drying at 40, 50, 60 and 70 °C.

Compound	RC 40 °C ppm*	RC 50 °C ppm*	RC 60 °C ppm*	RC 70 °C ppm*
2.5-hexadienal	ND	ND	0.004+/-0.001	0.013+/-0.0001
3-methyl, 1-butanol	0.008+/-0.0001	0.007+/-0.0001	0.023+/-0.0070	0.015+/-0.0031
hexanal	0.001+/-0.0003	0.003+/-0.0002	0.003+/-0.0001	0.004+/-0.0001
1-hexanol	0.011+/-0.00101	0.036+/-0.0004	0.018+/-0.005	0.016+/-0.0008
3-heptanone	0.0006+/-0.0001	0.002+/-0.0001	0.001+/-0.0002	0.001+/-0.0002
2,3,4-trimethylfuran	N/D	0.001+/-0.0003	0.002+/-0.0004	0.005+/-0.0001
Benzaldehyde	0.008+/-0.0001	0.004+/-0.0003	0.005+/-0.0004	0.015+/-0.0014
1-octen-3-ol	0.016+/-0.0004	0.010+/-0.0003	0.005+/-0.0003	0.010+/-0.0023
3-octanone	2.200+/-0.1252	2.601+/-0.0521	2.355+/-0.2930	2.326+/-0.1611
3-octanol	3.007+/-0.1470	3.126+/-0.1610	1.759+/-0.3240	1.370+/-0.3050
1,3-benzendiol, 5-methyl	0.005+/-0.0002	0.004+/-0.0003	0.004+/-0.0004	0.003+/-0.0011
1-hexanol, 2-ethyl	0.004+/-0.0007	0.005+/-0.0001	0.073+/-0.0020	0.005+/-0.0012
2-octenal	0.002+/-0.0001	0.004+/-0.0003	0.006+/-0.0002	0.006+/-0.0004
2-octen-1-ol	0.023+/-0.0053	0.021+/-0.0021	0.006+/-0.0013	0.007+/-0.0012
n-octanol	0.234+/-0.0122	0.175+/-0.0162	0.090+/-0.0021	0.080+/-0.0013
nonanal	0.003+/-0.0003	0.008+/-0.0014	0.004+/-0.0007	0.007+/-0.0012
nonanol	0.029+/-0.0011	0.042+/-0.0013	0.020+/-0.0042	0.016+/-0.0023
decanal	0.002+/-0.0001	0.005+/-0.0002	0.004+/-0.0004	0.003+/-0.0001
2-undecanone	N/D	0.004+/-0.0001	0.006+/-0.0003	0.007+/-0.0001
phenol,2,4-bis-(dimethyl)	0.021+/-0.003	0.032+/-0.0071	0.005+/-0.0023	0.005+/-0.0022

*Amounts are per 1ml extract

4. Conclusion

The VOC in *P. ostreatus* were investigated in fresh, boiled under reflux, dried and stored mushrooms for 10 days. The VOC content was fluctuating with 1-octen-3-ol the major volatile being lost while benzaldehyde content did not vary significantly during storage. 1-octen-3-ol and 3-octanol content decreased upon boiling under reflux. Different drying conditions affected the VOC profile where some compounds were newly formed such 2,5 – hexadienal,2,3,4-trimethylfuran and 2-undecanone while 1-octen-3-one was lost or could not be detected.The highest amount of 1-hexanol, nonanol, decanal, and phenol, 2,4-bis-(1,1-dimethyl) was noted at 50 °C but decreased with increasing temperature. The highest RC of most VOC was detected at 50 °C and hence it could be recommended for drying.

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COMPARISON OF DIFFERENT METHODS FOR EXTRACTION OF BIOACTIVE COMPOUNDS FROM LOVAGE (*Levisticum officinale* L.) ROOTS

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ABSTRACT:

Lovage (*Levisticum officinale* L.) is a perennial herb belonging to the *Umbelliferae* family. All parts of the plant are strongly aromatic and rich in bioactive compounds, and its seeds, leaves and roots are commonly used in food. The aim of the current research was to determine the best extraction method and time for obtaining lovage root extracts with high content of phenolic compounds and antiradical activity. For isolation of biologically active compounds several methods can be applied, and in current research for extraction of phenolic compounds from lovage roots the ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) were used. For comparison Soxhlet extraction (SOXE) was applied because in previous experiments about horseradish the method showed very good results. Using MAE content of phenolic compounds and antioxidant activity of extracts decreased by increasing extraction time, and the highest results were observed in samples extracted for 15 seconds. Whereas for UAE certain trend depending on extraction time was not observed, and the highest results in sample extracted for 60 seconds were obtained. In the present investigation, was found a great variability of extracts regarding the content of total phenolics and flavonoids, and antioxidant activity using several assays (DPPH scavenging activity, Trolox equivalent antioxidant capacity (TEAC), reducing/antioxidant power). In our experiment strong and moderate positive correlation between content of phenolic compounds and antioxidant activity was observed. For isolation of extracts with high content of phenolic compounds and antioxidant activity from lovage very important is extraction method and time and the best results were obtained by Soxhlet extraction 2h, but as a fast and convenient method MAE for 15 sec can be used.

Keywords: Antioxidant, phenolic, lovage, MAE, UAE.

1. INTRODUCTION

Plant phenolic compounds are one of the most important primary antioxidants, so it is important to investigate the quantities them in different plant species. Phenolic compounds commonly found in spices are biologically active substances having antiseptic, vitamin activity expression, and other properties (Rappoport, 2003; Daayf and Lattanzio, 2008). Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing (Marrelli et al, 2012). Based on these statements, it can be concluded that it is very important to develop the best method for extraction of these compounds from plants.

Lovage (*Levisticum officinale* L.) is a perennial herb belonging to the *Umbelliferae* family, with a characteristic earthy, celery-like flavour and smell (Szebeni-Galambosi, Galambosi and Holm, 1992). All parts of the plant being strongly aromatic, and its seeds, leaves and roots (fresh, powdered and as essential oils), are commonly used in Europe for flavoring foods and beverages and for their medicinal properties (Cu, Pu, Shi, Perineau, Delmas and Gaset, 1990). Lovage root has also been known for centuries as a medicine possessing spasmolytic, diuretic and carminative activities (Raghavan, 2000). Many plants of *Umbelliferae* family also contain several bioactive phytochemicals such as flavonoids and coumarins, which are reported to have curative, preventive, or nutritive value (Cherng, Chiang and Chiang, 2008).

For isolation of biologically active substances several methods are used, as conventional and Soxlet extraction. But for these methods has several disadvantages as long extraction duration and high volumes of solvents (Luque de Castro and Priego-capote, 2011). Actual is also question about “green chemistry”, and scientists develop methods that are envirometally friendly (Shivhare, Orsat and Vijaya Raghavan, 2009).

As one of the recently used alternative method for extraction of biologically active substances is ultrasound-assisted extraction (UAE). This method offers shorter extraction duration, easier manupulations, reduced use of electricity and solvents (Tabaraki and Nategi, 2011). During sonication, the cavitation process causes the swelling of cells or the breakdown of cell walls, which allow high diffusion rates across the cell wall in the first case or a simple washing-out of the cell contents in the second (Chemat, Tomao and Virot, 2008).

Recently scientits are also interested in microwave-assisted extraction (MAE), especially in the field of food science. MAE are based on the action of microwaves to the molecules with ion conductivity (Hemwimon, Pavasant and Shotipruk, 2007). Microwaves

can enter in the plant material and interaction with polar molecules results in increase of presure in cell and further interaption of cell walls and release of phytochemicals occur (Wu, Yan, Liu, Marcone, Aisa and Tsao, 2012). Using of MAE can reduce extraction duration and energy consumption significantly comparing to tradicional extraction methods (Luque de Castro and Priego-capote, 2011). Several factors can affect MAE efficiency, bet one of the most important one is extraction duration (Ballard, Mallikarjunan, Zhou and O'Keefe, 2010).

The aim of the current research was to determine the best extraction method and time for obtaining lovage root extracts with high content phenolic compounds and antiradical activity.

2. MATERIAL AND METHODS

2.1 Materials.

Fresh lovage roots (*Levisticum officinale* L.) were collected in Latvia during the period from September to November, 2012. For analyses the average sample of 300 grams was taken from 3 roots. Roots were washed, peeled and homogenized (for 5 minutes) together in order to obtain representative sample.

2.2 Extraction procedure.

For extraction of phenolic compounds the UAE, MAE and Soxhlet extraction (SOXE) was used. The extraction process was done in triplicate.

2.2.1 Microwave-assisted extraction (MAE).

A sample (3g) of lovage root was put into a 100 ml conical flask. After adding 30 ml of 95% ethanol, the flask was exposed to the microwave. The microwave extractor was operated at 900 W with an emission frequency of 2450 MHz under atmospheric pressure condition and the extraction was caried out 15 – 90 sek.

2.2.2 Ultrasound-assisted extraction (UAE)

A sample (3g) of lovage root was placed into a 100 ml conical flask, in whick 30 ml of 95% ethanol were added. Then the flask was sonicated 15 – 1800 sek.in an ultrasonic water bath. The output power was 250W and the frequency was 50 kHz.

2.2.3 Soxhlet extraction (SOXE)

Three grams of the sample were placed in the filter cartridge (paper No. 89) in a classical Soxhlet apparatus and extracted with 170 ml of 95% ethanol for 2 h. Extracts were cooled to room temperature.

2.3 Analytical methods

2.3.1 Determination of total phenolic content (TPC)

The TPC of the roots extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton, Orthofer and Lamuela-Raventos, 1999) with some modifications. The absorbance was measured at 765 nm and total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the lovage.

2.3.2 Determination of total flavonoid content (TFC)

The TFC was measured by a colorimetric method (Kim, Jeong and Lee, 2003) with minor modification. The absorbance was measured at 415 nm and total flavonoids were expressed as catehin equivalents (CE) 100 g^{-1} DW of the lovage.

2.3.3 Determination of DPPH$^{\cdot}$ radical scavenging activity

Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) radical as outlined by Yu, Haley, Perret, Harris, Wilson and Haley (2003). The absorbance was measured at 517 nm and the radical scavenging capacity (RSC) was expressed as Trolox mM equivalents (TE) 100 g^{-1} DW of the lovage.

2.3.4 Determination of ABTS$^{\cdot}$

The RSC of extract was also measured by ABTS$^{\bullet+}$ radical cation assay (Re, Pellegrini, Proteggente, Pannala, Yang and Rice-Evans, 1999). For the assessment of extracts, the ABTS$^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800±0.030 at 734 nm. The RSC was expressed as TE 100 g^{-1} DW of the lovage. The higher the Trolox equivalent antioxidant capacity (TEAC) value of a sample, the stronger the antioxidant activity.

2.3.5 Determination reducing power

The reducing power can be determined by the method of Athukorala, Kim and Jeon (2006). The absorbance is measured at 700 nm and reducing power were expressed as ascorbic acid equivalents (AAE) 100 g^{-1} DW of the lovage.

Additionally for all lovage roots moisture content was determined according to standard ISO 6496:1999 and all results are expressed to dry basis.

For all extracts TPC, TFC, DPPH$^{\bullet}$**,** ABTS radical scavenging activity and reducing power were determined using a spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). For all methods of measuring antioxidant activity the control sample contained all the reaction reagents except the extract. All determinations were performed in triplicate.

2.4 Statistical analysis

Experimental results were analyzed by Microsoft Excel 2010 and SPSS 17.00. Data were expressed as the mean values of triplicate tests standard deviation of the mean. Analysis of variance (ANOVA) and Tukey test were used to determine differences among samples. A linear correlation analysis was performed in order to determine relationship between the parameters. Differences were considered as significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Total phenolics and flavonoids content.

The TPC and TFC determined in lovage roots extracts obtained by different methods and extraction durations are shown in Table 1.

TABLE 1

Total phenolic and flavonoid content in lovage roots depending on extraction time and method.

Extraction		Total phenolic content, mg GAE $100g^{-1}$ DW	Total flavonoid content, mg CE 100 g^{-1} DW
MAE	15 sec	362.28 ± 0.81^{a}	458.15 ± 2.36^{a}
	30 sec	295.30 ± 0.46^{b}	424.90 ± 1.93^{b}
	45 sec	245.58 ± 0.28^{c}	369.58 ± 1.64^{c}
	60 sec	212.26 ± 0.59^{d}	332.51 ± 1.08^{d}
	90 sec	193.91 ± 1.51^{e}	$326.04\pm1.95^{d, e}$
UAE	15 sec	141.03 ± 2.22^{f}	209.85 ± 4.83^{f}
	30 sec	$184.30\pm0.43^{g, h}$	274.07 ± 2.15^{g}
	45 sec	197.82 ± 3.29^{i}	314.55 ± 6.05^{h}
	60 sec	308.29 ± 3.14^{j}	376.11 ± 4.29^{c}
	90 sec	182.25 ± 1.03^{g}	294.31 ± 1.24^{i}
	150 sec	166.79 ± 1.33^{k}	248.06 ± 2.31^{j}
	210 sec	172.77 ± 2.73^{l}	234.67 ± 5.49^{k}
	300 sec	$186.15\pm0.56^{h, m}$	$242.97\pm7.07^{j, k}$
	900 sec	189.60 ± 1.41^{m}	288.93 ± 6.75^{i}
	1800 sec	250.32 ± 1.17^{n}	$319.38\pm3.18^{e, h}$
SOXE	2 h	518.69 ± 1.48^{o}	932.50 ± 5.01^{l}

* Mean values within the same column followed by different letters significantly differ according to the LSD test ($p<0.05$).

Results of multivariate dispersion analyses showed that both extraction duration and method are significant factors affecting TPC and TFC ($p < 0.05$). Extracts obtained by SOXE showed significntly higher TPC. Aspé and Fernández (2011) analysed influence of conventional, SOXE, MAE and UAE to the TPC in *Pinus radiata* bark extracts and reported that SOXE showed the highest results that are in accordance with our results. Experimental results showed that for each method different tendencies depending on extraction duration can be

observed. Applying MAE, TPC un TFC decreased by increasing extraction duration, and the highest content of phenols in extracts obtained after 15 seconds are determined. Ballard, Mallikarjunan, Zhou and O'Keefe (2010) also repoted similar trend for MAE extracton of TPC from peanut skin with ethanol. Also using MAE for *Vitis vinifera* wastes TFC decreased by increasing extraction duration (Casazza, Aliakbarian, Mantegna, Cravotto and Perego, 2010). In UAE extracts fluctuating trend was determined and the highest results in sample extracted for 60 secunds was obtained. Song, Li, Liu and Zhang (2011) reported similar trend for TPC in extracts from *Ipomoea batatas* L. leaves obtained by MAE. Also several researchers reported opposite tendency that by increasing extraction time TPC in extracts increased, and it was found in olive tree leaves (Şahin and Şamli, 2013), blackberry leaves (Aybastier, Işık, Şahin and Demir, 2013), grape jam (Morelli and Prado, 2012) extracts.

3.2 Antioxidant activity

There are variations of antioxidants contained in lovage roots depending on extraction method and time (Table 2).

TABLE 2 Analysis of antioxidant capacity of lovage roots depending on extraction time and method.

		DPPH$^{\bullet}$, mM TE 100 g^{-1} DW	ABTS$^{\bullet+}$, mM TE 100 g^{-1} DW	Reducing power, mg AAE 100 g^{-1} DW
MAE	15 sec	17.44±0.01 ^{m*}	15.82±0.12 ^{g}	2055.30±3.10 ^{m}
	30 sec	12.10±0.02 ^{j}	15.63±0.12 ^{g}	1627.70±7.32 ^{k}
	45 sec	6.80±0.18 ^{c}	15.43±0.04 ^{g}	1469.56±8.19 ^{J}
	60 sec	6.46±0.02 ^{b}	13.26±0.28 ^{f}	1405.72±1.95 ^{h}
	90 sec	6.20±0.03 ^{a}	8.48±0.29 ^{d}	1333.76±1.78 ^{g}
UAE	15 sec	7.77±0.03 ^{d}	4.25±0.11 ^{a}	789.45±2.20 ^{a}
	30 sec	9.05±0.03 ^{f}	9.07±0.21 ^{d}	1025.13±3.72 $^{b, c}$
	45 sec	9.48±0.03 ^{g}	9.01±0.41 ^{d}	1257.87±8.42 ^{f}
	60 sec	14.27±0.17 ^{l}	10.24±0.21 ^{e}	1705.90±2.15 ^{l}
	90 sec	10.90±0.01 ^{i}	7.29±0.56 ^{c}	1244.94±2.36 ^{f}
	150 sec	9.19±0.01 ^{f}	4.51±0.09 ^{a}	1047.56±9.12 ^{d}
	210 sec	8.04±0.02 ^{e}	3.90±0.33 ^{a}	1022.22±6.40 ^{b}
	300 sec	9.52±0.04 ^{g}	4.82±0.32 ^{a}	1039.28±4.28 $^{c, d}$
	900 sec	10.42±0.03 ^{h}	5.95±0.17 ^{b}	992.60±9.91 ^{e}
	1800 sec	12.40±0.09 ^{k}	8.80±0.22 ^{d}	1442.27±9.78 ^{i}
SOXE	2 h	27.13±0.04 ^{n}	15.87±1.19 ^{g}	2489.79±17.49 ^{n}

* Mean values within the same column followed by different letters significantly differ according to the LSD test (p<0.05).

For lovage root the results of multivariate dispersion analyses showed that extraction

duration and method are significant factors affecting DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and reducing power ($p < 0.05$). In *Vitis vinifera* waste seeds and peels extracts higher antiradical power (DPPH$^{\bullet}$), for MAE extracts comparing UAE was repoted (Casazza, Aliakbarian, Mantegna, Cravotto and Perego, 2010). In our experiments the highest antioxidant activity was in extracts obtained by SOXE. Aspé and Fernández (2011) found the similar trend in anti-radical capacity of *Pinus radiata* bark extracts obtained by conventional, SOXE, MAE and UAE. Whereas Hemwimon, Pavasant and Shotipruk (2007) reported that highest antioxidant properties are for MAE extracts but the lowest for SOXE.

Results of investigations about influence of extraction duration are different and Brasilian scientists investigated antioxidants in grape jam and in range between 10-30 minutes, found that maximum was reached after 20 miutes of extraction (Morelli and Prado, 2012), but for olive tree leaves after 40 minutes with extraction range from 20 to 60 minutes (Şahin and Şamli, 2013). These results show that it is necessary to adjust extraction conditions for each plant matrix. UAE to the antioxidant activity of blackberry leaves Aybastier, Işık, Şahin and Demir (2013) showed that by increasing extraction duration antioxidant capacity (ABTS$^{\bullet+}$) increases.

Investigations about influence of UAE to the antioxidant activity of blackberry leaves Aybastier, Işık, Şahin and Demir (2013) showed that by increasing extraction duration antioxidant capacity (ABTS$^{\bullet+}$) increases.

Trends for changes of antioxidant properties depending on extraction duration are similar to those obtained for TPC and TFC. For extracts obtained by MAE, increasing of extraction duration resulted in higher antioxidant activity, but for UAE extracts clear tendency was not observed. The highest antioxidant activity was for extract obtained by MAE for 15 sec.

3.3 Relationship between phenolic compounds and antioxidant capacity

Phenolic compounds have radical scavenging activity. The Pearson`s coefficients between the phenolic compounds levels and antioxidant capacities, are presented in Table 3. The antiradical capacity of an extract is often related to its polyphenolic constituents. In our experiment strong and moderate positive correlation between content of phenolic compounds and antioxidant activity was observed.

TABLE 3: Pearson`s coefficients lovage roots between total antioxidant capacity, total phenolic and total flavonoid content

	TPC	TF	DPPH	ABTS
TPC	1			
TF	0.928**	1		
DPPH	0.771**	0.584**	1	
ABTS	0.788**	0.911**	0.523**	1
Reducing power	0.961**	0.960**	0.669**	0.830**

**Correlation is significant at the 0.01 level (2-tailed).

Strong correlation between phenolic compounds and antiradical activity was also found in experiments about seabuckthorn (Hippohae rhamnoides L.) leaves (Kumar, Dutta, Prasad and Misra, 2011), lychee (*L. chinenesis* Sonn.) flowers (Liu, Lin, Wang, Chen and Yang, 2009) and canola meal (Hassas-Roudsari, Chang, Pegg, and Tyler, 2009).

4. CONCLUSION

Phenolic compounds has strong antioxidant properties, and for isolation of these compounds from lovage roots both extraction method and duration are significant. For higher content of phenols and antioxidant properties Soxhlet extraction 2h can be chosen, but from alternative methods as fast and effective MAE for 15 sec.

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MECHANISMS UNDERLYING THE EFFECTS OF LACTIC ACID BACTERIA (LAB) ON PHENOLIC COMPOUNDS

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ABSTRACT

Phenolic compounds (PC) are widely studied due to their effects on food quality and health. They not only give sensorial characteristics such as flavor, astringency and color; but produce health effects through their antioxidant, antimicrobial, anti-proliferative and anti-inflammatory properties, as well. Also, PC positively affect the growth and metabolism of Lactic acid bacteria (LAB), which are indigenous to raw fruits or vegetables and play an important role in fermentation. LAB show probiotic effect and are increasingly being added to the food systems to improve the products qualities with health promoting properties. In return, LAB can transform PC into small phenolic compounds through decarboxylation, dehydroxylation or cleavage; causing important changes in the aromatic profile of food products, thus creating a potential for improving the quality and complexity of PC-rich foods, or obtaining high added-value antioxidants from PC-rich food waste.

Recent evidence points to the contribution of PC and their metabolites to the maintenance of gastrointestinal health by interacting with epithelial cells, and largely by modulating the gut microbial composition. They may act as prebiotic that affect factors of survival for beneficial gut bacteria—mainly LAB—and thus, inhibiting the growth of enteric pathogens. A modulatory effect of the presence of PC in the gut flora in terms of increased *Lactobacillus* spp. and decreased pathogenic bacterial groups; as well as to the transformation of PC into more bio-available degradation products following intake by humans and animals. For example, phenolic acids such as those found in cereals, which mostly exist as bound phenolics, reach the colon relatively untouched by host digestive enzymes, but are readily absorbed there through enzymatic degradation by the intestinal microorganisms such as LAB Also, polyphenol-rich foods supplemented with probiotic LAB may improve the intestinal microflora, as well. However, such factors that may influence the capacity of LAB to metabolize the phenolic compounds are not well known. This review will provide an update

on the mechanisms of PC degradation by lactic acid bacteria, and the potential applications of this biotransformation in Food Technology and Health Sciences.

Keywords: phenolic compounds, Lactic acid bacteria, enzymatic degradation, intestinal microflora, bioavailability

1. INTRODUCTION

In the last years, PC has been gained more interest among researchers and food manufacturers due to the awareness of their antioxidant properties, their great abundance in the diet, and their important role in the prevention of various diseases such as cancer, and cardiovascular and degenerative diseases (Manach, et al, 2004). PC are classified into different groups including phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives), stilbenes, lignans and flavonoids according to their phenol ring function and structural diversity. Diversity does not only come from aromatic ring and hydroxyl group but in nature they also have organic acid and sugar conjugates mostly glycosides which influence the effects of PC on foods (Scalbert and Williamson, 2000; Ross and Kasum, 2002). The association of PC with food quality is due to mainly volatile phenols which are produced from hydroxylation of alcohols or metabolism of microorganism such as yeast and LAB and/or tannins related to astringency of foods by precipitating proteins (Rodríguez et al, 2009).

LAB preferably use the PC as substrate in their metabolism or in return they can degrade PC and produce some metabolites such as volatile phenols which gives foods their aromatic properties. This information is very crucial for food process technology for enhancement of the product quality in case of obtainment of high value added products and treatment of by-products (Landete et al, 2007; Rodríguez et al, 2009).

The mechanism in the body is more complex than as in the food. In the human body, most of the PC, which are naturally found in the form of esters, glycosides and polymers, cannot be metabolized directly and most of them reaches the human colon; absorbed and metabolized by the help of intestinal microflora (Manach et al, 2004; Scalbert and Williamson, 2000). Colonic microflora digest PC into their aglycones, which are further metabolized into low molecular aromatic acids (Rechner et al, 2004; Lee et al, 2006; Gonthier et al, 2003). This conversion is essential for the absorption and modulates the biological activity of PC (Selma et al, 2009).Specific bacterial strain, especially LAB, can be responsible for the specific

metabolism of these compounds in the body. PC can serve as substrate (as prebiotic) in their metabolism and induce the production of some organic acids which may inhibit the growth of pathogenic microorganisms in the colon (Tzounis et al, 2008). On the other hand, the antimicrobial properties of PC can alter the composition of gut microflora by inhibiting the sensitive ones (those mostly pathogenic bacteria) and promoting the others (those more resistant ones).

The physiological benefits of LAB may be enhanced in the presence of prebiotics (for instance fructo-oligosaccharides). Alternatively, probiotic strains might be selected for their ability to generate prebiotic oligosaccharides, which are then preferentially utilized by the producing probiotics (Rastall et al, 2005). Similarly, PC themselves also acts as prebiotic compounds to probiotic LAB providing the symbiotic relationship between PC and LAB. This relationship might be associated with improved outcomes related to the human health (Rastall et al, 2005).

2. THE INTERACTION MECHANISMS BETWEEN PHENOLIC COMPOUNDS AND LACTIC ACID BACTERIA

The interaction mechanisms between PC and LAB have potential effect on the quality of PC rich foods and their health promoting properties. The metabolism of phenolic compounds by LAB not only is limited to the degradation of PC into new metabolites but also results in the increase in biomass of LAB and can acts as growth stimulatory factor speeding up the other metabolisms of cells (Hervert-Hernandez et al, 2009). The catabolism activity of LAB depends on the chemical structure, degree of polymerization and the type of linkage which presents in PC (Sanchez-Patan et al, 2012). Moreover, different PC are metabolized by different LAB strains on different time periods. In return, the growth promoting effect of PC on LAB was also found to be dependent on the structure, the microbial strain and the assay dosage (Puupponen-Pimiä et al., 2001; Almajano et al, 2008; Hervert-Hernández et al, 2009). Because of their abundance in many foods and their persistence in human gastrointestinal tract (GIT) (Adlerberth et al, 1996), only few LAB species specifically *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus hilgardii, Lactobacillus acidophillus and Lactobacillus johnsonii* were well studied for their PC metabolism activities.

LAB strains such as *L. plantarum* and *L. brevis* possess many phenolic acid decarboxylase (PAD) and reductase enzymes for degradation of PC as presented in Table 1.

TABLE 1: Metabolism of some PC by LAB

Parent Compound	Compound produced	Enzymes involved	Species involved	References
p-coumaric acid	p-vinyl phenol	phenolic acid decarboxylase (PAD)	*L.plantarum, L.brevis*	Rodriguez et al, 2008c; Curiel et al, 2010
	ethyl phenol	reductase	*L.plantarum*	Rodriguez et al, 2008d
	phloretic acid	Uncharacterized reductase enzyme	*L.plantarum*	Barthelmebs et al, 2000
Caffeic acid	p-vinyl catechol	phenolic acid decarboxylase (PAD)	*L.plantarum, L.brevis*	Rodriguez et al 2008d; Curiel et al, 2010
	ethyl catechol	Reductase	*L.plantarum*	Rodriguez et al, 2008d
Ferulic acid	p-vinyl guaiacol	Phenolic acid decarboxylase (PAD)	*L.plantarum, L.brevis*	Rodriguez et al 2008d; Curiel et al, 2010
	ethyl catechol	Reductase	*L.plantrum*	Rodriguez et al 2008d
	hydroferulic acid	Uncharacterized reductase enzyme	*L.plantarum*	De las Rivas et al, 2009
Gallic acid	pyrogallol	Gallate decarboxylase enzyme	*L.plantarum L.brevis*	Rodriguez et al 2008d; Curiel et al, 2010
Tannic acid	gallic acid	Tannase	*L.plantarum*	Rodriguez et al, 2008a
	gyrogallol	Gallate decarboxylase enzyme	*L.plantarum*	Rodriguez et al, 2008a
Protocatechuic acid	catechol	Decarboxylase enzyme	*L.plantarum L.brevis*	Rodriguez et al 2008d; Curiel et al, 2010
Quinic acid	catechol	Uncharacterized enzyme	*L.plantarum*	Whiting and Coggins, 1971
Shikimic aicd	catechol	Uncharacterized enzyme	*L.plantarum*	Whiting and Coggins, 1971
Epicatechin-3-*O*-gallate	gallic acid	Galloyl esterases	*L.plantarum*	Sánchez-Patán et al, 2012
Gallic acid	pyrogallol	Decarboxylase enzyme	*L.plantarum*	Sánchez-Patán et al, 2012
Gallic acid	catechol	Benzyl alcohol dehydrogenases	*L.plantarum*	Sánchez-Patán et al, 2012
Oleuropein	elenoic acid, hydroxytyrosol and tyrosol	β-glucosidase and esterase	*L. plantarum*	Ciafardini et al, 1994: Marsillio et al,1996

As seen from Table 1, both *L. plantarum and L. brevis* were able to degrade food phenolic acids, hydroxycinnamic acids, into their vinyl derivatives by using their PAD enzymes (Cavin et al, 1997b; Barthelmebs et al, 2000; JECFA, 2001; Landete et al, 2008; Rodríguez et al, 2008d; Curiel et al, 2010). However, while *L. plantarum* further metabolized them into their ethyl derivatives by reductase, *L. brevis* was not able to modify vinyl derivatives to further

form ethyl derivatives (Chatonnet et al, 1992, 1995; Couto et al, 2006; Curiel et al, 2010). This information is very important for the food technologist since some ethyl derivatives have low sensory threshold and act as off-flavor by affecting negatively to food quality (Chatonnet et al, 1992).

Similarly, while *L. plantarum* has ability to metabolize hydroxybenzoic acids in foods by using their PAD (Whiting and Coggins, 1971; Rodriguez et al, 2008d; Curiel et al, 2010), *L. brevis* was only able to degrade protocatechuic acid and gallic acid into catechol and pyrogallol, respectively. Pyrogallol was reported as the most potent antioxidant among simple phenols (Ordoudi and Tsimidou, 2006) by suggesting that the metabolism of *L. brevis* strains might improve its antioxidant properties when present in a food substrate (Curiel et al, 2010).

On the other hand, oleuropein, which is the most abundant PC found in olive leaves and olive oil and responsible for the development of bitterness, was hydrolyzed to its aglycones by the β-glucosidase produced by *L. plantarum* (Ciafardini et al, 1994). Following this information, Marsillio et al (1996) showed that aglycone released from oleuropein further metabolized to elenoic acid, hydroxytyrosol and tyrosol by esterase activity of *L. plantarum*.

In return, PC can act as growth-stimulatory factor and results in increase in the biomass of cells (Hervert-Hernandez et al, 2009). The study of Hervert-Hernandez et al (2009) showed that both tannic acid and grape pomace phenolic extract had stimulatory effect on growth of *L. acidophilus* suggesting that PC would be supplying energy to cells or enhancing the nutrient consumption (sugar) (Alberto et al, 2001; Alberto et al, 2004). Similarly, quercetin (flavonol type PC) promoted quicker growth of *L. plantarum* (Landete et al, 2008) because of the affinity of quercetin to the lipid bilayer of *L. plantarum'*s cell membrane (Nakayama et al, 1998). The promotion in the growth of *L. plantarum* was also shown in the presence of flavanol and flavanol rich extracts (Lopez de Felipe et al, 2010). Catechin (flavanol type PC) allowed more substrate uptake of *L. plantarum* by affecting the transportation system, altering biophysical properties of cell membrane, and improved the fermentation performance which caused increase in the cell biomass (Lopez de Felipe et al, 2010).

3. THE ROLE OF INTERACTION BETWEEN PHENOLIC COMPOUNDS AND LACTIC ACID BACTERIA ON HUMAN GUT HEALTH

Human gut microbiota is composed of 10^{12} cells per gram and show commensal relationship with human host. While some colonic bacteria cause diseases, some release protective effect

by modulating the microbial balance. They have important roles on food metabolism to digest and absorb certain starches, fiber, oligosaccharides by releasing specific enzymes that human cannot able to secrete (Gibson, 2004). These enzymes can be responsible for hydrolyzing the glycosides, amids, esters, lactones and also can carry out the ring cleavage reduction and decarboxylation reactions (Selma et al, 2009). The bioavailability and potential bioactivity of a large proportion of dietary polyphenols are largely dependent on the activity of the colonic microbiota. LAB as a part of the human gut microbiota have capability to increase digestion of PC by breaking down into more easily absorbed phenolic compounds and provide them to release more antioxidative effect for human gut health (Rechner et al, 2004).

In many dietary fibers rich foods, phenolic acids such as hydroxycinnamic acids (hydroxynametes ferulic acid, sinapic acid and *p*-coumaric acid) are found in their ester forms bonded to the polymeric non-starch polysaccharides in plant cell wall and cannot be absorbed by human body. In human gut, phenolic acid esters are deesterified from dietary fibers and transformed to ferulic acid by the help of ferulic acid esterases produced by LAB species prior to absorption (Andereason et al, 2001). Released ferulic acids are easily absorbed and show powerful antioxidant effect as proved by both in vitro (Maillard et al, 1996; Nardini et al, 1995) and *in vivo* studies (Itagaki et al, 2009; Young et al, 2008). The distribution of cinnamoyl esterase activity in the intestine of rats and humans was shown by using synthetic hydroxy cinnamoyl esters as model substrates in the study of Andereason et al, (2001). This study demonstrated the release of human colonic esterase(s) (mostly of microbial origin) of sinapic acid and *p*-coumaric acid from rye and wheat brans suggesting that hydrolysis by intestinal esterase(s) is very likely the major route for release of antioxidant hydroxycinnamic acids in vivo. *L. acidophilus* strains were also examined for their capability to produce ferulic acid esterase (Szwajgier and Jakubczyk, 2010). *L. acidophilus* were able to transform ferulic acid to p-coumaric acids and probably caffeic acids from polymeric nonstarch polysaccharides (Szwajgier and Jakubczyk, 2010) suggesting that the alterations in the chemical structure of PC by *L. acidophilus*, can modify its absorption through the gut and the antioxidant activity exhibited into the body (Szwajgier and Jakubczyk, 2010).

L. johnsonii is another LAB found in GIT of many mammals including humans (Zang et al, 2011). *L. johnsonii* isolated from rat ileum was shown to produce enzymes having an ability to degrade common dietary phytophenols (ferulic, caffeic, and *p*-coumaric acid) as a mode of self protection (Lai et al, 2009; Birs, 2012).

The study of Jakešević (2011) showed that the addition of *L. plantarum* to rosehips of the rose species, *Rosa pimpinellifolia*, decreased lipid peroxidation and oxidative stress by increasing the concentration of PC (total PC content) in cecum part of mice gut. As a result, protocatechuic acid, which were shown to have high antioxidant capacity, was observed as a main degradation product of cyanidin-3-*O*-glucoside in the cecum content (Jakešević, 2011).

In the study of Tabasco et al (2011), it was reported that some *L. plantarum, Lactobacillus casei*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains were able to grow in the presence of a flavan-3-ol extract from grape seeds. In particular, *L. plantarum* showed the potential to catabolize flavan-3-ols not only by galloyl esterase, decarboxylase, and benzyl alcohol dehydrogenase activities but also by other possible enzymatic activities that resulted in the formation of a new unidentified metabolite. Identification of this new metabolite was performed by incubation of *L. plantarum* with individual monomeric flavan-3-ols and dimeric A- and B-type procyanidins (Sánchez-Patán et al, 2012). The results of this study showed that while *L. plantarum* strain was capable of rapidly degrading (–) epicatechin-3-*O*-gallate but not A- or B-type dimeric procyanidins, it was able to produce large changes in the phenolic profile of the cranberry extract mainly due to the catabolism of hydroxycinnamic and hydroxybenzoic acids. Also, *L. plantarum* cleaved the heterocyclic ring of monomeric flavan-3-ols, giving rise to 1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl) propan-2-ol, activity exhibited by only a few human intestinal bacteria (Sánchez-Patán et al, 2012).

In return, PC may act as prebiotic that affect factors of survival for beneficial gut bacteria—mainly LAB— shows symbiotic effects and thus, inhibiting the growth of enteric pathogens. Some in vitro studies demonstrated that PC especially, epicatechin and catechin exerts harmful and pathogenic affects or encourages the growth of potentially beneficial bacteria, *Bifidobacterium* spp. and *Lactobacillus* spp. (Lee et al, 2006; Tzounis et al, 2008). The improvement of the intestinal microflora can even be amplified more by supplementation of polyphenol-rich foods with probiotic LAB.

In conclusion, while the interaction mechanisms between PC and LAB were mostly investigated in food systems, extended studies are needed to understand their role in human gut microbiota and to reveal their further health effects.

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ANALYSIS OF VOLATILE COMPOUNDS IN GLUTEN-FREE FLOUR

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ABSTRACT:

Celiac disease is a permanent enteropathy caused by the ingestion of gluten, a protein occurring in wheat, rye and barley. Gluten-free products often have low quality, exhibit poor mouth feel and flavor. The development of gluten-free products is a challenge and very demanding call for food technologists. It is necessary to develop new high quality, tasty, inexpensive gluten-free products. Quality, appearance, flavor of the product depends on properties of used raw materials. The aim of this research was to investigate the volatile compounds in gluten-free flour. Six gluten-free flour types – amaranth, soy, extruded maize, yellow maize, rice and buckwheat – were studied. An investigation of volatile compounds in gluten-free flour is done using solid-phase microextraction (SPME) and gas-chromatography mass-spectrometry (GC-MS). The results showed that the total amount of volatile compounds isolated from each type of gluten-free flour is different. 30 volatile compounds were detected in gluten-free flour and among them identified 6 aldehydes, 1 furan, 10 alcohols, 6 acids, 2 alkene, 2 terpene, 1 ester, 1 phenylpropene, and 1 cyclic ester. Alcohols, aldehydes and acids are the major volatile compounds in gluten-free flour.

Keywords: gluten-free flour, volatile compounds, amaranth, maize, rice, buckwheat

1. INTRODUCTION

Celiac disease is one of the most common human genetic disorders; its prevalence is approximately 1–2% worldwide and apparently increased reports of celiac disease cases (Catassi and Fasano, 2008; Niewinski, 2008; Green and Cellier, 2007; Heap and van Heel, 2009). This condition has been recognized globally in Europe, North and South America, North Africa and Australia as well as in the Middle East and India, suggesting that celiac disease has a truly international face (Catassi and Fasano, 2008; Niewinski, 2008; Fasano and

Catassi, 2001; Alaedini, Peter, Green, 2005). Various novel approaches to celiac disease treatment have been explored. The only effective treatment is avoid peptides derived from gluten proteins, wheat endosperm and similar proteins from taxonomically-related species such as rye, barley, but also spelt, durum, kamut, triticale, emmer, and einkorn (Hamer, 2005; Catassi and Fasano, 2008; Kagnoff, 2007). Gluten-free cereal foods and the gluten-free diet can and should be nutrient dense and healthy. Gluten-free grains include an abundance of healthy whole-grain options, including buckwheat, rice, corn, amaranth, quinoa, teff, millet, sorghum and oats (Thompson, 2009).

Gluten is a major protein component of some cereals which is responsible for flour processing characteristics (good water absorption capacity, cohesiveness, viscosity and elastic properties) in bakery industry (Torbica, Hadnadev, Dapčevic, 2010; Arendt and Nunes, 2010). Due to the lack of gluten proteins in gluten-free cereals it is very difficult to obtain an acceptable yeast-leavened product, such as bread, because of the absence of a proper network necessary to hold the carbon dioxide produced during proofing, therefore the development of gluten-free bread with good quality and high nutritional value is a serious and challenge task to both the cereal technologists and the bakers. Therefore, many researchers have investigated the substitution of gluten by ingredients able to mimic its functional properties (Torbica, Hadnadev, Dapčevic, 2010; Blanco, Ronda, Perezs, Pando, 2011). The majority of commercially available gluten-free breads are inferior in quality to their gluten-containing counterparts. The basic gluten-free bread often present poor quality with a crumbling texture, dry and friable crumb (crumb which is wet after baking and sticks together, next day becomes dry, rough and crumbly), lack of flavor and mouth feel, poor color, relatively short shelf life and other post baking defects. Home-made gluten-free bread is prepared for several days, it is very important to keep sensory quality during the storage period. Also gluten-free breads are often characterized by low nutritional quality as they are mainly starch based and contain low amounts of vitamins, minerals and in particular dietary fiber (Torbica, Hadnadev, Dapčevic, 2010; Phimolsiripol, Mukprasirt, Schoenlechner, 2012). In recent years, various technological parameters and formulations has been investigated to improve quality of gluten-free bread by using flour mixtures (Sciarini, Ribotta, Leon, Perez, 2010) or starches (Milde, Ramallo, Puppo, 2012) or adding additives such as hydrocolloids (Peressini, Pin, Sensidoni, 2011), enzymes (Renzetti, Dal Bello, Arendt, 2008), gums and emulsifiers (Demirkesen, Mert, Sumnu, Sahin, 2010) or proteins (Crockett, Ie, Vodovotz, 2011).

The flavor of a food is created by aromatic volatile compounds that are biosynthesized during normal metabolic processes in plant and possibly further modified by cooking or processing. The natural habitat determinants, fertilization, storage conditions as well as the processing also have an influence on the forming of volatile compounds and could therefore affect the sensory quality. Food produced with thermal process contains many more volatiles than the raw material (Dresow and Böhm, 2009; Belitz, Grosch, Schieberle, 2009). To improve quality and flavor of gluten-free products it is necessary to start with investigation of volatile compounds in gluten-free flour, and then continue with dough and final product.

Cereals are the source of carbohydrates to supply food energy to the diet; the most used are wheat, maize, rice, rye and barley. Because of buckwheat and amaranth unique chemical structures, they are classified as pseudocereals. Legumes (such as chickpeas, soybean and peanut) are next to cereals in terms of their economic and nutritional importance as human food sources (Riahi and Ramaswamy, 2003; Arendt, Renzetti, Dal Bello, 2009). Preparation and storage of products from soybeans is often inhibited by rancidity and bitter aroma defects caused mostly by volatile aroma active carbonyl compounds, e.g., (Z)-3-hexenal, (Z)-1,5-octadien-3-one and 3-methyl-2,4-nonandione. The rancidity-causing is lipid peroxidation. One way to increase quality is to use defatted soybean protein, complete removal of lipids is used as an additional precautionary measure in order to obtain an off-flavor-free product (Belitz, Grosch, Schieberle, 2009).

The aim of this research was to investigate the volatile compounds in gluten-free flour.

2. MATERIAL AND METHODS

Experiments were carried out in the Laboratory of Packing Materials Investigation at the Department of Food Technology in the Latvia University of Agriculture.

2.1 Flour characterization

Four gluten-free flour types – yellow maize, extruded maize, rice and buckwheat from Joint Stock Company “Ustukiu Malunas” (Lithuania) – were studied. Amaranth seeds and soybean from company Rapunzel (Germany) were ground in a laboratory mill Hawos (Hawos Kornmühlen GmbH, Germany) obtaining fine flour for further studies.

2.2 Determination of volatile compounds

Volatile compounds were extracted from flour samples using solid-phase microextraction (SPME) with an 85 μm Carboxen/Polydimethylsiloxane (CAR/PDMS) coating (Supelco, Inc.). For SPME extraction 4.00±0.03 g of flour were weighed into a 20 ml vial. Extraction time was 65 min at 40±1 °C (pre-incubation without the fiber for 15 min, 40 °C). Volatile compounds from fiber were thermally desorbed in the injector of a gas chromatograph PerkinElmer 500 GC/MS with a capillary column Elite-Wax ETR (60 m x 0.25 mm i.d.; DF 0.25 μm). In this research GC oven temperature programme started at 40 °C, hold – 7 min, programmed from 40 to 160 °C at 6 °C min^{-1} and from 150 to 200 °C at 10 °C min^{-1}, hold 15 min; carrier gas (He) – 1 ml min^{-1}; split 2 : 1. Mass spectrometer in Electron impact Ionization (EI+ 70 eV) mode with inlet line temperature at 250 °C and source temperature 250 °C was used. Acquisition parameters in full scan mode: scanned m/z 40-400. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98 (Sabovics, Straumite, Galoburda, Kronberga, 2010).

3. RESULTS AND DISCUSSION

Totally 30 volatile compounds were detected and characterized by GC–MS analysis in the studied gluten-free flour. Table 1 shows the volatile compounds identified in the gluten-free flour samples. On the basis of functional groups, these volatile compounds were divided into 9 groups: aldehydes, furans, alcohols, acids, alkenes, terpenes, esters, phenylpropenes and cyclic esters.

TABLE 1. Peak area (area count x 10^6) of volatile compounds detected in gluten-free flour

Volatile compounds	Retention time (min)	Flour type					
		Yellow maize	Extruded maize	Rice	Buck-wheat	Amaranth	Soy
Aldehydes							
Hexanal	14.21	50.60	309.80	103.05	33.00	n.d.	22.96
Heptanal	16.93	11.34	1.36	n.d.	n.d.	n.d.	n.d.
Octanal	21.42	n.d.	n.d.	12.32	n.d.	n.d.	n.d.
Nonanal	22.81	3.61	26.36	11.30	8.62	n.d.	n.d.
Benzaldehyde	26.21	1.98	8.05	8.61	22.06	17.02	11.63
2-hydroxy-benzaldehyde	29.35	n.d.	n.d.	2.85	40.60	n.d.	n.d.
Subtotal		*67.53*	*345.56*	*138.13*	*104.27*	*17.02*	*34.59*
Furans							

Volatile compounds	Retention time (min)	Flour type					
		Yellow maize	Extruded maize	Rice	Buck-wheat	Amaranth	Soy
2-pentylfuran	18.30	n.d.	3.07	11.25	n.d.	n.d.	n.d.
Subtotal		*n.d.*	*3.07*	*11.25*	*n.d.*	*n.d.*	*n.d.*
Alcohols							
4-penten-2-ol	9.58	32.87	n.d.	n.d.	6.34	12.13	4.10
1-pentanol	18.81	9.36	7.32	n.d.	6.17	5.54	7.80
1-hexanol	21.75	n.d.	13.57	n.d.	n.d.	n.d.	n.d.
1-octen-3-ol	23.93	3.12	2.65	n.d.	3.13	0.80	5.37
1-heptanol	24.10	n.d.	3.72	n.d.	n.d.	n.d.	n.d.
2-nonanol	24.29	4.40	n.d.	n.d.	n.d.	n.d.	n.d.
2-octen-1-ol	24.98	n.d.	n.d.	5.30	n.d.	n.d.	n.d.
2-furanmethanol	28.59	n.d.	23.41	n.d.	6.49	3.22	4.79
Benzylalcohol	32.12	1.77	4.55	n.d.	n.d.	0.66	2.40
Phenol	33.95	n.d.	n.d.	n.d.	n.d.	0.96	n.d.
Subtotal		*51.52*	*55.21*	*5.30*	*22.12*	*23.31*	*24.46*
Acids							
Hexyl ester pentafl-uoropropanoic acid	21.62	45.51	n.d.	n.d.	6.60	18.26	50.98
Acetic acid	24.22	n.d.	n.d.	n.d.	90.44	3.31	49.85
Hexanoic acid	31.43	10.27	31.29	4.50	32.76	6.44	7.09
2-ethylhexanoic acid	32.81	n.d.	n.d.	0.57	n.d.	1.40	1.42
Heptanoic acid	32.89	1.42	2.43	n.d.	4.03	n.d.	n.d.
Octanoic acid	34.42	1.39	3.80	0.80	5.01	0.66	n.d.
Subtotal		*58.58*	*37.53*	*5.87*	*138.85*	*30.06*	*109.34*
Alkene							
Tridecene	19.86	8.90	n.d.	n.d.	9.84	n.d.	n.d.
(Z)-7-Methyl-2-decene	30.78	n.d.	n.d.	n.d.	n.d.	1.70	n.d.
Subtotal		*8.90*	*n.d.*	*n.d.*	*9.84*	*1.70*	*n.d.*
Terpene							
α-phellandrene	16.00	7.74	n.d.	n.d.	n.d.	4.56	7.03
Caryophyllene	27.53	n.d.	n.d.	1.23	n.d.	n.d.	n.d.
Subtotal		*7.74*	*n.d.*	*1.23*	*n.d.*	*4.56*	*7.03*
Ester							
Vinylhexanoate	28.77	n.d.	2.80	n.d.	n.d.	n.d.	n.d.
Subtotal		*n.d.*	*2.80*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Phenylpropene							
Estragole	28.92	2.29	n.d.	1.17	3.26	n.d.	n.d.
Subtotal		*2.29*	*n.d.*	*1.17*	*3.26*	*n.d.*	*n.d.*
Cyclic ester							
delta-Valerolactone	28.24	n.d.	n.d.	n.d.	n.d.	n.d.	5.58
Subtotal		*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*5.58*

n.d. – not detected

Within the present research it was established, that yellow maize flour contained 16 volatile compounds, rice flour – 12, extruded maize and buckwheat flour – 15, amaranth flour – 14 and soy flour – 13. In all types of studied gluten-free flour were identified two common volatile compounds (benzaldehyde and hexanoic acid). *Lasekan and Lasekan* (2012) reported more volatile compounds in buckwheat and amaranth comparing with the current research. Also *Sabovics et al* in yellow maize flour detected 27 volatile compounds, major compounds were hexanal, 1-hexanol and 1-octen-3-ols (Sabovics, Straumite, Galoburda, Kronberga, 2010; Kruma and Sabovics, 2012).

Zhou et al (2001) indicated that the composition of the volatile components of rice was complicated and no single volatile compound contributed to the characteristics of rice aroma. However, the compound, 2-acetyl-1-pyrroline has usually been identified as the most important volatile constituent contributing to the aroma in several aromatic rice varieties (Zhou, Robards, Helliwell, Blanchard, 2001). *Yasumatsu et al* (1966) determined that the key volatile compounds in rice are propanol, pentanol, acetaldehyde and hexanol (Yasumatsu, Moritaka, Wada, 1966). In rice flour totally there were found 22 volatile compounds, major compounds were:hexanal, 1-hexanol and 1-octen-3-ols (Sabovics, Straumite, Galoburda, Kronberga, 2010; Kruma and Sabovics, 2012).

Analyzing the collected data, in amaranth flour there were identified 6 alcohols, 5 acids, 1 aldehydes, alkene and terpene, in soy flour – 5 alcohols, 4 acids, 2 aldehydes, 1 terpene and cyclic esters. *Lasekan and Lasekan* (2012) reported that the flavor volatile profile of amaranth is largely dominated by alcohols, aldehydes, ketones, benzene derivatives, terpenoids, alkanes and furanoids. Amaranth revealed between 20 to 54 volatile compounds, the main volatile compounds in raw seeds appeared to be 2,4-dimethyl-1-heptene and 4-methylheptane (Lasekan and Lasekan, 2012).

In the current study volatile compounds detected in buckwheat flour are as follows: 5 acids, 4 alcohols and aldehydes, 1 alkene and phenylpropene. According to the study of *Janeš, Kantar, Kreft, and Prosen* (2009) the compounds with the highest contribution to the buckwheat aroma are: 2,5-dimethyl-4-hydroxy-3(2H)-furanone, (E,E)-2,4-decadienal, phenylacetaldehyde, 2-methoxy-4-vinylphenol, (E)-2-nonenal, decanal, hexanal and salicylaldehyde (2-hydroxybenzaldehyde). Buckwheat flour revealed 40 aroma compounds, major compounds groups are hydrocarbons, alcohols, acids and esters. Nonanal, octanal and hexanal are important aroma compounds of buckwheat (Lasekan and Lasekan, 2012).

In extruded maize flour – 6 alcohols, 4 aldehydes, 3 acids, 1 furan and ester, in rice flour – 5 aldehydes, 3 acids, 1 alcohols, terpene, furans and phenylpropene.

The volatile compounds identified in gluten-free flour provide different odor. According to data found in literature the odor description of volatile compounds in gluten-free flour is shown Table 2.

TABLE 2. Odor description of volatile compounds in gluten-free flour

Volatile compounds	Odor description	References
Hexanal	grass, green, tallow, fatty, green tomato, grass-like	Serot et al, 2002; Bryant and McClung, 2011; Dresow and Böhm, 2009; Jensen et al, 2011; Paraskevopoulou et al, 2012
Heptanal	green, fat, rancid, citrus, soapy-fruity, resinous, fatty, rancid, fruity	Bryant and McClung, 2011; Dresow and Böhm, 2009; Jensen et al, 2011
Octanal	orange, citrus fruit, citrus, floral, fatty, soapy-fruity, fruity	Bryant and McClung, 2011; Dresow and Böhm, 2009; Paraskevopoulou et al, 2012; Serot et al, 2002
Nonanal	citrus, fatty, fat, soap, rancid, boiled potato	Dresow and Böhm, 2009; Paraskevopoulou et al, 2012; Jensen et al, 2011
Benzaldehyde	bitter almond-like, almond, caramel, flowery	Dresow and Böhm, 2009; Jensen et al, 2011; Belitz et al, 2009
2-pentylfuran	green-bean, green, raw nuts, mushroom, butter, unpleasant, beany, grassy, cooked, floral, fruit, nutty	Dresow and Böhm, 2009; Paraskevopoulou et al, 2012; Jensen et al, 2011; Bryant and McClung, 2011
1-pentanol	balsamic, fruit	Jensen et al, 2011
1-octen-3-ol	mushroom-like, mushroom, fungus	Dresow and Böhm, 2009; Paraskevopoulou et al, 2012; Jensen et al, 2011; Bryant and McClung, 2011; Belitz et al, 2009
2-nonanol	moss, fresh	Serot et al, 2002
2-octen-1-ol	green	Serot et al, 2002
Acetic acid	vinegar, acid, pungent	Jensen et al, 2011
Hexanoic acid	sweat, prepared food, soup, cake, raw potato	Jensen et al, 2011; Dresow and Böhm, 2009
α-phellandrene	terpene-like, medical, dill-like, herbaceous	Belitz et al, 2009
Caryophyllene	fresh, fruity	Paraskevopoulou et al, 2012
Estragole	anise, licorice, sweet	Paraskevopoulou et al, 2012

In the yellow maize flour the major volatile compounds were hexanal (odor – grass, green, grass-like) of the aldehydes group, hexyl ester pentafluoropropanoic acid of the acids group and 4-penten-2-ol of the alcohols group. But in rice flour major compound was hexanal, which comprises 63.24% of total peak area. *Sabovics et al* (2011) identified that the major volatile compound of rice and maize is hexanal, also *Yasumatsu et al* (1966) mentioned

hexanal as the key volatile compound in rice. Also in the present research hexanal was identified in rice and yellow maize flour. The major volatile compounds in extruded maize flour were hexanal and hexanoic acid (odor – sweaty, prepared food, soup, cake, raw potato), their total amount of all volatile compounds was 69.75% and 7.05%, respectively, in amaranth – 23.82% hexyl ester pentafluoropropanoic acid and 22.20% benzaldehyde (odor – bitter almond-like, almond, caramel, flowery). In soy flour the major volatile compounds were hexyl ester pentafluoropropanoic acid and acetic acid (odor – vinegar, acid, pungent), but in buckwheat acetic acid, 2-hydroxy-benzaldehyde and hexanal. *Lasekan and Lasekan* (2012) reported nonanal, octanal and hexanal as important aroma compounds of buckwheat, but in the present research only hexanal was identified in buckwheat.

CONCLUSION

Thirty volatile compounds were detected in gluten-free flour and identified 6 aldehydes, 1 furans, 10 alcohols, 6 acids, 2 alkene, 2 terpene, 1 ester, 1 phenylpropene and 1 cyclic ester. In all types of flour were found two common volatile compounds (benzaldehyde and hexanoic acid). Alcohols, aldehydes and acids are the major volatile compounds in gluten-free flour. In the yellow maize, extruded maize and rice flour the major volatile compounds were hexanal, amaranth and soybean – hexyl ester pentafluoropropanoic acid, but buckwheat – acetic acid.

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STUDIES OF MICROSTRUCTURE OF KERNELS OF *MACADAMIAINTEGRIFOLIA* AND ITS HYBRIDS THROUGH MRI, X-RAY TOMOGRAPHY AND CONFOCAL MICROSCOPY

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ABSTRACT

The aim of this study was to develop techniques that enable examination of macadamia kernels to provide a better understanding of physic-chemical properties of kernels during postharvest processing. It was expected that such techniques could be applied for quality monitoring in the macadamia industry. One of the objectives was investigate the browning centre symptoms that usually occur in nuts-in-shell. The techniques applied included confocal microscopy X-ray tomography and magnetic resonance imaging (MRI). Five different varieties of macadamia nuts (A38, 246, 816, 842 and Daddow) were selected to include distinct characteristics such as drop pattern, growing location. Analysis of the microstructure of kernels by confocal microscopy showed the distribution of possible brown pigment compounds as well as the distribution of lipids, carbohydrates and proteins inside macadamia cells. Physical properties data including shell density and seed to volume ratio were obtained by X-ray tomography. Magnetic resonance diffusion tensor imaging used in this study showed marked differences in microstructure which indicate that different varieties exhibit different microstructures expressed as fraction of anisotropy and apparent diffusion coefficient that appear to be related to the occurrence of the brown centre. Hence, the findings of this study have potential to improve the existing postharvest techniques used in the macadamia processing industry. They will benefit to the industry in terms of improved quality control and cost reduction.

Keywords: *Macadamia integrifolia*, microstructure, magnetic resonance imaging, confocal microscopy, X-ray tomography.

1. INTRODUCTION

Macadamia is a native plant to Australia. It is evergreen tree. Australia exports about 40% of the total world macadamia production. One problem of quality defect in macadamia nut is browning centre which is not well understood. Microstructure can be assessed by techniques such as X-ray tomography, magnetic resonance imaging and confocal microscopy.

Magnetic resonance imaging (MRI) is non-destructive and an extension of standard two-dimensional nuclear magnetic resonance (NMR) methods. It is based on the principle of the magnetic moment of atomic nuclei with external magnetic field. Radiation emitted from the nuclei in the form of free induction decay is received as a frequency and time dependent signal. The mathematical description of the signal provides information about the chemical and physical states and spatial distribution of the nuclei and their neighbours (Simoneau et al., 1993). Magnetic resonance imaging (MRI) is a useful technique that provides the mapping of anatomy or tissue volume which based on the application of NMR. Diffusion-tensor MRI (DT-MRI) is a relatively recent MRI modality used for relating image intensities to the relative mobility of tissue water molecule. The data obtained offer quantitative anisotropy and orientation information.

DT-MRI data are acquired by repeating the acquisition while altering the magnitude or orientation of the diffusion-sensitizing gradient. Hence, the diffusion weighted imaging (DWI) data acquired are generally multidimensional (Hasan et al., 2011). Anisotropic media have different properties depending on direction. They are described by the ellipsoid where the radius defines the diffusion in a particular direction. The geometric nature of the diffusion tensors can quantitatively characterise the local structure in tissue (Westin et al., 2002). The MRI technique is able to provide the information on diffusion in particular direction. However it is important to relate the measure of diffusion anisotropy to the structural geometry in the quantification. The most popular are the relative anisotropy (RA) and the fractional anisotropy (FA), (Westin et al., 2002). The anisotropy is determined by FA value; the closer FA to zero the more anisotropic diffusion of the vector sample (Basser and Pierpaolic, 1996). In addition apparent diffusion coefficient (ADC) is another quantified scalar value. It involves diffusion tensor measurement which indicates signal intensity of diffusion weight image (Masutani et al., 2003). It is also an indication of molecular diffusivity under motion restriction such as fluid viscosity (Masutani et al., 2003).

No significant MRI work on macadamia nuts was reported so far. Given its application on many biological materials, MRI appears useful for study of macadamia nuts in shell (NIS). Like MRI, X-Ray tomography is a non-invasive method, which allows visualisation of body features without compromising the system integrity. X-Ray tomography is used to elucidate micro structure. Dhondt et al. (2010) demonstrated that ex-vivo scanning at submicron resolution allows the quantification and visualisation of the cellular organisation of plant tissue (*Arabidopsis* shoot) and can be achieved by X-ray tomography.

Confocal microscopy is a novel technique that has been used for many years in cell histology and cytochemistry. This method is able to locate the compounds of interest within the cell sample, which leads to a better understanding of chemical reactions inside the cell. There are few publications on microstructure of macadamia nuts using microscopy (Walton and Wallace, 2005, Wallace et al., 2012). However the work was performed on dried macadamia kernels. Freshly harvested nuts could lead to different outcome.

The aim of this study is to better understand the relationship between cellular and subcellular structure and physical properties of macadamia nuts in order to improve their quality after harvest.

2. MATERIALS AND METHODS

2.1 Sample procurement

Macadamia samples were obtained as NIS in different seasons from plantations in various locations of New South Wales and Queensland. After being received, they were sorted and graded.

2.2 Microscopy

2.2.1 Slide preparation and staining

The studies involving microscopy were performed at the UNSW Histology Unit. Freshly harvested macadamia kernels were cut into 20 μm sections with a microtome at a temperature of -80 °C, then the tissue was frozen with optimal cutting temperature medium (OCT) compound. The cut section was then placed on a glass slide and air dried vertically overnight. Dried cut section was placed in 95% ethanol for 10 min. Prior to staining the sample was removed and left for 1 h at ambient temperature to let ethanol evaporate completely. After the cut slides were stained with the periodic acid staining (PAS) compound, ninhydrin and iodine, the slides were fixed in 95 % ethanol for 10 min at -20 °C.

Only Sudan black slides were unfixed and mounted with the antifade reagent. Regarding adhesive agents used, the agent used for cellulose slide was an aqua mount, while invitrogen antifade aqua mount was used for Sudan black. For other stains including PAS, ninhydrin and iodine, Digital Picture Exchange (DPX) was used as adhesive. At a final step nail polish was painted around cover slips to prevent slides from drying.

2.2.2 Image analysis

Photos of stained sections were taken with a confocal microscope (Olympus BX60, Japan). Image-Pro version 6.2 software was used for image analysis.

2.3 X-ray tomography

Microtomographic acquisitions were performed on a SkyScan 1172 Scanner. Resolution of the radiograms was 34.56 μm/pixel. The X-ray tube voltage was set in between 40-60 kV, and the Hamamatsu 10 megapixel camera of the scanner was used with 4×4 binning. Reconstruction was performed using SkyScan's Nrecon software, using its noise reduction features: ring artifact reduction, beam-hardening correction and misalignment compensation. The fresh nuts were stored in a refrigerator, and taken out for the measurement. They were immediately weighed before being placed in the scanner for acquisition. The sequence of image processing operations mostly consisted of classical filter. The morphological closing used 18-connectivity, the blur function was a convolution with a Gaussian kernel of size 5×5×5 pixels, and a width of 1. The gradient function computes the derivative along the X, Y and Z directions independently, by first interpolating the images as a spline function. The result that is used for the active surface algorithm is actually a 3D vector field, meaning that each pixel is a set of three values, the gradient showing along the three main directions.

2.4 Nuclear Magnetic Resonance Imaging (MRI)

NMR analyses were conducted with a Bruker Avance 500 MHz (11.74 tesla) Spectrometer equipped with triple axis gradients capable of generating magnetic field gradients of 1.5 tesla/m. The DTI sequence had: echo time = 20 ms, rep. time = 2500 ms, 6 Averages, was a spin echo sequence, diffusion gradient duration = 5 ms, diffusion gradient separation = 8.52 ms, twelve diffusion directions were arranged on the vertices of an icosahedron, with b-values of 7,161 s/mm$_2$, 14,323 s/mm$_2$ and 21,484 s/mm$_2$.

2.5 Statistical analysis

Samples were analysed in triplicate and results were subjected to statistical analysis, which included determination of mean data set and standard deviation as well as analysis of variance (ANOVA) at $p \leq 0.05$ significance level. The differences were tested using Duncan Multiple Range Test (DMRT) at $p \leq 0.05$significance level.

3. RESULTS AND DISCUSSION

3.1 Microscopy

3.1.1 Cell structure

Confocal microscopy revealed a well-developed structure of the macadamia embryo and cotyledons. The kernels consist of 2 cotyledons surrounded by epidermis consisting of a single cell layer. The mature embryos are fused together with separated epidermis. The adjacent cells at the rim appear columnar. By contrast, the centre has mainly cuboidal cells. Parenchyma storage cells are generally globose and are the predominant cell type (Figure 1).
The epidermis of all varieties is of similar size. Figure 1 shows that there are two layers of epidermic cell that separate the cotyledon from each other. This explains why the kernel is easily split under compression force, as the seam connection has a tendency to break.

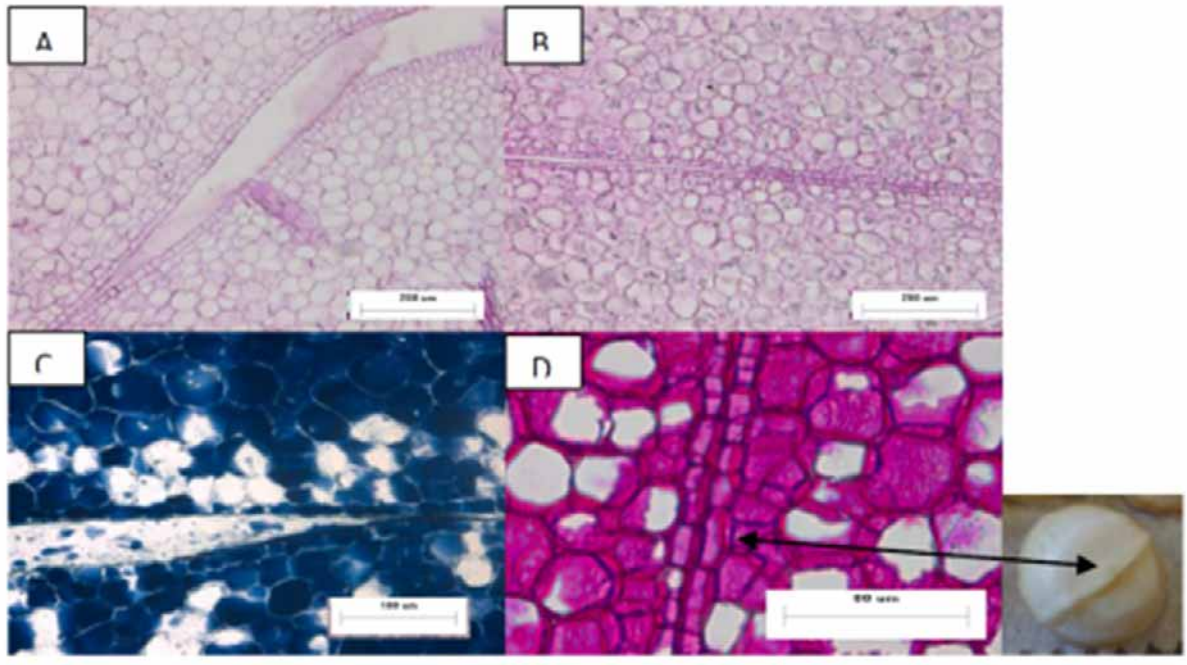

Figure 1 Seam connection between two cotyledons of macadamia kernel on the variety 246. A: (10×), B: (10×), C: (20×), D: (40×) magnification.

3.1.2 Carbohydrate analysis by Periodic acid-Schiff (PAS) staining

The purpose of this staining was to locate carbohydrate macromolecules within the cell and to establish their relation to kernel discoloration. PAS staining of all varieties resulted in staining areas of different intensity. It can be seen that the variety 842 and brown kernel of mixed varieties showed darker large stained areas compared to the other 4 varieties. A 38 variety showed the least stained area. PAS indicates the presence of carbohydrate compounds characterised by pink or purple stain (Figure 2).

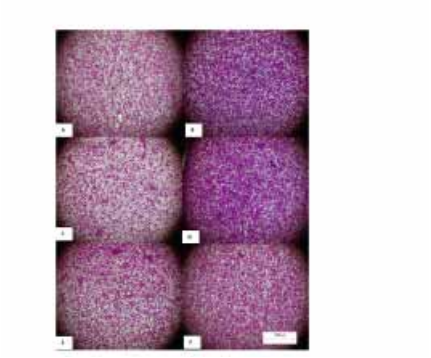

Figure 2 PAS staining observed under 2X magnification. A: Daddow, B: Brown kernel, C: A 38, D: 842, E: 816, F: 246

Figure 2 shows that different varieties have uneven distribution of carbohydrates in the kernel structure. Brown kernels had a higher carbohydrate compound level present in the cell compared to the other fresh varieties except 842. Those could also be carbohydrate related compounds as PAS staining reacts with carbonyl group in sugar compounds. It seems plausible that brown kernel could be the result of complex carbohydrate compound formation (Figure 2). If the presence of carbohydrate is one of the factors leading to brown kernel, the variety 842 is susceptible to brown kernel formation due to a high concentration of carbohydrate inside the cells which agrees with the high incidence of internal discoloration of 842 found in experiments. While performing ninhydrin staining, intense pink staining was formed randomly in the cutting section. This indicates a protein complex inside variety 246 that plays an important role in kernel browning. The brown kernels have high esterified phenols compared to white section (Figure 3). This clearly demonstrates that the COOH groups of amino acids were esterified with OH group of phenols resulting in their higher content in brown kernels. This phenomenon is described in Fraenkel-Conrat & Olcott (1945).

Figure 3 Cells of variety 246 that were intensely stained by ninhydrin A (10x), B (20X,) C (20X) magnification.

3.1.3 Lipid analysis by Sudan staining

Sudan stains lipid in blue. In Figure 4, the A 38 variety shows the lowest proportion but an even distribution of blue colour. The staining intensity of each variety was not the same. In contrast to PAS staining, brown kernels showed lesser oil concentration, while Daddow exhibited the most intense Sudan staining. This indicated that oil in macadamia kernel was evenly distributed, but with different intensity in each variety.

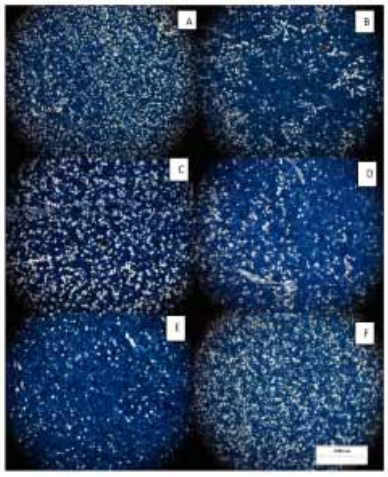

Figure 4 Sudan staining observed under 2X magnification. A: A38, B: 246, C: 816, D: 842, E: Daddow, F: Brown kernel

In addition an observation of macadamia kernels under microscope made by Walton and Wallace (2005) showed that different cultivars exhibit different ultrastructure including thickness of the cuticle convolution and air space in the cuticle. However their work was done with macadamia nut dried at 58 °C, a high temperature which could have resulted in kernel deterioration.

3.2 X-Ray tomography

The purpose of this investigation was to study the ultrastructure of macadamia shell as well

as its kernel. In addition, X-Ray tomography will give information related to physical properties of macadamia nut in an intact NIS such as shell, kernel and NIS density (Figure 5). Shell densities of each variety were very close between the dried and fresh samples of the same variety (Figure 6). In addition to that, sample of the same variety, but different batches gave nearly identical value of shell density. However, the drying process affected kernel to nut volume ratio (Figure 5). The comparison of 816a which was fresh sample (0.46) and 816T which was dried in the cabinet dryer (0.41) shows that the reduction of seed volume was due to kernel shrinkage.

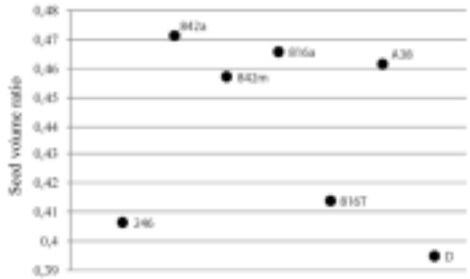

Figure 5 Seed volume ratio presenting as kernel to NIS volume ratio. **D** = Daddow variety, **842a** = 842 variety of third batch, **842m** = 842 variety of first batch, **816T** = 816 variety dried with cabinet dryer, **816a** = 816 variety of third batch

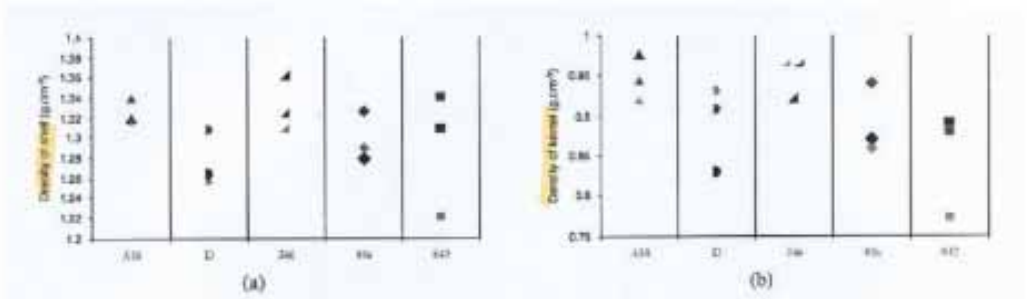

Figure 6 Density of the shell (a), density of kernel (b). Each dot point represents each data value. Similar symbol represent same variety.

From the experiment, it clearly appears that the shell density influence drying rate of NIS. Average shell density from lower to higher was Daddow < 842 < 816 < 246 < A38 while drying date of cabinet drying from faster to slower was Daddow >842 >816 > 246 > A38. According to Ahmadpour and Do (1997) low density is accompanied with high surface area and micropore volume.

3.3 Nuclear Magnetic Resonance Imaging (MRI)

The purpose of this experiment was to study the micro structure of macadamia kernel in order to understand the features that are likely to influences the nut quality. Micro-imaging by MRI indicated that different varieties of macadamia kernel exhibited different microstructures. In this study image based display of diffusion tensor data is presented.

The fraction anisotropy (FA) index and the apparent diffusion coefficient (ADC) are shown in Figures 7 and 8 respectively. FA and ADC value have no unit as they are compared values. Daddow had the lowest value of FA which indicated that diffusion is unrestricted in all direction compared to other varieties (Basser & Pierpaolic, 1996). This reveals that diffusion direction influence browning phenomenon in macadamia nuts. Being anisotropic in structure allow Daddow variety to be dried faster as water will diffuse more rapidly in the direction aligned with the internal structure. In contrast, in other varieties that exhibit random diffusion direction, water will diffuse slower leading to the accumulation and reaction of solutes inside the cell including protein/carbohydrate reaction as well as esterification of polyphenolics. Apparent diffusion coefficient is shown is Figure 8. It reveals that molecular diffusivity of all varieties was similar. This indicates that varieties studied had similar principal diffusivity but different principal diffusion directions.

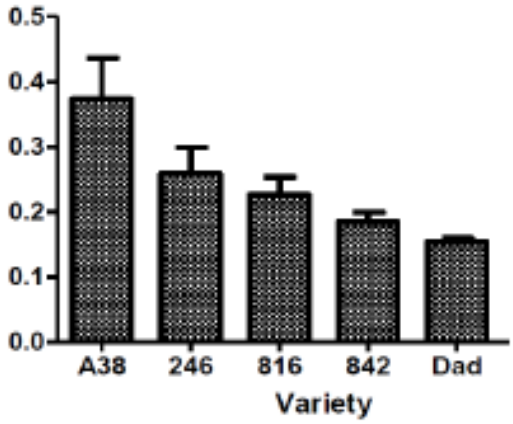

Figure 7 Fraction anisotropy index of macadamia varieties

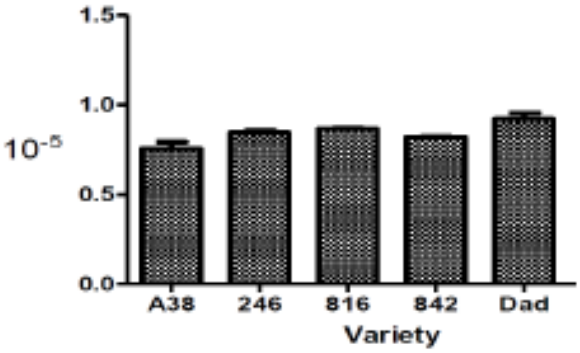

Figure 8 Apparent diffusion coefficient (ADC) of macadamia varieties.

By using histological approaches, X-ray tomography and MRI, microstructure of macadamia could be characterised and the relationship between drying pattern and formation of brown kernels in particular areas of the kernels better understood.

4. CONCLUSION

Daddow exhibits anisotropic microstructure as well as lower shell and kernel density. All these characteristic increase drying rate compared to other varieties. This results in being less susceptible to brown kernel formation.

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SINGLE KERNEL CORN DRYING MODEL FOR CFD APPLICATION IN SPOUTED BED DRYER

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ABSTRACT

Spouted Bed has been used extensively in processes due to the benefit from its high surface contact area. This characteristic coupled with the recirculating flow are attractive for its application in drying. However due to its complex flow, the performance of Spouted Bed Drying cannot be readily predicted. In the last decade, CFD approach has been applied to model the drying and flow of grain inside SBD. However the drying model used in these studies has been based on simple combination of constant and single parameter of falling rate model. Constant rate model, falling rate model, diffusivity model and the combination of these model will be compared. The drying kinetic data were collected in a thin layer drying apparatus to approximate the effect of single kernel drying inside spouted bed dryer. Non-linear regression will be used in the analysis to enable comparison between these different models on the same basis.

Keywords: drying, grain, CFD, thin layer, model, spouted bed

1. INTRODUCTION

Drying is one of the oldest method of food preservation and an established post-harvest process in grain production. Grain drying commonly achieved by contact of drying fluid that might be heated depending on the goal of drying and the climate or weather condition. Grain drying is a complex process and need to be balanced with the grain quality produced due to exposure to moisture gradient and high temperature as well as physical changes due to shrinkage. Thus while a rapid and continuous drying process are preferred, ultimately drying parameter and in turn the rate of drying are mainly influenced by the nature of product dried. This raised the need of drying model to help predicting and studying the drying process to achieved efficient and effective drying

Spouted Bed Dryer (SBD) is a dryer with a unique flow characteristic that inherently provided a tempering process which enables the use of high temperature with minimal

product deterioration (Inprasit and Noomhorm, 2001; Montes et al., 2010; Thuy et al., 1999) which shortened the length of active drying. However, the complexity of its hydrodynamics raises difficulty in scaling up process and thus hindering a wide-spread adaptation of the dryer commercially. In the last decade, Computational Fluid Dynamics (CFD) has been applied extensively in drying studies to help on analysis of performance and characteristic of complex dryers. These studies championed CFD as a tool to assist in scaling up and dryer performance studies, ultimately as a substitute for a pilot plant unit (Jamaleddine and Ray, 2010). However studies of CFD application on SBD are mostly on the hydrodynamics and the studies on modelling drying in SBD are limited. In these studies, the moisture movement between the drying medium and the solid are modelled using a simple combination of constant rate period and falling rate period, inferring from drying characteristic of solid body (Szafran and Kmiec, 2004; Zhonghua and Mujumdar, 2007). While the combination was reported to produce a good agreement with experimental result and proven the merit of CFD in complex drying simulation model, a mismatch between an increase of accuracy on the temperature and flow field calculated using CFD model and the simple drying model are obvious. Drying model that used a simple average value for the flow field (Bie et al., 2007; Madhiyanon et al., 2002) also managed to predict a good agreement on moisture content. Thus a study is needed to assess the suitability of single kernel drying model for application in CFD.

2. PROPOSED MODEL

Drying model has been studied for a long time. The model varies between a single equation predicting moisture content on material dried in a specific dryer to a complex set of equations which simulated the changes of moisture and temperature not only in the material but also in the drying medium. Predictive drying model can be separated into three types:

a) The concept of drying curves, which recognized drying stages and developed a model based on the characteristic of drying stages.
b) The distributed parameter, which employs coupled heat and mass transfer equation.
c) The empirical method which is obtained by solving simple and multivariable linear regression.

The third method will not be considered in this work since the application of the model is limited to specific condition or dryer that the work was conducted. The CFD simulation

model can be categorised in the second type. But the model also need another input in the form of mass transfer rate between the medium and the product. This can be explained using the single kernel drying model.

There are three single kernel models proposed on this work. The first model (A) was used by Szafran and Kmiec (2004) in their work. Model A is based on the assumption that drying occurs in two distinct stages. The constant rate is followed by the falling rate. The switch between the stages occurs simply when the drying rate of one stages is greater than the other. The drying kinetics can be illustrated as in Fig 1.

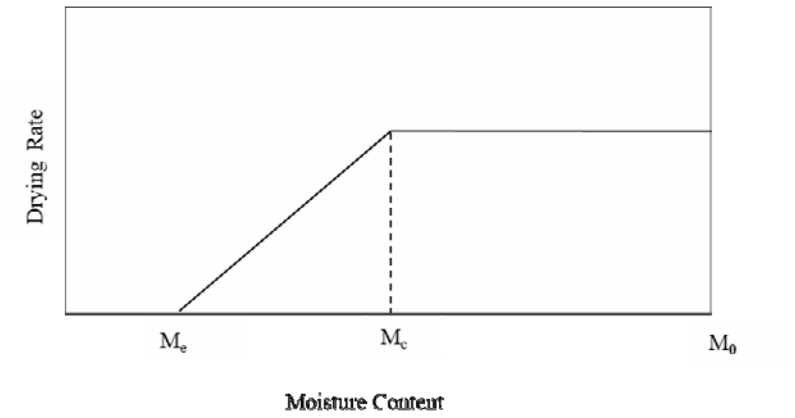

Fig 1. Constant and falling rate drying stages.

The moisture prediction can be calculated by integration within appropriate boundary conditions. Defining M_c and t_c as the critical moisture and time when the model was switching between the stages. The solution can be written as

$$\begin{aligned} M &= M_i - Ct && \text{for } t \le t_c \\ M &= (M_c - M_e)e^{-k(t-t_c)} && \text{for } t > t_c \end{aligned} \qquad (1)$$

The second (B) and third model (C) were derived from solution of Fick's Law of diffusion. If the movement of the moisture inside a single kernel is assumed to be mainly due to diffusion then the distribution of moisture inside the kernel can be calculated by solving this equation:

$$\frac{\partial M}{\partial t} = D\,\nabla^2 M \qquad (2)$$

with D as the single parameter that characterises the drying process. The model can then be extended to encompass a process where the moisture movement is due to many other mechanisms by lumping all the effects into the parameter and defining the parameter as effective diffusivity. In order to solve the equation, two boundary conditions (at the surface and center of the kernel) and one initial condition are defined. The common initial condition

is to assume uniform moisture content at the beginning. Model B and C emerged due to different choice on the surface boundary conditions. The common approach is to use the constant moisture content at surface which is equal to the equilibrium moisture content. This approach based on the assumption that the internal moisture movement is a lot slower than the external thus the surface is always in equilibrium with the drying medium. The partial differential equation can then be solved with separation variable method and after applying the appropriate boundary and initial condition yield (Crank, 1975)

$$\frac{M - M_e}{M_i - M_e} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{e^{\frac{-n^2 \pi^2 D_{eff} t}{r^2}}}{n^2} \qquad (4)$$

Model C was derived by taking surface flux as a boundary condition. While this resulted in a more complicated solution, it will have closer theoretical representation to the real drying. The solution can be written as (Crank, 1975)

$$\frac{M - M_e}{M_i - M_e} = 6Bi_m{}^2 \sum_{n=1}^{\infty} \frac{e^{-\frac{\beta_n{}^2 Deff\, t}{r^2}}}{\beta_n{}^2 (\beta_n{}^2 + Bi_m (Bi_m - 1))} \qquad (5)$$

with

$$Bi_m = \frac{h_m r}{D_{eff}} \qquad (6)$$

and β_n as the series constant to be calculated from the relation below:

$$\beta_n \cot \beta_n + Bi_m - 1 = 0 \qquad (7)$$

The left hand term of equation 4 and 5 is the normalised moisture content and defined as the moisture ratio. The effective diffusivity was assumed to be a function of temperature following an Arrhenius type relation as follows:

$$D_{eff} = D_0 e^{-\frac{E_a}{RT}} \qquad (8)$$

3. MATERIALS AND METHOD

Waxy corn was chosen as material to generate data on drying kinetics. The kernel radius was calculated from measuring the diameter at three different axis and taking the geometric average mean. The calculation was repeated for 100 kernel samples.

Corn kernels were rewetted by adding the required amount of water. They were then stirred twice a day and stored in cold storage to enhance moisture movement. Before drying, the kernels were allowed to equilibrate with the ambient condition for 2 hours.

Drying kinetic data was generated using a tunnel dryer. The samples were arranged on a tray to be single kernel depth to ensure uniform contact with the drying air. Sample weight was logged at one minute interval. Temperature and relative humidity was also logged at 5 minutes interval. The average values were used in the calculation. These data were collected using PC-based data logging software.

Moisture content of the sample were determined at the end of the drying run using oven method (ASAE Standards, 1997). Six drying runs at three different temperatures ranging from 40 ^{o}C to 60 ^{o}C were used to provide drying kinetics data. The model was then used to calculate the moisture ratio for the corresponding time of the drying run. Sum of square difference of the moisture ratio was minimised to calculate effective diffusivity and convective mass transfer coefficient. Moisture equilibrium was calculated based on the model proposed by Krokida et al.(2003).

Two constant parameters describing the value of effective diffusivity as a function of temperature were optimised for all the models. Three additional parameters for convective mass transfer coefficient at different temperatures were also calculated for model A and C. Those parameters were calculated by non-linear regression.

4. RESULT AND DISCUSSION

In this studies, solution for model B was calculated up to term 10, while model C was calculated up to term 9. The terms was dropped as the value falls below 0.005. As they are decaying terms then the error of the dropped terms will be less than 2%. The idea of calculation until the second term still valid as long as the model was used for predicting drying end point. Comparison of moisture ratio value calculated by computing the first two term compared to the full term (as explained above) at 60 ^{o}C can be seen in Fig 2. This result is important on looking at prospect for CFD application since the time step used in CFD model of SBD is 1 ms (Szafran and Kmiec, 2004), thus an accurate prediction on the early part of drying is needed.

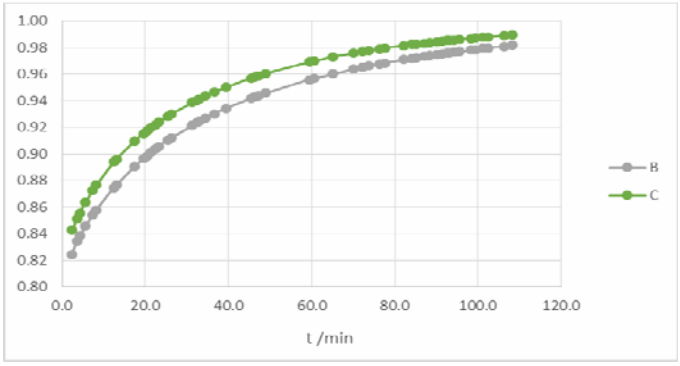

Fig 2. Ratio of computation with two terms to full terms at 60 ^{o}C

Model A grossly predicted the moisture content. It modelled the initial rate of drying at significantly lower level than the experimental data. From Fig 2, it can be seen that falling rate stages was predicted a higher rate of drying than the experimental data. Coupling this with the need to ensure that the switch happened at the point where the rate of drying of both stages are equal, the model was limited on the level of constant rate of drying that it can used. The result can be improved by letting the critical moisture level to float freely, but this would mean that the critical moisture content location need to be solved before the model can be applied in CFD.

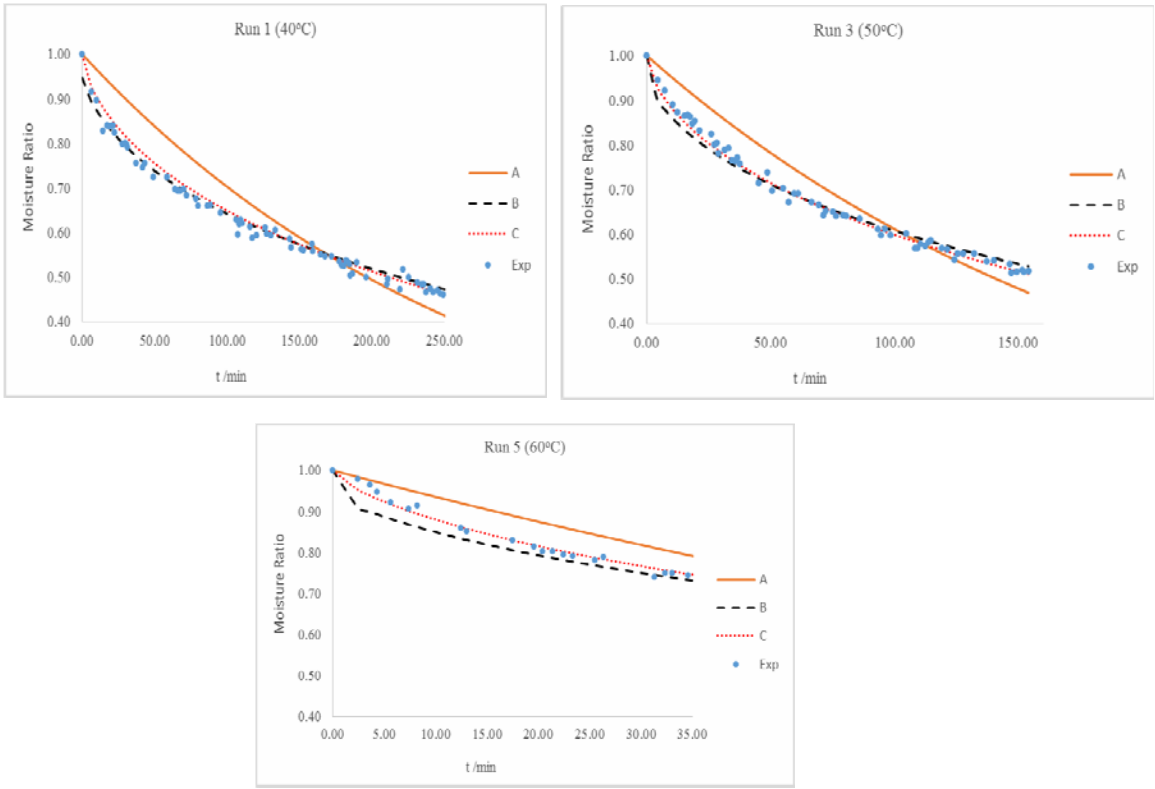

Fig 3. Drying kinetic fit

Models B and C were found to predict the moisture content with acceptable accuracy, especially around the tail of the drying run. At the beginning of the drying, the model B was found to under predict the moisture content which is due to its assumption of rapid surface drying and temperature deviation from average value. Model C also under predicted the early moisture content for 40 ^{o}C and 50 ^{o}C, this deviation was caused by neglecting the temperature deviation from the average value since the model was developed using a uniform constant temperature. It can be seen on Fig 3 that the deviation increase as the drying temperature increases.

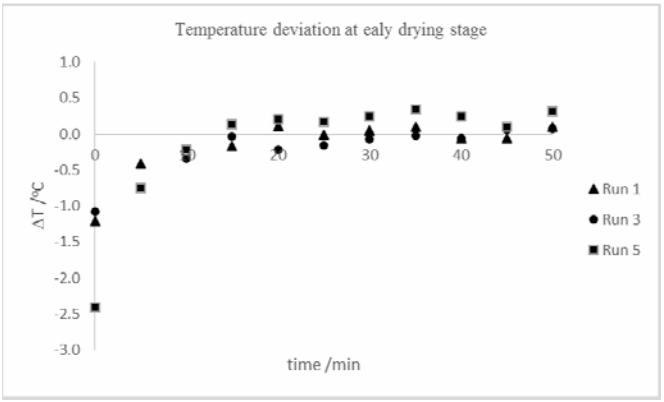

Fig 4. Early drying temperature deviation

Model B is used widely as the solution of diffusional model for thin layer drying. However, Fig 2 shows that at grain size of 3.8 mm, the model under predicted the moisture content at the early drying stage. Moreover, the difference increases with the increase of drying temperature. Thus the model should be applied cautiously on high drying temperature scenario, especially if it coupled with similar size grain.

Model C was found to provide the best prediction, however this was achieved with some added complexity in the solution. Equation 6 needed to be solved before the final solution could be calculated. Fortunately, the solution for these coefficient followed an almost linear fashion at a high value of Bi_m which is generally the case where CFD is applied. The plot of the coefficient solution can be seen in Fig 4.

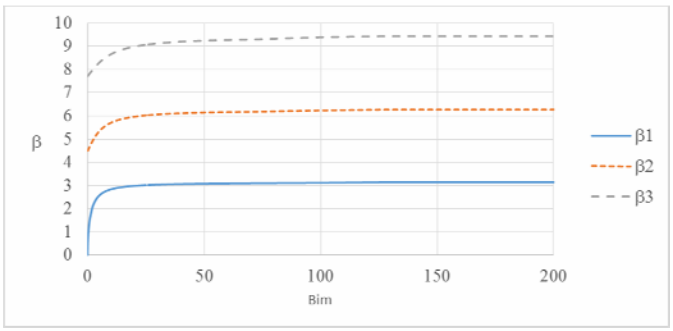

Fig 4. Solution for β coefficient

The coefficient calculation difficulty mainly due to the nonlinear nature of the characteristic equation. However as the value of Bi_m increases the changes in β_n solution decreases, thus the coefficient can easily be solved using Newton Rawson method with appropriate initial guess. Looking at Fig 4, the solution can be said to resemble a negative exponential function. Thus the initial guess can be approximated with equation 8.

$$\beta_{trial} = \pi(n - kBi_m) \qquad (9)$$

The constant k can be optimised to provide a faster convergence rate at specific Bi_m range. But in this work an arbitrary value of 0.2 was chosen and it was found that the solution for a wide range of Bi_m can be computed after 10 iterations.

Model A predicted a smaller convective mass transfer coefficient compared to model C. The effective diffusivity for model A was calculated by equation the falling rate part of the model to the first term of model B. The result showed that each modelling provided a vastly different Ea value and D_0 value, however the D_{eff} value calculated from model A and model B at the corresponding temperature did not have the same contrast (Fig 5).

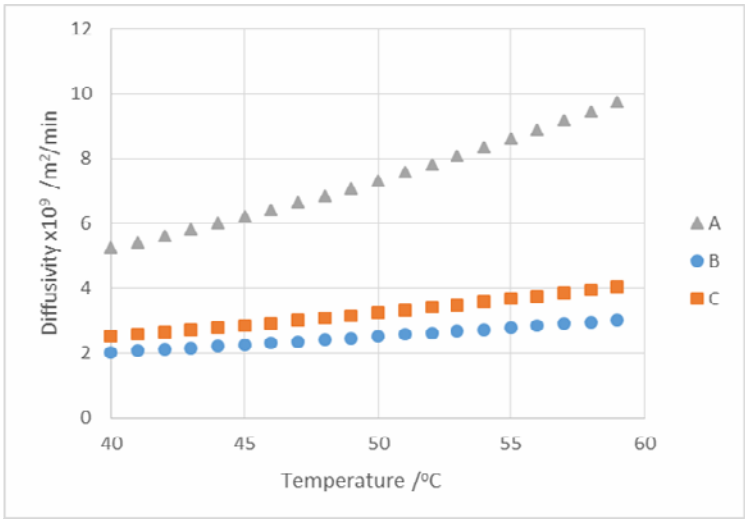

Fig 5. Effective diffusivity as function of time for different model

The effective diffusivity parameter agreed with result published by Hacıhafızoğlu et al. (2009) that employed similar method.

TABLE 1. Summary of results of model fitting

	A	B	C
E_a x10^4 kJ/kmol	2.8	1.8	2.2
D_o m^2/min	2.8x 10^{-4}	2.2x10^{-6}	1.1x10^{-5}
SSerr	1.1965	0.1686	0.0818
R^2	0.82	0.98	0.99
df	372	375	372

TABLE 2. Summary of result of convective mass transfer coefficients

Model	40 ^oC	50 ^oC	60 ^oC
A	4.5x10^{-6}	6.2x10^{-6}	8.5x10^{-6}
C	3.1x10^{-2}	4.2x10^{-2}	4.31x10^{-2}

5. CONCLUSION

Model C was found to provide the best fit for experimental data. The result also indicates that the value of effective diffusivity calculated from this model will differ from model B, especially at higher temperature. Therefore effective diffusivity measured from other work should be applied with caution. The added complexity of computation for model C can be minimised if the working range of Bi_m are identified. Model A cannot provide a good prediction if the transition period between the two stages took a significant time. The limits on the number of terms calculated on model B and C need to be evaluated carefully since at early drying stages < 30 min, the first and second term only provide less than 95% of the value, especially contrasted with 1 ms integration step on CFD model.

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APPENDIX

Notation

Bi_m biot mass number

D diffusivity coefficient (m^2/min)

E_a activation energy (kJ/kmol)

h_m convective mass transfer coefficient (m/min)

M moisture content (dry basis)

R gas constant

r radius (m)

T temperature (K)

t time (min)

k, C constant

Subscript

c critical

eff effective

i initial

FOSSIL FUEL DEFICIT - CONSERVATION TILLAGE AND ON FARM BIOFUEL PRODUCTION TO COPE WITH THE PROBLEM

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ABSTRACT

The limited resources of fossil fuels along with the highly fluctuating prices, call for investigation to find diesel alternatives. Biofuels from vegetable oils, seems the easiest accessible substitutes as they can be used in conventional diesel engines without lot of modifications. There are though two mainstream attitudes on this approach. The one points that it is immoral to divert environmental resources from food production to energy production when the global population increases and the other claims that without mechanization and fuel to power it, food production will finally be decreased. Conservation tillage adoption may contribute in significant fuel savings by eliminating tillage operations. If they would be combined with on farm biofuel production, they would certainly require less land to be devoted for this purpose. In the present work, based on data of a long term tillage experiment, it was calculated the percentage of land that would be required to cultivate with a biofuel crop (sunflower for instance) in order to cover the fuel requirements of an arable farm, for three alternative tillage methods: conventional (CT), reduced (RT) and no-tillage (NT). The results indicated that in CT, the 11% of the land would be enough to provide the biofuel for all the field operations (except irrigation). In RT, due to lower fuel consumption, the 7.5% of the land would be sufficient. That means that a 3.6% yield reduction is justified. In NT, only the 3.5% of the land is required to produce the biofuels justifying a 7.7% yield reduction. This sets the limits of yield reduction that can be acceptable. However we have to add in this balance the environmental effects of using conservation tillage like erosion reduction, increasing soil organic matter and biodiversity maintenance.

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Keywords: Biofuels, Food production, Conservation tillage, No-tillage, Sunflower

1. INTRODUCTION

Agricultural mechanization along with irrigation, biological improvements and chemical input development has been the main factors enhancing the agricultural production during the past decades. Agricultural mechanization was based on the use of fossil fuels to power the farm machinery. However, the limited resources of fossil fuels along with the highly fluctuating prices, call for investigation to find diesel alternatives. Especially E.U. is highly dependent on imported fossil fuels to cover its energy requirements (European Commission 2006). Existing knowledge and reports indicate that biomass derived liquid fuels such as vegetable oils and alcohols are the main alternatives we have at the moment (Biofuels Technology Platform 2008). They can be used in conventional diesel engines without lot of modifications (Ishii, Y and R. Takeuchi, 1987).

Vegetable oils are produced by oil seed producing crops after extraction. Extraction can be carried out by cold pressing or by chemical extraction (CIGR 1999). The later is more efficient industrial method while cold pressing is simple and can be used at farm level. In that way farmers have the chance to produce their fuels by their own. However there are two mainstream attitudes on this approach. The one points that it is immoral to divert environmental resources from food production to energy production when the global population increases (Rosillo-Calle and Hall, 1987) and the other claims that without mechanization and fuel to power it, food production will finally be decreased (Spiertz and Ewert, 2009).

Conservation tillage adoption may contribute in significant fuel savings by eliminating tillage operations (Sharma et al., 2011). If they would be combined with on farm biofuel production, they would certainly require less land to be devoted for this purpose. In addition there are a lot of environmental benefits of using conservation tillage like erosion reduction (Blanco-Moure et al., 2012), increasing soil organic matter (Sombrero and de Benito, 2010) and biodiversity maintenance (Perez-Brandán et al. 2012) and conserving soil water (Cullum, 2012). Also in the last years a discussion about the benefits of using biofuels to the environment has started. Several papers have questioned the benefits from the energy crops to the environment, to the CO_2 emissions and the effects to the food supply (Grahn at al., 2009).

Energy analysis of the cropping systems can offer the basis for assessing the benefits of an energy crop. In the present work, based on data of a long term tillage experiment, it was calculated the percentage of land that would be required to cultivate with a biofuel crop

(sunflower for instance) in order to cover the fuel requirements of an arable farm, for three alternative tillage methods

2. MATERIAL AND METHODS

2.1. Experiment description

Data from a long term tillage experiment carried out in the Farm of University of Thessaly in central Greece were used for the analysis. The experiment started out in 1997 compared five alternative methods of soil tillage in a variety of arable crops. In years 2011 and 2012, fifteen years after the beginning the experimental field was cultivated with sunflower. Three of the compared tillage methods were chosen for the present analysis.

Conventional tillage (CT). This method represents the usual tasks carried out by local farmers for seedbed preparation. It involves deep ploughing at a depth of at least 25 cm during the autumn and repetitive passes, according to the conditions, with disk harrows and field cultivators for seedbed preparation at spring.

Reduced tillage (RC). It is a less common method in Greece though it's widely used in other parts of the world like USA and Australia. Primary tillage is accomplished with one pass at a medium depth of 20 cm of a heavy cultivator with spring type shanks while seedbed preparation is completed as in the conventional method though fewer passes are required.

No-tillage (NT). The lesser adopted method in Europe and completely unknown in Greece. In the USA, about 30% of the cultivated land is considered to be under a form of no-tillage practice. In this method not any tillage operations are applied in the soil until planting. The crop is planted over the previous year crop and weed residue by using special robust seeders designed to work on hard conditions. In the present experiment, due to lack of such a seeder, a conventional pneumatic machine were used which however gave sufficient results. In no-till applications the weeds are usually destroyed with a total phase herbicide (e.g. glyphosate) before planting or immediate after, but for instance it wasn't necessary. The sunflower variety used in the present case (PR64LE29) allowed post-emergence application (for all the treatments) of a mixture of herbicides (*fluazifop-P-butyl* & *tribenuron methyl*) that controlled both narrow and broadleaf weeds. Along with the rapid development of the sunflower plants it was capable for the crop to compete sufficiently the weed populations.

Besides tillage, the rest of the cultivation practices were the same for all the treatments. The operations and inputs were recorded as shown in Table 1. Harvesting was done with an

experimental plot harvester (HEGE 125) owned by the University Farm. Seed samples were used for cold pressing in a screw type press (Taby Press 1.5Hp) at the Lab. As no differences were obtained between tillage treatments, mean oil content for the seed was estimated.

TABLE 1. Schedule of tasks and inputs performed over the two years.

Year :	**2011**			**2012**		
Tillage treatment:	***CO***	***RC***	***NT***	***CO***	***RC***	***NT***
Soil Tillage (passes)						
Ploughing	1			1		
Heavy cultivator		1			1	
Disk harrow	2	2		2	2	
Field cultivator	1					
Sowing						
Row spacing (m)	0,75			0,75		
Population (seeds/ha)	90.700			78.000		
Seed used (kg/ha)	8,67			7,46		
Fertilisation						
N - P - K	93 - 51 - 51			95 - 63 - 63		
Herbicides						
fluazifop-P-butyl (kg/ha)	0,037			0,037		
tribenuron methyl (kg/ha)	0,312			0,312		
Irrigation						
Pumping depth (m)	80			80		
water supply (m^3/h)	50			50		
Traveller sprinkler (m^3/ha)	350			200		
Drip Irrigation (m^3/ha)	3260			3680		
Yield						
Seed weight (kg/ha)	4.120	3.954	3.255	2.875	2.506	2.132

2.2 Energy inputs estimation

Based on the records the direct and indirect energy (Pimentel 1992) consumption was estimated. Direct is the energy consumed in the farm, in the form of energy products, like fuel, lubricants and human labour. Indirect is the energy consumed outside the farm to produce any input (machinery, chemicals) used in the farm. Any material brought into the farm is considered as “input” while any product sold to the market was added to “output”. Products used in the farm were considered “neutral”.

Indirect energy was estimated as the energy sequestered to the machinery during manufacturing. Machinery characteristics and data from Bowers (1992) were used for this purpose. The methodology is described in details by Cavalaris et al. (2008).

Direct energy was estimated from power and fuel consumption measurements in the field with the help of instrumentation added to the tractors (Papathanassiou et al. 2002) which allowed on-the-go measurement of draft and PTO power consumption. Fuel and energy consumption during the operations was estimated from the collected data. The energy content

of diesel was considered 38.66 MJ/L. A fuel production and handling energy of 9.12 MJ/L were also taken into account (Leach 1976). The procedure is described by Cavalaris et al. (2008). The consumed energy by lubricants was also encountered as a 4% of the fuel energy (Fluck 1992). Finally, the energy spent for repair and maintenance was added. It was estimated as a percentage of the energy spent to produce the machine, using R&M coefficients (Bowers 1992)

Consumable goods were used in several stages of crop growth. For most of them there were energy sequestered values in the literature (Leach 1976, Pimentel 1992, Bowers 1992)

A five tone capacity wagon toed by a 82 kW tractor with an average travel speed of 20 km/h was assumed for product transportation. The average distance from the field to the barn was assumed to be 5 km. Direct and indirect energy consumption was estimated (Cavalaris et. al. 2008).

Irrigation water was pumped from underground reservoirs and it was distributed by aluminum pipes and applied in the crop by traveler irrigators with gun sprinklers or by drip irrigation. The pumping plant was powered by electrical motor. Direct and indirect energy consumption for irrigation was estimated as described by (Cavalaris et. al. 2008).

For harvesting, both direct and indirect energy was estimated by literature data (Leach 1976).

A screw type small size press with an electric motor of 1.1 kW (1.5 hP) and a capacity of 6-7 L of oil/h was used for oil extraction. An electric power meter connected in series to the press was used to measure power consumption during pressing. At the same time the processed seed and the produced oil were measured to estimate the energy per kg of seed processed or per liter of oil produced. The indirect energy for manufacturing, repairing and maintaining the press was added.

2.3 Energy output estimation

Sunflower outputs were only the harvested seed as the stalks were left in the field. Cold pressing of seed gave in average 33.5% oil and 66.5% cake. After sedimentation and filtering the oil was ready to use as an alternative fuel to diesel in the tractor engine. The energy content for oil was 36.8 MJ/kg and for cake 15 MJ/kg (Riva and Sissot 1999).

2.4 Energy budgets

For the energy budgets, mean values of inputs and outputs from the two years (2011 & 2012) were estimated. The net energy which is the energy output minus the energy input measured

in MJ and the energy efficiency coefficient which is obtained by dividing the energy output by the energy input were calculated to evaluate the system. When an amount of vegetable oil was used to cover the fuel requirements of the enterprise, this amount was considered neutral and was excluded from the outputs. The discussed scenarios considered that the enterprise produces by its own the fuel required to power the autonomous vehicles moving in the field (tractor and harvester). Irrigation equipment used electric energy and so wasn't considered to be able to be powered from vegetable oils.

3. RESULTS AND DISCUSSION

At first, as can be seen on the "energy budget" field in Table 2, the net energy for all the tested methods and scenarios are positive. This means that it is possible for the sunflower to be used as an energy crop. It should also be mentioned that the positive budget was obtained by considering as output only the seed as the stalks remained in the field.

By examining the energy inputs in Table 2 it is found that irrigation, fertilization and soil tillage are the most important factors representing 96% of total energy requirements in the field. Irrigation is the most important from the three covering almost 70% of total energy inputs in the conventional method. Unfortunately, sunflower can not give sufficient yields in Greece under dryland cultivation. It is interesting to mention that the high energy requirements are present because the water is pumped from underground reservoirs at a deep depth which is a common case for Greece. There are however areas which present a high underground water horizon or give the opportunity to use ground water resources from streams and canals. In that case, the energy consumption for irrigation could be even tenfold lower.

Second in importance energy input is the use of fertilizers (Table 2). In conventional tillage it represents 18,5% of total energy inputs. Even though nowadays industry efficiency of producing nitrogen fertilizers has increased (IFA, 2010), the use of nitrogen fertilization still appears as an intensive energy demanding practice. Reducing the amount of fertilizers will probably lead to reduction of yields.

TABLE 2. Energy budgets for sunflower im the three methods of tillage for two alternative scenarios for substitution of diesel fuel with vegetable oil.

	100% diesel - 0% oil			70% diesel - 30% oil			0% diesel - 100% oil		
Energy Budget	**CT**	**RC**	**NT**	**CT**	**RC**	**NT**	**CT**	**RC**	**NT**
Energy Inputs (MJ/ha)									
Tillage	4.326	2.193	0	3.161	1.605	0	441	232	0
Sowing	601	601	601	496	496	496	250	250	250
Fertilization	8.973	8.973	8.973	8.937	8.937	8.937	8.853	8.853	8.853
Pesticide application	81	81	81	64	64	64	23	23	23
Irrigation	33.528	33.528	33.528	33.528	33.528	33.528	33.528	33.528	33.528
Harvest	1.117	1.117	1.117	915	915	915	445	445	445
Transportation	61	56	47	52	48	40	31	28	24
Oil extraction				117	73	28	390	243	94
Total	48.686	46.548	44.346	47.269	45.665	44.007	43.961	43.603	43.216
Yield (kg/ha)									
Oil for trade	962	888	741	929	868	733	854	821	715
Oil for biofuel production				32	20	8	107	67	26
Cake	2.081	1.922	1.603	2.081	1.922	1.603	2.081	1.922	1.603
Total (seed yield)	3.043	2.810	2.344	3.043	2.810	2.344	3.043	2.810	2.344
Energy Outputs (MJ/ha)									
Oil	35.385	32.678	27.255	34.203	31.940	26.971	31.444	30.219	26.309
Cake	31.220	28.831	24.047	31.220	28.831	24.047	31.220	28.831	24.047
Total	66.606	61.509	51.301	65.423	60.772	51.018	62.664	59.050	50.355
Energy Budget									
Net Energy (MJ/ha)	17.920	14.961	6.955	18.155	15.107	7.010	18.703	15.448	7.139
Energy Efficiency	1,37	1,32	1,16	1,38	1,33	1,16	1,43	1,35	1,17
Fuel Scenarios									
Yield reduction	0,0%	7,7%	23,0%	0,0%	7,7%	23,0%	0,0%	7,7%	23,0%
Yield percentage required to cover the fuel demands				3,3%	2,3%	1,0%	11,1%	7,5%	3,5%
Total	0,0%	7,7%	23,0%	3,3%	9,9%	24,0%	11,1%	15,2%	26,4%

Soil tillage is the third most important factor of energy consumption in the field (Table 2). In conventional tillage it represents 9% of total inputs. Compared with CT, RC can reduce energy requirements at the half and NT uses no energy for seedbed preparation. Total energy consumption is reduced from 48,686 MJ/ha to 46,548 MJ/ha (4.5%) with RC and to 44,346 (9%) with NT. Further more savings can be obtained if vegetable oil produced in the farm is used to power the farm machinery. For example, even with conventional method, total energy requirements are 43,961 MJ/ha (9.5% reduced) when machinery is powered with 100%

vegetable oil. The savings rise up to 11% (total energy requirements 43,216 MJ/ha) if vegetable oil is used in combination with no-tillage. However, the use of soil conservation tecniques resulted in sunflower yield reduction which in turn affected negatively the energy outputs. Moreover, using part of the seed to produce fuel oil means a further reduction of outputs. The yield reduction due to the application of conservation tillage was 7.7% for RC and 23% for NT. In the case of powering farm machinery with 70-30% diesel - biofuel mixtures, 3,3% of the seed has to be devoted for this purpose when using the conventional tillage method. In RC, 2.3% of the seed has to be used to produce fuel but there is also a 7.7% reduction in yield giving a total of 9.9% less product. To remain competitive with CT method, yield reduction in RC mustn't be greater than 3.3-2.2 = 1.1%. In NT, only 1% of the produced seed would be sufficient to give the fuel required to cover 30% of the energy for machinery operations. To be competitive with CT, yield reduction in NT shouldn't be greater from 3.3-1=2.3%. The observed yield reduction however was 23%. When diesel fuel is substituted 100% by biofuel, 11.1% of the produced seed must be devoted to produce the fuel requirements for the CT method. This percent is reduced to 7.5% with RC, which means that there is an 11.1-7.5=3.6% margin for yield reduction that is however still smaller from the 7.7% observed in the experiment. In NT, 3.5% of the seed produced would be sufficient to power 100% of the farm machinery operations. The margin for yield reduction in NT compared to the CT method is 11.1-3.5=7.7%, significant smaller from the 23% obtained in the experiment.

From the presented analysis it can be concluded that if an amount of food resources (eg. sunflower seed) should be devoted to produce the necessary fuels to sustain agricultural production high, it is preferable to apply conventional tillage techniques that despite the higher energy demands, give higher yields. Of course conservation tillage techniques offer significant environmental benefits but in that case, the yield reduction shouldn't be greater from the limits discussed above. In the present experiment however, yield reduction was greater from the estimated limits. The lack of an appropriate no-tillage planter could be a possible cause for this. There are however many other experiments that report less or even no yield reduction when applying conservation tillage (Farooq et al., 2011, Sharma et al. 2011). More tillage experiments contacted in different regions with different soil and climate conditions would certainly offer a more complete aspect of the perspectives. The present work doesn't intend to give an answer at the immorality of using food resources for energy

production. It however sets some important limitations to achieve this, in combination with conservation tillage techniques, with the less elimination in human food supplies.

4. CONCLUSIONS

Sunflower gave a positive energy balance and can be used as an energy crop for biofuels Reduced tillage offered energy savings of 4.5% while no-tillage offered savings of 9% compared with conventional. Average yield was 7.7% lower in RC and 23% lower in NT compared to CT. When using 70-30 diesel – biofuel mixture to power farm machinery, 3.3% of the produced seed or land cultivated must be devoted to this purpose in CT. In RC the percent is 2.3% and in NT 1%. The differences from CT give the marginal yield reduction in the conservation tillage methods. When powering farm machinery with 100% farm produced biofuel, 11.1% of the produced seed or land cultivated must be devoted to this purpose in CT. In RC the percent is 7.5% and 3.3% in NT. The differences from CT give again the marginal yield reduction. Conventional tillage due to higher yields sustains the higher food productivity even if an amount is used to produce the necessary fuels in the field. Yield reduction in conservation tillage methods shouldn't be greater than the limits mentioned above.

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COMPARISON BETWEEN THE OHMICALLY-HEATED AND CONVENTIONALLY-HEATED MEAT BALLS

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ABSTRACT:

Meat ball is an emulsion meat product that is popular in many countries especially in Asia. In the present work, the pork meat balls were heated from approximately 25 °C to 74 °C by ohmic method at two heating rates consisting of 24.5 °C/min and 4.9 °C/min. The ohmic heating rate of 4.9 °C/min was identical to that of the conventional method, boiling in hot water. The attributes of ohmically-heated and conventionally-heated samples were compared in aspects of moisture content, yield strength, microstructure and color. The main objective was to investigate the effects of applying ohmic heating on the properties of meat ball products. The results indicated that the differences in heating method and heating rate leaded to the dissimilarities of product qualities. The ohmically heated samples were significantly firmer and more uniform in microstructure, and brighter in colors than their counterpart whereas their moisture contents were lower. The findings of this work would be useful for both industry and researchers that are interested to apply the ohmic heating technique to the cooking process of meat balls.

Keywords: Meat ball, Ohmic heating, Pork, Physical property

1. INTRODUCTION

Meat ball is an emulsion meat product that is popular in many countries especially in Asia (Tseng et al., 2000). In the production of meat ball, cooking is a vital step that has significant effect on the quality of meat ball. Generally, the meat balls are cooked by boiling them in hot water (Shirsat et al., 2004a); however, this method relies on the heat convection and conduction mechanisms that cannot raise the temperature of the coldest point of meat ball to the specified level within the short time due to the low thermal conductivity of meat ball. Because of this limitation, the meat ball cannot be thermally processed by ultra-high

temperature scheme in aseptic processing if the conventional heating method is applied. In addition, the long heat exposure time of meat balls at high temperature in conventional heating usually leads to the quality deterioration of products particularly at the surface.

Ohmic heating is a thermal processing technique that is deemed as a potential method to cook the meat ball products within the shorter time than the conventional method. Nevertheless, the rate of temperature increase of food products during ohmic heating is directly related to their electrical conductivities (Shirsat et al., 2004b). According to the previous work (Engchuan & Jittanit, 2012), it appeared that the electrical conductivities of meat ball samples were in the range between 0.677-3.156 S/m indicating that they can be effectively heated by ohmic method.

Although it was proved that meat balls can be heated up within a short time by ohmic method because of their high electrical conductivities, the quality of ohmically-heated meat balls comparing with those of conventionally-heated products is another aspect that is imperative and interesting for both food industry and researchers prior to applying ohmic method commercially. Owing to the deficiency of published data about the quality of ohmically-heated meat balls, in the present study, the pork meat balls were cooked by two heating schemes consisting of ohmic heating and boiling in hot water; after that, the attributes of ohmically-heated and conventionally-heated samples were compared in aspects of moisture content, yield strength, microstructure and color. The aim was to investigate the effects of applying ohmic heating to the properties of cooked meat ball products. The outcome of this research would be useful for both industry and academics that desire to apply the ohmic heating technique to the cooking process of meat balls.

2. MATERIALS AND METHODS

2.1 Sample preparation

The preparation procedure of meat balls in this study was identical to that in the earlier work (Engchuan & Jittanit, 2012). The “Betagro” Lean pork rump (manufactured from Betagro group, Bangkok, Thailand) bought from supermarket was minced by a “Robot Coupe” food processor model R201 Ultra (Robot-Coupe Australia Pty Ltd., NSW, Australia) for 1 minute and then added with salt (NaCl) at 1.5% of the pork weight. Afterward the mixture was minced another time for 2 minutes before pouring additional salt for 1% and mincing again for 2 minutes. Next, the sugar, STPP, tapioca starch, pepper powder and garlic were filled into the mixture prior to 2 minute mincing at the amounts of 2, 0.2, 3, 0.5 and 0.5% of the pork

weight respectively. The well-minced mixture was kept in a refrigerator setting temperature at 4 °C for 30 minutes before making meat balls (weight of 14.6 g each). It is noted that for the meat ball preparation the salt was added in two stages in order to make it uniformly distributed. The uniform distribution of salt inside the minced sample was essential because it affected on the amount of protein extracted from the meat structure that would act as the emulsifier for meat ball.

The fresh meat balls were cooked by two different heating techniques consisting of (1) ohmic heating and (2) heating in boiling water until the core temperature reached 74 °C and then cooled down to approximately 25 °C by putting them in cold water. There were two heating rates that were applied in ohmic heating comprising 24.5 °C/min and 4.9 °C/min. The ohmic heating rate of 4.9 °C/min was comparable to that of the conventional method, boiling in hot water, applied in the present work. The attributes of ohmically-heated and conventionally-heated samples were compared in aspects of moisture content, yield strength, microstructure and color.

2.2 Heating apparatus

For the conventional heating, a "FISHER" hot plate stirrer model 210T (Fisher Scientific (M) Sdn Bhd, Malaysia) was used for boiling water while for the ohmic heating, a static ohmic heating device was applied. The schematic diagram of this ohmic heating system is depicted in Fig. 1. The cylindrical ohmic cell was made from acrylic pipe whereas the electrodes were stainless steel grade 316L. The diameter of electrodes was 0.043 m while the distance between electrodes was 0.036 m. For the average heating rate of 24.5 °C/min, the electrical field strength of 20 V cm^{-1} at frequency of 50 Hz was applied. Moreover, the ohmic heating experiment at the mean heating rate of 4.9 °C/min was conducted by controlling the meat ball temperature profiles to be similar to that of the conventional heating experiment by manually adjusting the applied electrical field strength using the voltage variable transformer. The meat ball and surrounding water temperatures were measured using two of type-T thermocouples. The thermocouple was located at the center of meat ball when measuring the meat ball temperatures during conventional and ohmic heating processes. The measured temperature data were recorded at 5 s time interval by a "Yokogawa" data logger model DX 1012 (Yokogawa Electric Corporation, Tokyo, Japan). The applied voltage and current were

measured by a "Fluke" digital multimeter model 8808A (made by Fluke Corporation, WA, USA) that was linked to a computer with a RS-232 cable in order to record the data.

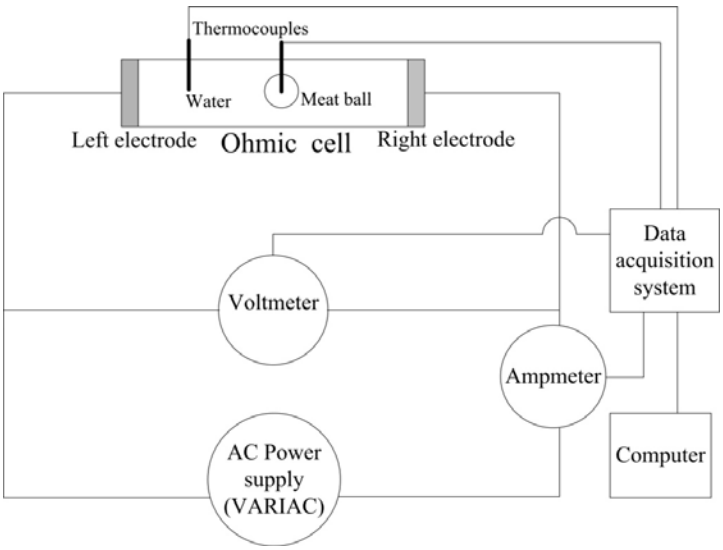


FIGURE 1. The schematic diagram of ohmic heating system.

2.3 Quality comparison

The meat ball specimens that were cooked by (1) conventional method, (2) ohmic method at heating rate of 4.9 °C/min and (3) ohmic method at heating rate of 24.5 °C/min were determined and compared their attributes in aspects of moisture content, yield strength, microstructure and color.

2.3.1 Moisture content

The moisture contents of samples were determined in three replications by the oven method using 2 g of meat ball and 105 °C drying air temperature. The procedure followed the method of Jittanit et al. (2010). The weight loss after drying in the oven was used to calculate the moisture content that was expressed in wet basis (wb).

2.3.2 Yield strength

The determination of yield strength of meat ball samples in the present work was adapted from Tseng et al. (2000). The meat ball samples were cut to be cylindrical shape with 25 mm diameter and 25 mm height prior to measuring their yield strength by the "Stable Micro Systems" texture analyzer model TA.XT Plus (Stable Micro Systems Ltd, Surrey, UK). The yield strength was the maximum force measured during the plunger penetrated into the sample until the breaking of sample happened. The yield strength values represent the firmness of the meat ball structure. This test was performed in triplicate by applying a

spherical plunger diameter of 5 mm. The test speed was constant at 1.5 mm/s while the trigger force was set at 50 g.

2.3.3 Microstructure

The specimens were cut from the interior of the meat balls to be the W × L × H dimension of 0.5 × 0.5 × 0.1 cm, mounted on aluminum specimen stubs using an electrically conductive double-sided adhesive tape prior to the microstructure observation by the "Hitachi" scanning electron microscope (SEM) model SU-1500. The magnification of 200 times and accelerating voltage of 15 kV were applied using the signals of secondary and backscattered electrons (Totosaus and Perez-Chabela, 2009).

2.3.4 Color

The colors of specimens cut from the interior of the meat balls were measured with a Hunter Laboratory MiniScan XE colorimeter (Hunter Associates, Reston, VA) in the L*, a*, b* scale. L* represents lightness ($0 \leq L \leq 100$), while a*(+), a*(-), b*(+) and b*(-) denote redness, greenness, yellowness, and blueness correspondingly. The colorimeter was calibrated with a standard white tile using illuminant D65 and the 10° standard observer. The measurement was carried out in three replications. The values of hue angle and chroma were also calculated (Zell et al., 2009).

3. RESULTS AND DISCUSSION

The values of moisture content, yield strength and color of the meat ball specimens that were cooked by (1) conventional method, (2) ohmic method at heating rate of 4.9 °C/min and (3) ohmic method at heating rate of 24.5 °C/min are presented in Table 1. Ozkan et al. (2004) stated that the moisture content in cooked meat product has significant influence on taste. It appeared that the moisture contents of meat ball samples cooked by conventional method were slightly higher than those cooked by ohmic method. This result is alike that of Shirsat et al. (2004a) which indicated that the conventionally-cooked meat emulsion batter contained more moisture content than the ohmically-cooked counterparts if all of them were cooked to the similar end point temperature of 73 °C. Furthermore, two different heating rates by ohmic method did not result in the significant difference between moisture contents of samples. The higher moisture was contained by the conventionally-heated meat balls because the conventional heating relied on the heat convection from the hot water to the sample surface and heat conduction from the outer to the center of sample; so, the surface of meat balls

exposed to heat from the boiling water since the beginning until the end of heating process. Accordingly, the sample surface would be harder than the other parts of sample and blocked the movement of the evaporated moisture from the inner of meat ball to outside. On the other hand, for the ohmic method the heat was uniformly generated all over the sample; as a consequence, the moisture loss from meat ball during ohmic heating process was more than that of conventional heating since the sample surface was not as hard as the conventionally-cooked sample surface.

Barbut et al. (1996) stated that the rigid structure of meat batter is formed when the proteins initiate to denature and contribute in protein-protein interactions. The yield strengths of cooked meat balls in this work were in the range between 9.73 – 13.53 N which are higher than those of cooked surimi gels measured by Alvarez et al. (1997) that fell among 1 – 5 N. It is obvious that the texture of ohmically-heated meat ball (at heating rate of 4.9 °C/min) was stronger than that of ohmically-heated (at heating rate of 24.5 °C/min) and conventionally-heated meat ball respectively. The conventionally-cooked meat balls had the softer texture because they contained more amount of moisture inside them; so, the strength of protein matrix inside the samples would be less. Moreover, the larger pores within the samples could be another cause of the less strength of conventionally-heated meat batter product (Barbut et al., 1996). Shirsat et al. (2004c) stated that the textural properties of cooked meat emulsion batters are influenced by the product's ability to bind water. They also pointed out that the different rates and methods of heating could affect on the water binding in meats. Carballo et al. (2000) claimed that the condition of thermal treatment affected on the texture of meat batters. The results in this study indicated that the meat balls cooked by ohmic method at slower rate were significantly firmer than those cooked at faster heating rate. It is because the slower heating rate provided the longer time for the protein inside meat balls to develop firmer gel structure and subsequent protein matrix. It must be noted that the strength of meat ball structure is a crucial characteristic that can have significant effect on the consumer acceptance. Hsu & Yu (1999) pointed out that consumers prefer a hard texture meat ball. Therefore, the texture of ohmically-cooked meat balls should be more desirable than that of conventionally-cooked samples.

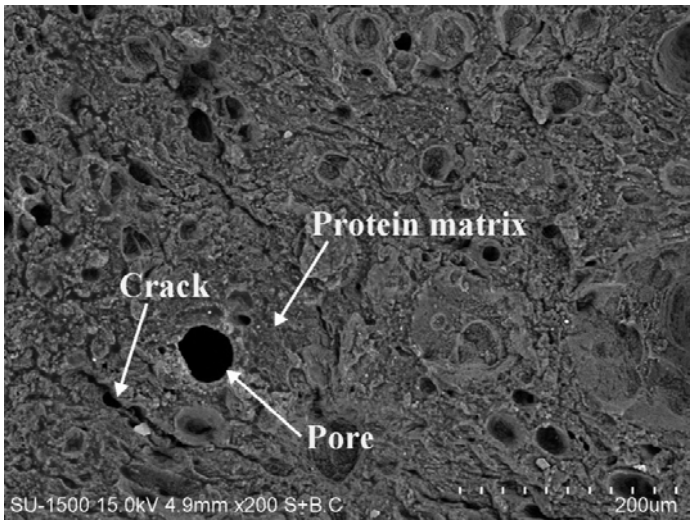
The color is another attribute of meat ball product that consumers usually consider before consumption. Hsu & Yu (1999) asserted that consumers favor a brightly colored meat ball. According to the results of color measurement, the color parameters of meat balls heated by ohmic method at heating rate of 4.9 °C/min were insignificantly different from those

ohmically heated at the rate of 24.5 °C/min except for the lightness that was slightly higher in case of applying the slower heating rate. Zell et al. (2010) found that the meat samples that were heated by ohmic method at different heating rates would expose to heat for a dissimilar period of time leading to the dissimilarities of protein gel occurred inside the meat ball and the reflection of the product surface. The redness and hue angle of conventionally-cooked meat balls were significantly higher than those heated by ohmic method. This result resembles to the finding of Zell et al. (2009) that compared between the colors of ohmically-cooked and conventionally-cooked beef muscle specimens. It implies that the browning reactions especially maillard reaction occurred in conventionally-cooked meat balls more than ohmically-cooked samples. It is because for the ohmic heating the temperature distribution within the sample was rather uniform due to its heat generation mechanism whereas for the conventional heating that relied on the heat convection and conduction, the outer layer of meat balls would be hotter than the core area since the beginning until the end of heating process. As a result, the browning reactions would be accelerated at the outer layer of the conventionally-cooked meat balls due to the high temperatures. It must be noted that although in the present work the sample temperature changes during ohmic heating in case of slower rate (4.9 °C/min) was controlled to be almost identical to that of conventional cooking, these temperatures were measured at the center of meat balls. Therefore, the temperatures at the other positions inside the meat balls could be dissimilar between two heating methods.

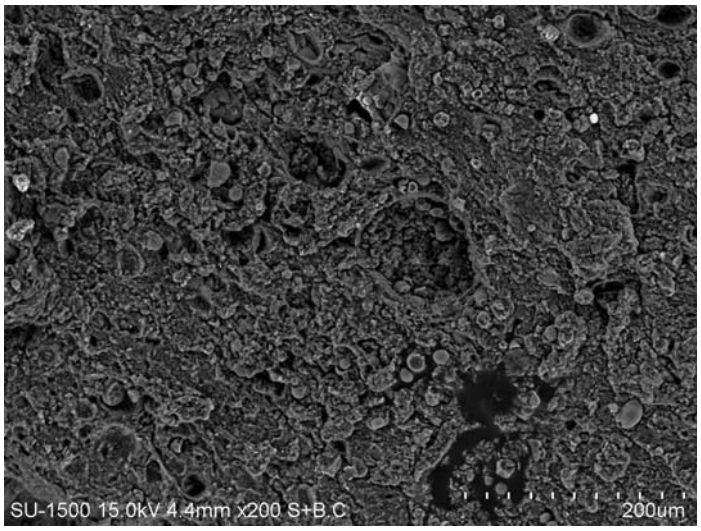
The micrographs of samples observed by SEM were illustrated in Fig 2 (a) to (c). These figures show that the microstructure of the conventionally-cooked meat ball had the looser and less uniform structure, more quantity of pores and cracks than that of the ohmically-cooked samples. The explanation is that for the ohmic heating, the heat was generated within the samples rather homogeneously; as a result, the protein network was developed simultaneously throughout the sample resulting in the uniform microstructure of samples. On the other hand, during the boiling of meat balls in the hot water, the heat was transferred from the hot water to the sample core via convection and conduction mechanisms; hence, the apparent temperature gradients within the sample must occur leading to non-uniform microstructure, mechanical stress and subsequent cracks inside the sample. Furthermore, the less temperature gradient inside the samples during ohmic heating caused the rapid and uniform moisture evaporation inside the sample resulting in the small and somewhat consistent pores throughout the samples. Conversely, the moisture evaporation from the inner

of sample and the expansion of air volume during conventional cooking were slower and less uniform; thus, the larger and inconsistent pores appeared.

a)



b)



c)

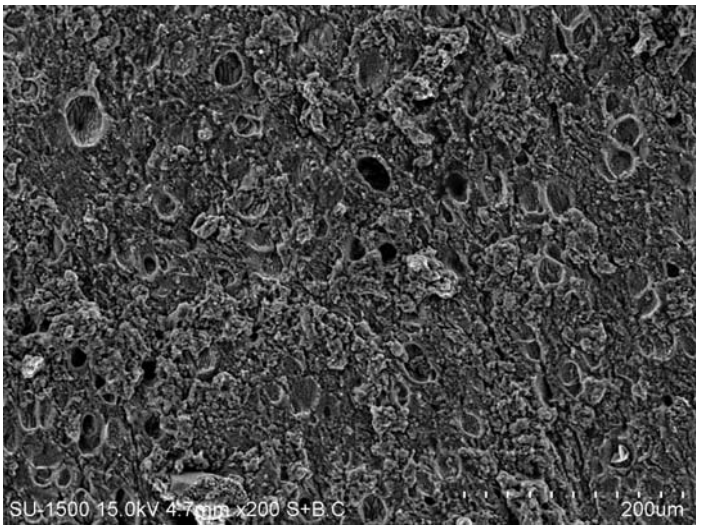


FIGURE 2. Scanning electron microscopy at 200x magnification of cooked meat balls: a) conventional; b) ohmic (4.9 °C/minute); c) ohmic (24.5 °C/minute).

TABLE 1 The moisture content, yield strength and color of the meat ball samples cooked by different processes.

Cooking method	MC (%wb)	Yield strength (N)	Color				
			L*	a*	b*	Hue angle	Chroma
Conventional heating	$73.1^{a} \pm 0.27$	$9.73^{a} \pm 0.56$	$67.78^{a} \pm 0.67$	$1.72^{a} \pm 0.17$	$14.97^{a} \pm 0.35$	$83.44^{a} \pm 0.52$	$15.07^{a} \pm 0.36$
Ohmic heating (4.9 °C/minute)	$72.5^{b} \pm 0.31$	$13.53^{c} \pm 0.92$	$69.82^{b} \pm 0.98$	$0.28^{b} \pm 0.03$	$15.19^{a} \pm 0.42$	$88.96^{b} \pm 0.10$	$15.19^{a} \pm 0.42$
Ohmic heating (24.5 °C/minute)	$72.4^{b} \pm 0.20$	$12.02^{b} \pm 1.05$	$68.18^{a} \pm 0.48$	$0.29^{b} \pm 0.15$	$15.74^{a} \pm 0.05$	$88.94^{b} \pm 0.54$	$15.75^{a} \pm 0.04$

Note: MC = moisture content, L* = lightness, a* = redness, b* = yellowness; Moisture content, yield strength and color are mean ± standard deviation; Means with the same superscript within same column are insignificant different ($P > 0.05$).

4. CONCLUSION

The results in this research indicated that the differences in heating method and heating rate caused the dissimilarities of some features of meat balls. The moisture content and browning of meat balls cooked by conventional method were significantly higher than those cooked by ohmic method; however, those of ohmically-heated meat balls applying rapid and slow heating rates were alike. The texture of ohmically-heated meat ball using slower heating rate was stronger than that of ohmically-heated at faster heating rate and conventionally-heated meat balls respectively. The conventionally-cooked meat ball had the less uniform structure, more quantity of pores and cracks than that of the ohmically-cooked samples. The uniformity of temperatures inside the samples during ohmic heating is the key reason for the differences between the attributes of conventionally and ohmically cooked meat balls.

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COMPARATIVE INVESTIGATION OF THE EFFECT OF EXTRACTION METHODS ON PHENOLIC CONTENT OF ORANGE PEEL

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ABSTRACT

The extraction of phenols from orange peel was performed by microwave assisted extraction (MAE), ultrasound assisted extraction (USE) and conventional solvent extraction (CSE). The CSE conditions were determined (ethanol 80%; solid to solvent liquid 5:50; extraction temperature 35°C, extraction time 30 min and three extraction cycles). The total phenols and flavonoids contents of orange peels were evaluated using the Folin-Ciocalteu assay and colorimetric aluminum chloride method, respectively. The contents of phenols and flavonoids of the orange peel extract obtained by CSE (35°C, 30 min) were compared to those obtained by MAE (170 W, 10s) and by USE (30 W, 30 min). The MAE allows obtaining the highest total phenols (2.363 ± 0.014 g GAE/100 g dry peel) and flavonoids (1.265 ± 0.023 g rutin/100 g dry peel) contents. Overall, this study provides supporting evidence that MAE is an efficient and environment-friendly method for the preparation of natural extracts aimed at replacing synthetic antioxidants.

Key words: Orange peel, phenolic compounds, flavonoids, extraction parameters, conventional solvent extraction, ultrasound assisted extraction, microwave assisted extraction.

1. INTRODUCTION

Citrus peels represent a rich source of natural flavonoids unique to citrus, which are relatively rare in other plants. It contains high amounts of flavanone glycosides, lower amounts of polymethoxylated flavones, traces of flavonols, glycosylated flavones and hydroxycnnamic

acids (Bocco et *al.,* 1998. Kawaii et *al.,* 1999). Flavonoids are generally antioxidants and act as free radical scavengers as they are potential reducing agents and protect from oxidative reactions taking place inside the body (Routray and Orsat, 2012). They act against many chronic diseases such as cancer, inflammation, cardiovascular, and neurodegenerative disorders (Bocco et *al.,* 1998; Ghasemi et *al.,* 2009). Currently, their extraction from citrus peels has attracted considerable scientific interest to use them as low cost natural antioxidants mainly in foods to prevent the rancidity and the oxidation of lipids (Anagnostopoulou et *al.,* 2006), to increase their shelf life (Abd El-aal et *al.,* 2009; Tumbas et *al.,* 2010), and to replace synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which might be liver- damaging, carcinogenic (Ak and Gülçin, 2008) and more generally toxic (Moure et *al.,* 2001). Therefore, extraction represents an important contributing element to the development of processed foods and nutraceutical food ingredients (Stalikas, 2007). The common methods of extraction of flavonoids include conventional methods such as hot water bath extraction (Soltoft et *al.,* 2009), soxhlet extraction methods (Pan et *al.,* 2010; Zhu et *al.,* 2010), ultrasound extraction (Ma et *al.,* 2009; Khan et *al.*, 2010; Chemat et *al.,* 2011), and microwave extraction (Inoue et *al.,* 2010; Ahmad et *al.,* 2012) or modern methods such as supercritical and subcritical extractions (Giannuzzo et *al.,* 2003; Lee et *al.,* 2010) and pressurized liquid extraction (Li et *al.*, 2012). Scientists explor other possible methods which are more environment friendly, using less energy and less solvent while producing higher yields.

The objectives of this research were (1) to determine the conventional solvent extraction parameters of phenolic compounds from orange peels: the effect of repeated extraction, different types of organic solvents, the concentration of the solvent and temperature of extraction; (2) to conduct a comparison between three extraction methods: microwave assisted extraction (MAE), ultrasound assisted extraction (USE) and conventional solvent extraction (CSE).

2. MATERIAL AND METHODS

2.1. Plant material and sample preparation

About 20 kg of fresh oranges (*Citrus sinensis*) of "Maltaise" variety were collected in March 2012 from Manzel Bouzalfa (Nabeul, Tunisia) in their commercial maturity. All fruits were of eating quality, and without blemishes, or damage. On arrival at the laboratory, the orange

fruits were immediately washed by tap water and peeled. The remaining orange peels account for approximately 40% of the total fruit. The peels were stored at -80°C before any further treatments. Orange peels were dehydrated by using a freeze dryer (CHRIST Alpha 1-2 LD, France) for 72h (at -50°C and 0.001 mbar) and then finely ground using a coffee grinder (Moulinex®, France) to achieve a standard size of particles of ~ 0,315 mm. Large particles remaining on the sieve were further ground. The process was repeated until all the material passed through the metal sieve. The orange peels powder were placed in vacuum packaging bags and stored in a freezer maintained at 4°C before their use for experiments.

2.2. Chemicals and reagents

Potassium persulfate was purchased from Fluka, Switzerland. Rutin, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azimobis (3 ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), and gallic acid monohydrate (GA) (purity ≥ 98.0%) were purchased from Sigma-Aldrich, Germany. Sodium hydroxide (NaOH) and methanol were obtained from Carlo Erba-SDS, France. Ethanol (purity ≥ 95.0%) was from VWR, Belgium, and acetic acid was obtained from VWR, Belgium.

2.3. Extraction methods

2.3.1. Conventional solvent extraction (CSE)

Five grams of *citrus sinensis* peels powder were extracted with 50 ml of 80% ethanol. The mixture was shaken at 200 rpm in darkness by using a mechanical stirrer for 30 min at 35°C. The crude extract was centrifuged at 8000g for 10 min and the supernatant was filtered through a 0.2 μm solvent filter. The residue was further extracted two times with 50 ml of the same solvent under the same extraction conditions. Combination of the three extracts were collected and stored at 4°C.

2.3.2. Ultrasound assisted extraction (UAE)

Five grams of *citrus sinensis* peels powder were extracted with 50 ml of 80% ethanol in the ultrasound sonicator (VibraCell 75115, Bioblock-Fisher, France) for 30 min at 35°C and an ultrasonic power level of 30 W. The mixture was shaken in darkness at the same time with

magnetic stirrer. The crude extract was centrifuged at 8000g for 10 min and the supernatant was filtered through a 0.2 μm solvent filter. The residue was further extracted two times with 50 ml of the same solvent under the same extraction conditions. Combination of the three extracts were collected and stored at 4°C.

2.3.3. Microwave assisted extraction (MAE)

Five grams of *citrus sinensis* peels powder were put into 500 ml tightly closed bottle with 50 ml of 80% ethanol and placed in the central position of the microwave oven (WAVEDOM LG, France) Samples were heated at 170 for 10s to obtain an extraction temperature of 35°C. Temperature of the extract was measured by using a K-thermometer. After MAE, the extracts were cooled to room temperature and immediately centrifuged at 8000g for 10 min and the supernatant was filtered through a 0.2 μm solvent filter. The residue was further extracted two times with 50 ml of the same solvent under the same extraction conditions. Combination of the three extracts were collected and stored at 4°C.

2.4. Analytical methods

2.4.1. Determination of total phenol content (TPC)

Total phenols in *citrus sinensis* extracts were evaluated by using the Folin-Ciocalteu assay, which was adapted from (Wrolstad et *al.,* 2002) with some modifications. Briefly, 40 μl of orange extract was mixed with 200 μl of Folin-Ciocalteu reagent and 3.160 mL deionized water. The mixture was shaken by vortex and kept at room temperature for 3 min, and then 600 μl of 20% sodium carbonate (w/v) was added. The mixture was heated in a water bath at 40°C for 30 min. Absorbance was measured at 765 nm using a spectrophotometer (Genesys 10uv screnning, Thermo Electron Corporation, France). The blank consisted of Milli-Q water. A standard curve was established using gallic acid at concentrations of 50, 100, 200, 300, 400 and 500 mg/l diluted in ethanol (R^2=0.9922). Total phenol content was expressed as mg of gallic acid equivalent (GAE) per 100 g of dry matter (DW) ± standard deviation for three triplicates.

2.4.2. Determination of total flavonoids content (TF)

Total flavonoids were determined following the modified procedure of Sultana et al, (2008). 0.5 ml of aqueous extract was placed in a 5 ml volumetric flask, then 2.5 ml of distilled water

were added followed by 0.15 ml of 5% $NaNO_2$. After 5 min, 0.15 ml of 10% $AlCl_3$ were added. 5 min later, 1 ml of 1M NaOH were added and the volume made up with distilled water. The solution was mixed and absorbance was measured at 510 nm using a spectrophotometer (Genesys 10uv screnning, Thermo Electron Corporation, France). A standard curve was determined by using rutin at concentrations of 40, 80, 120, 160 and 200 mg/l diluted in methanol (R^2=0.9985). TF amounts were expressed as rutin equivalent g/100g of dry matter ± standard deviation for three triplicates.

3. RESULTS AND DISCUSSION

3.1. Determination of the conventional solvent extraction parameters

3.1.1. Effect of organic solvents

Solvent selection is based on the solubility parameters and the polarity of the target components and the solvent. According to the solubility models proposed by Kassing et al. (2010), components dissolve best into each other if the solubility parameters (logP) are equal. The comparison of the theoretical values of hydrophobicity (Log P) of phenolic compounds as well as those of some organic solvents, using Software Pallas30 ® has showed that both methanol (-0.870; -0.760) and ethanol (-0.770; -0.600) at a concentration ranging from 70% to 80% are more suitable for extraction of citrus phenolic compounds.

Several studies have shown that methanol is the most effective solvent for the extraction of phenolic compounds. Zia-ur-Rehman (2006), showed that the highest yield of extraction of citrus peel phenols is obtained using methanol (19.87%), followed by acetone (15%), diethyl ether (12.75%) and ethanol (11.00%) (m/v: 10:100; 24h, room temperature, agitation). This result is confirmed by Ma et al. (2008); Ma et al. (2009); and Li et al. (2012). Whereas Li et al. (2006), found that the extraction yields of total phenols extracted by methanol and ethanol are almost the same (ethanol, m/v: 2:16; 80°C, 3h, agitation). In addition, in industrial applications, methanol should be avoided because its toxicity and its environmental problem. It was replaced by other organic solvents which are not toxic, such as ethanol, n-butanol, isopropanol, acetone, ether diethyl and petroleum ether (Bartnick et al, 2006), but ethanol is the most widely used. The ethanol was thus selected for next investigations on citrus peel phenols extraction.

3.1.2. Effect of ethanol concentration

The total phenols content (TPC) of citrus peels obtained by using ethanol at 70 and 80% are respectively 1.888±0.018 g GAE/100g DW and 1.969±0.003 g GAE/100g DW (3 extractions). Therefore, ethanol at 80% allows a significant increase of the total phenol content and it was selected for the next extractions. The ethanol concentration at 70-80% was the most used range in literature for citrus peels phenol extractions. Khan et al. (2010) reported that 80% is the optimum concentration of ethanol used for extraction of flavanones from orange peels (ethanol; 0.25 g/ml; 40°C, 30 min; 95 bar). While Li et *al.* (2012), Tumbas et *al.* (2010), Cheigh et *al.* (2012) and Inoue et *al.* (2010) showed that the optimal solvent concentration was 70%. Consequently for the next investigations, three extractions (30 minutes, 35°C; ratio m/v: 5g:50 ml; 80% ethanol, with mechanical agitation) will be performed for phenols citrus peel extractions.

3.1.3. Effect of time and repeated extraction

Conventional solvent extraction (CSE) (ethanol 80%, solvent to solid ratio: 5g:50ml) was carried out at 35°C for different periods varying from 5 to 80 min. The extraction temperature was fixed at 35°C to avoid thermal degradation of antioxidants and 5 successive extractions were performed. Figure 1 present the total phenol content according to the extraction time. Increasing the extraction time from 5 to 20 min significantly influenced the extraction of phenolic compounds. The extraction of phenolic compounds was rapid for the first 20 min (total phenols content reached 1.550 g GAE/100g DW after 20 min and 1.598 g GAE/100 g DW after 30 min of the first extraction). However, when the extraction time was above 20 min, the total phenols content reached a level, which represents the saturation of the solvent in phenols.

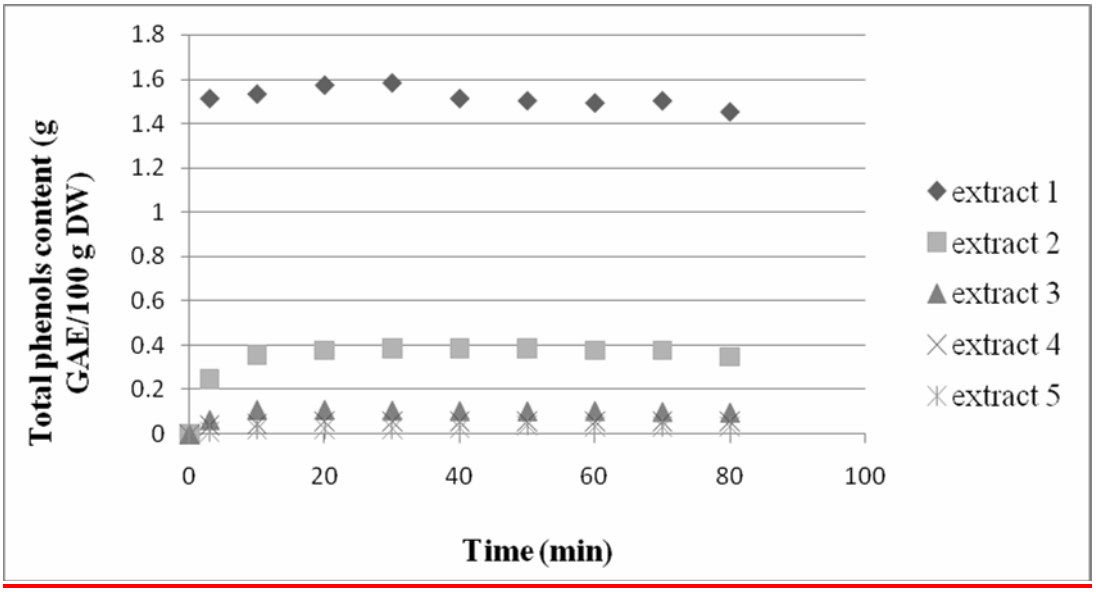

Figure 1: Equilibrium curves of conventional solvent kinetic extraction
(m/v: 5g: 50ml, 35°C, mechanical agitation at darkness and 5 extraction cycles)

Figure 1 show also that total phenols content (TPC) decreases in response to successive extractions. Three extractions allow obtaining the maximum of orange peel phenolic compounds (0.109±0.001 g GAE/100 g DW for the third extraction; 0.055±0.001 and 0.026±0.002 g GAE/100 g DW for the fourth and fifth extraction). So the amounts recuperated at the fourth and the fifth extractions are negligible and three extractions were sufficient to recuperate almost all of phenolic compounds of citrus peel powder.

Zia-ur-Rehman et al. (2006), reported also that it is necessary to realize a double extraction of phenolic compounds from citrus peels (methanol, ethanol, acetone and diethyl ether; m/v: 10:100; room temperature, overnight, and with stirring). Li et al. (2006) observed that a single extraction (ethanol; m/v: 2:16; 80°C; 3h; and with stirring) of 3h results in higher recovery of total phenols of citrus peels (mandarin, lemon, grapefruit and sweet orange) than a double extraction (2×1.5h).

3.2. Comparison of different extraction methods

The total phenolic and flavonoid contents of the orange peel extracted by conventional solvent extraction, ultrasonic assisted extraction and microwave assisted extraction were summarized in Table 1.

TABLE 1: Total phenolic and flavonoid contents in the orange peel extracted by different extraction methods (m/v: 5g/50ml, 35°C, ethanol 80%, and 3 successive extractions).

Extraction method	Total phenol content*	Total flavonoid content**
Conventional solvent extraction 30 min, (mechanical agitation)	1.968 ± 0.003	1.013 ± 0.005
Ultrasound assisted extraction (30 min, 150W)	2.117 ± 0.030	1.103 ± 0.024
Microwave assisted extraction (170W, 10s)	2.363 ± 0.014	1.265 ± 0.023

* g GAE/100 g of citrus peel powder

** g rutin equivalent/100 g of citrus peel powder

As can be seen in Table 1, the contents of total phenolics (2.363 ± 0.014 g GAE/100 g citrus peel powder) and flavonoids (1.265 ± 0.023 g rutin/100 g citrus peel powder) in the citrus peel obtained by the microwave assisted extraction method were higher than those obtained by ultrasonic assisted extraction method, followed by conventional solvent extraction.

Hayat et *al.* (2009) compared three techniques of extraction of phenolic acids from mandarin peels: conventional solvent extraction, ultrasound assisted extraction and microwave assisted extraction. The results indicated that MAE has the highest content of ferulic acid (0.239 g/100g DW) compared to ultrasonic extraction (UAE) (0.235 g/100g). The efficiency of the conventional solvent extraction was found to be the lowest (0.205 g/100g DW). Li et *al.* (2012) showed also that the low yield of extraction is obtained by conventional extraction (5.78%), while it is 7.79% for ultrasonic extraction. Khan et *al.* (2010) reported that the quantities of naringin and hesperidin from ultrasound assisted extraction were considerably higher (0.250, 0.070 g/100g DW) than those obtained by conventional extraction (0.145; 0.051 g/100g DW), respectively.

Conventional solvent extraction is a very friendly method and gives reasonable recovery (Li et *al.,* 2006). However, it poses some disadvantages like possible loss of initial quality of the extract because of the combination of long hours of extraction (2, 3, 20 and 24h) and high applied temperature (40, 80 and 100°C) which affect the recovery of mainly thermolabile components (Wang and Weller 2006). Routray and Orsat (2012) reported that UAE can be a technique of choice for thermolabile components as the operating temperature can remain low

during the process, thus maintaining extract quality. But Hayat et *al.* (2009) reported that solid and liquid particles are vibrated and accelerated under ultrasonic action. Since, generally the ultrasonic energy is not homogeneously distributed, which may induce the low precision of ultrasonic extraction. Microwave assisted extraction cause the rise of solvent temperature and internal pressure, which then increases the solubility of the compound of interest. This subsequently leads to rupture of cell walls and fast release of bioactive compounds (Camel, 2001). But, the recovery obtained by MAE is low when the solvent lacks a significant dipole moment for microwave energy absorption. The methods are therefore limited in terms of solvents and nature of the solid material.

4. CONCLUSION

The total phenolic contents of citrus peels were affected by the type of the solvent and its concentration, the operating temperature, the extraction time and the repeated extraction. The extraction parameters were determined: ethanol (80%), 30 minutes, 35°C, ratio m/v: 5g:50 ml, with mechanical agitation, 3 successive extractions. The microwave assisted extraction (MAE), the ultrasound assisted extraction (UAE) and conventional solvent extractions (CSE) were used for total phenol and flavonoids determinations of citrus peels. The high total phenolic and flavonoid contents were obtained by MAE (2.363 ± 0.014 g GAE/100 g dry peel; 1.265 ± 0.023 g rutin/100 g dry peel). Overall, microwave assisted extraction of phenolic compounds from abundant citrus peels and by using food grade solvent has a strong potential for industrial development as an efficient and environment-friendly process for the preparation of extracts rich in natural antioxidants aimed at replacing synthetic antioxidants.

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USE OF EDIBLE FILM ADDED WITH ANTIMICROBIAL AGENTS IN SHELF-LIFE EXTENSION OF COLD SMOKED GIANT PERCH (*Lates calcarifer*) PRODUCT

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ABSTRACT

This research aims to study suitable edible film added with antimicrobial agents for shelf-life extension of cold smoked giant perch. The performance of edible antimicrobial films, namely, chitosan film added with nisin (CNF), carrageenan film incorporated with cinnamon extract (CCF) and pectin film incorporated with liposome containing antimicrobial extract from Thai herbs (PLF) on microbiological inhibition and sensory evaluation of cold smoked fish was observed. Each edible antimicrobial film was wrapped on cold smoked giant perch, and the wrapped fish was then stored at 4 °C. It was found that CNF was able to extend shelf life of the product by up to 40 days. CCF and PLF could extend shelf life of cold smoked giant perch for 30 and 25 days, respectively, while control sample (unwrapped cold smoked giant perch) had shelf life only 20 days. The cold smoked giant perch wrapped by CNF was the most accepted in sensory evaluation. Therefore, CNF was selected to wrap on cold smoked giant perch for testing against pathogen and spoilage microorganisms including, total plate count, Lactic acid bacteria, *Listeria* spp. and *Staphylococcus aureus* during storage (4 °C) for 40 days. The result showed that the film was effective in inhibiting growth of pathogen and spoilage microorganisms compared to control sample. CNF was able to extend shelf-life of the product to 40 days.

Keywords: edible film; antimicrobial agents; cold smoking; shelf-life; giant perch

1. INTRODUCTION

Giant perch (*Lates calcarifer*) is freshwater fish which is popular in Thai and export markets due to its texture and sweetness of the flesh. The increasing demand of giant perch

resulted in expansion of their aquatic farms in both rivers and artificial ground ponds. Giant perch that was raised in artificial ground pond has a tint smell of earth in their flesh, which reduces the price of selling the fresh fish. Therefore, most of the fish from artificial ground ponds is processed to other types of fish product such as smoking, fish paste, fish balls and dried mixed culinary products. Among these products, smoked fish is most popular in Thailand. For local markets, hot smoking is preferred more than cold smoking due to longer shelf-life of the product. On the other hand, cold smoked giant perch is more in demand in restaurants and hotels. However, the supply of cold smoked giant perch is always in shortage due to short shelf-life and contamination of pathogen and spoilage microorganisms of the product. Microbial contamination reduces the shelf-life of foods and increases the risk of food borne illness, therefore adequate preservation technologies must be applied in order to preserve its safety and quality. Traditional methods of preserving foods from the effect of microbial growth such as refrigeration can reduce the growth of microorganisms, but it cannot kill microorganisms. The consumer demands high quality, convenient, innovative, regular and safe food products with natural flavors and taste as well as an extended shelf-life. Moreover, less chemically preserved products are required by consumers. This has led to investigation of using natural antimicrobial compounds to replace chemical preservatives.

Nisin is a bacteriocin that has been listed in GRAS. It can inhibit gram positive bacteria including *Bacillus*, *Mycobacterium*, *Clostridium*, *Lactococcus*, *Listeria*, *Staphylococcus* and S*treptococcus*. The antimicrobial mechanism is reaction with cell membrane resulting changes of selective properties of the cell membrane (Ouattara et al., 2000). Spices and herb extracts are natural compounds that have been widely studied for their natural antimicrobial activities, and it has been found that some examples have potential for inhibition of microorganisms in foods (Smith-Palmer et al., 1998; Holley and Patel, 2005; Arora and Kaur, 1999; Burt, 2004; Alanis et al., 2005). There are important phytochemical in those extracts such as cinnaldehyde from cinnamon, eugenol from clove oil (Ouattara et al., 1997) or allicin in garlic (Siripongvutokorn et al., 2005). Moreover, those natural extracts are classed as generally recognized as safe (GRAS) food additives (Cowan, 1999)

Unfortunately, most natural compounds are biologically unstable because they are sensitive to the environment (water, oxygen, light) that destroys their antimicrobial activities (Nychas et al., 2003; Hao et al., 1998). Currently, novel methods have been introduced in order to improve their stabilities and their bioavailability, by using encapsulation to prevent reactivity with the environment and minimal compounds loss during storage. The use of

edible films in food protection and preservation has recently increased since they offer several advantages over synthetic materials, such as being biodegradable and environmentally friendly (Tharanathan, 2003). The examples of biodegradable films are chitosan, carrangeenan and pectin films. When those films come into contact with food, moisture from food releases antimicrobial agents to food surface for inhibition of food spoilage and food pathogen microorganisms (Han, 2005; Cutter, 2006). Moreover, edible film has abilities to retard moisture, oxygen**,** aromas and solute transportation (Han, 2005).

Therefore, the objective of this research is to study the suitable edible film added with antimicrobial agents on safety and shelf-life extension of cold smoked giant perch for high-end markets.

2. MATERIALS AND METHODS

2.1 Raw materials

Giant perch fish was received from the Chaipattana foundation, Thailand. The giant perch was prepared by filleting, trimming and washing. Then the giant perch fillets were stored at 4 °C before pretreatment and cold smoking.

2.2 Pretreatment and cold smoking

The clean fillets (7.5 × 15 × 1.0 cm) were soaked in crude lemongrass extract solution added with salt at 5% w/v concentrations for 30 min (fish to brine ratio of 1:1 w/w). After soaking, the fish was transferred to a smoking chamber at smoking temperature at 45 °C for 2 hours.

2.3 Edible film added with antimicrobial agents

There were 3 antimicrobial edible films used to study their activity on retarding growth of microorganisms and extend shelf-life of cold smoked giant perch:

- chitosan film added with nisin (CNF) (Mu et al., 2008);
- carrageenan film incorporated with cinnamon extract (CCF) (Tharaket, 2008);
- pectin film incorporated with liposome containing antimicrobial extract from Thai herbs (PLF) (Mekkerdchoo et al., 2009).

Cold smoked fillet (7.5 × 15 × 1.0 cm) was wrapped around with each film and packed in LDPE bag (15 ×25 cm), the bag was sealed and stored at 4 ± 1 °C for further sampling. The control cold smoked fillet was not wrapped with any film and packed in LDPE bag, sealed and then stored at 4 ± 1 °C for further sampling every 5 days.

2.4 Microbial assessment

All cold smoked fillets were sampled every 5 days to test total bacteria count (AOAC, 1995), *Listeria* spp., *Staphylococcus aureus* (FDA-BAM, 2001) and lactic acid bacteria (ISO 15214: 1999(E)) until number of bacteria is over than 6 log CFU/g. For each microbial test used 25 g of cold smoked fillets samples and the assessment was done in duplicates.

2.5 Sensory evaluation

Sensory evaluation of treated sample was done on every 5 days during storage at 4±1 °C by using acceptance test 9-point hedonic scale with 30 panelists who know about cold smoked fish products. The mean values of score in each description were compared using Duncan's New Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Appearance of antimicrobial edible films used

Figure 1-2 show appearance of antimicrobial films and smoked giant perch fish fillets wrapped in edible film.

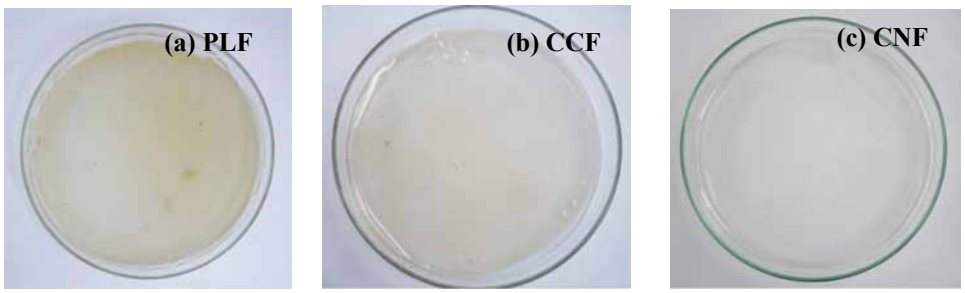

Figure 1 Edible film added with antimicrobial agents (a) PLF (b) CCF (c) CNF

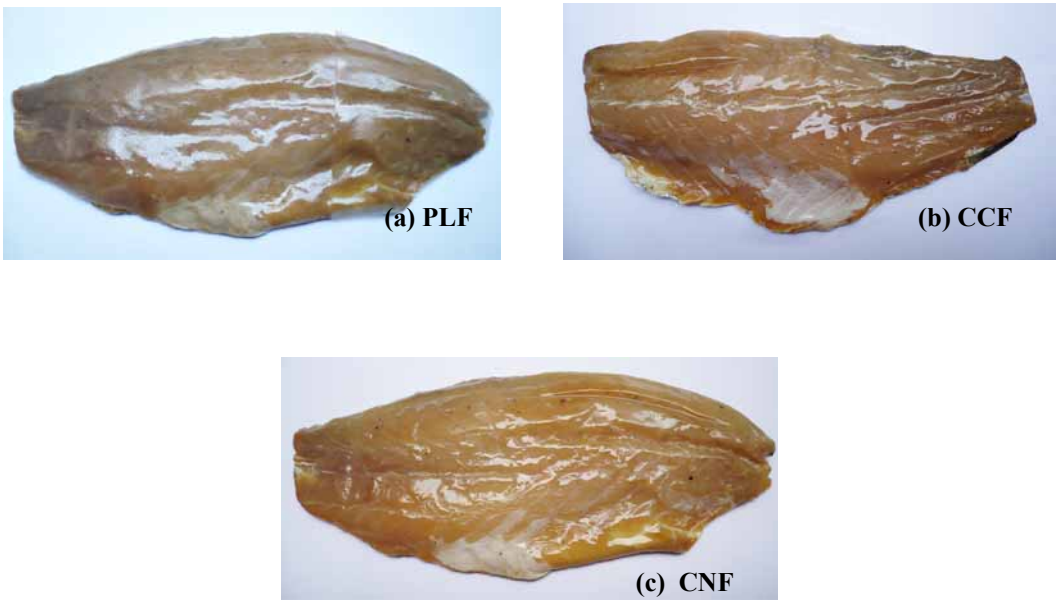

Figure 2 Smoked giant perch fillet wrapped with edible film added with antimicrobial agents (a) PLF (b) CCF (c) CNF

3.2 Antimicrobial activity of edible films

The performance of edible film added with antimicrobial agents on extension shelf life of cold smoked giant perch is evaluated by using the film to wrap cold smoked giant perch fillet. The result of total bacteria is shown in Figure 3.

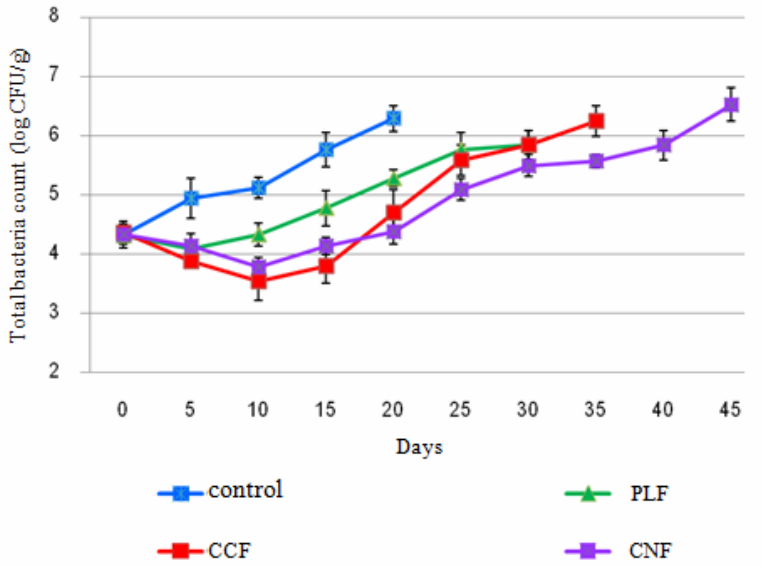

Figure 3 Total bacteria count in control smoked giant perch and treatment samples wrapped with different antimicrobial films in LDPE bag stored at 4 ± 1 °C

From Figure 3, it can be seen quite clearly that the entire fillet sample wrapped with edible film added with antimicrobial agent can slow the growth of microorganisms when compared to control fillet. At the first 10 days of storage CNF and CCF can reduce the number of total bacteria count. However, the number of bacteria increased again slowly after 10 days storage at 4 ± 1 °C for CNF and CCF. For PLF can retard the growth for first 5 days then the bacteria start to increase. The rate of increasing of bacteria is fastest in control sample followed by PLF, CCF and CNF. For control sample, only 20 days of storage, number of bacteria is over 6 log CFU/g, while CNF can maintain the number of bacteria under 6 log CFU/g up to 40 days.

Chitosan is polycationic. On its chain, there is positive charge that will react with phosphoryl group (negative charge) of phospholipids components on cell membrane of microorganisms resulting in leaking of important liquid out of bacterial cell (Li et al., 2006). For nisin, it is a bacteriocin that has been listed as safe by GRAS. It can inhibit gram positive bacteria including *Bacillus*, *Mycobacterium*, *Clostridium*, *Lactococcus*, *Listeria*, *Staphylococcus* and S*treptococcus*. The antimicrobial mechanism of nisin is its reaction with cell membrane resulting changes of selective properties of the cell membrane (Ouattara et al., 2000). Therefore, this combination effect of chitosan and nisin promotes the bacteria inhibition of CNF. Cinnamon and all Thai herbs contain antimicrobial agent such as cinnamic aldehyde (cinnamon) eugenol (clove), allicin (garlic), tannin and phenolic compounds (pomegranate skin extract). However, their activity is not as powerful as nisin.

For the use of edible films added with antimicrobial agents, antimicrobial performance is the primary consideration, but sensory acceptance from consumers is also important. The sensory evaluation of smoked giant perch fillets wrapped with different edible films during the time of safe storage showed in Table 1-3. The acceptance score is from 0-9 and when the score is less than 6 means not accepted.

3.3 Sensory evaluation of cold smoked giant perch fillet wrapped with different antimicrobial edible films

Sensory evaluation of cold smoked giant perch filler wrapped with different antimicrobial edible films was performed by using 30 panelists. The results are shown in Table 1-3.

Table 1 Average score of sensory evaluation of smoked giant perch warped with CNF in LDPE bag during storage at 4 ± 1 °C

Days	Appearance	Colour	Odour	Taste	Texture	Overall liking
0	7.30±0.52^{a}	7.25±0.26^{a}	7.34±0.45^{a}	7.34±0.28^{a}	7.29±0.59^{a}	7.22±0.24^{a}
5	7.29±0.48^{a}	7.26±0.32^{a}	7.35±0.26^{a}	7.31±0.31^{a}	7.28±0.28^{a}	7.21±0.35^{a}
10	7.19±0.48^{b}	7.25±0.38^{a}	7.22±0.20^{ab}	7.25±0.26^{ab}	7.24±0.34^{a}	7.18±0.42^{a}
15	7.18±0.15^{b}	7.18±0.39^{b}	7.18±0.32^{b}	7.24±0.51^{ab}	7.18±0.25^{ab}	7.15±0.19^{ab}
20	7.16±0.65^{bc}	7.17±0.29^{b}	7.17±0.45^{b}	7.22±0.28^{ab}	7.15±0.21^{ab}	7.11±0.35^{ab}
25	7.10±0.50^{c}	7.14±0.52^{b}	7.16±0.26^{b}	7.19±0.43^{ab}	7.10±0.19^{bc}	7.01±0.25^{b}
30	7.15±0.49^{bc}	7.15±0.26^{b}	7.18±0.33^{b}	7.20±0.41^{ab}	7.14±0.43^{ab}	7.08±0.28^{ab}
35	7.09±0.52^{c}	7.14±0.26^{b}	7.14±0.13^{b}	7.18±0.45^{ab}	7.00±0.11^{c}	6.95±0.29^{b}
40	7.05±0.42^{c}	7.10±0.34^{c}	7.15±0.25^{b}	7.12±0.33^{b}	6.97±0.20^{c}	6.88±0.30^{c}

Different superscripts on each column mean that the values are significant different ($p \leq 0.05$).

Table 2 Average score of sensory evaluation of smoked giant perch warped with CCF in LDPE bag during storage at 4 ± 1 °C

Days	Appearance	Colour	Odour	Taste	Texture	Overall liking
0	7.24±0.52^{a}	7.25±0.25^{a}	7.16±0.19^{a}	7.33±0.35^{a}	7.25±0.44^{a}	7.21±0.24^{a}
5	7.21±0.64^{a}	7.24±0.26^{a}	7.16±0.25^{a}	7.31±0.19^{a}	7.24±0.28^{a}	7.20±0.43^{a}
10	7.19±0.50^{a}	7.22±0.35^{a}	7.15±0.31^{a}	7.30±0.24^{a}	7.22±0.35^{a}	7.18±0.35^{a}
15	7.15±0.51^{ab}	7.13±0.45^{b}	7.13±0.29^{a}	7.31±0.29^{a}	7.23±0.34^{a}	7.16±0.26^{ab}
20	7.12±0.56^{ab}	7.20±0.32^{ab}	7.11±0.27^{ab}	7.28±0.35^{ab}	7.18±0.50^{b}	7.14±0.29^{b}
25	7.11±0.42^{ab}	7.18±0.15^{ab}	7.10±0.41^{ab}	7.27±0.29^{ab}	7.17±0.42^{b}	7.13±0.37^{b}
30	7.08±0.61^{b}	7.15±0.22^{ab}	7.08±0.26^{b}	7.20±0.46^{c}	7.17±0.38^{b}	7.13±0.33^{b}

Different superscripts on each column mean that the values are significant different ($p \leq 0.05$).

Table 3 Average score of sensory evaluation of smoked giant perch warped with PLF in LDPE bag during storage at 4 ± 1 °C

Days	Appearance	Colour	Odour	Taste	Texture	Overall liking
0	7.15±0.20^{a}	7.30±0.45^{a}	6.84±0.28^{a}	6.80±0.25^{a}	7.10±0.48^{a}	6.94±0.26^{a}
5	7.16±0.35^{a}	7.28±0.15^{a}	6.75±0.26^{a}	6.78±0.26^{a}	6.95±0.28^{a}	6.85±0.59^{ab}
10	7.01±0.35^{ab}	7.20±0.16^{ab}	6.41±0.24^{b}	6.61±0.32^{ab}	6.94±0.28^{a}	6.45±0.52^{b}
15	6.78±0.41^{b}	7.16±0.45^{ab}	6.28±0.11^{bc}	6.35±0.23^{b}	6.91±0.40^{a}	6.29±0.58^{b}
20	6.74±0.32^{b}	7.00±0.42^{c}	5.75±0.36^{c}	5.90±0.33^{bc}	6.83±0.22^{ab}	6.11±0.51^{bc}
25	6.75±0.29^{b}	7.00±0.19^{c}	5.65±0.34^{c}	5.78±0.29^{c}	6.74±0.19^{ab}	5.79±0.31^{c}

Different superscripts on each column mean that the values are significant different ($p \leq 0.05$).

Sensory evaluation showed that the overall score of the appearance and odour of CNF wrapped smoked giant perch fillet is higher than CCF and PLF wrapped sample, while score of texture of CCF wrapped sample is a little bit higher than CNF and PLF wrapped sample. PLF wrapped smoked fillets sample has the lowest score due to the odour of garlic and clove that resulted in a lower acceptance score. When storage time is increased, the sensory evaluation scored is dropped as each treated sample was developing off characteristics that lower sensory evaluation score. However, CNF can maintain sensory evaluation score above 6.88 for up to 40 days. Therefore, CNF is selected to test against spoilage and pathogen microorganism including *Listeria* spp, *S. aureus* and lactic acid bacteria. The results are showed in Figure 4a-4c.

3.4 Performance of CNF against pathogenic and spoilage microorganisms

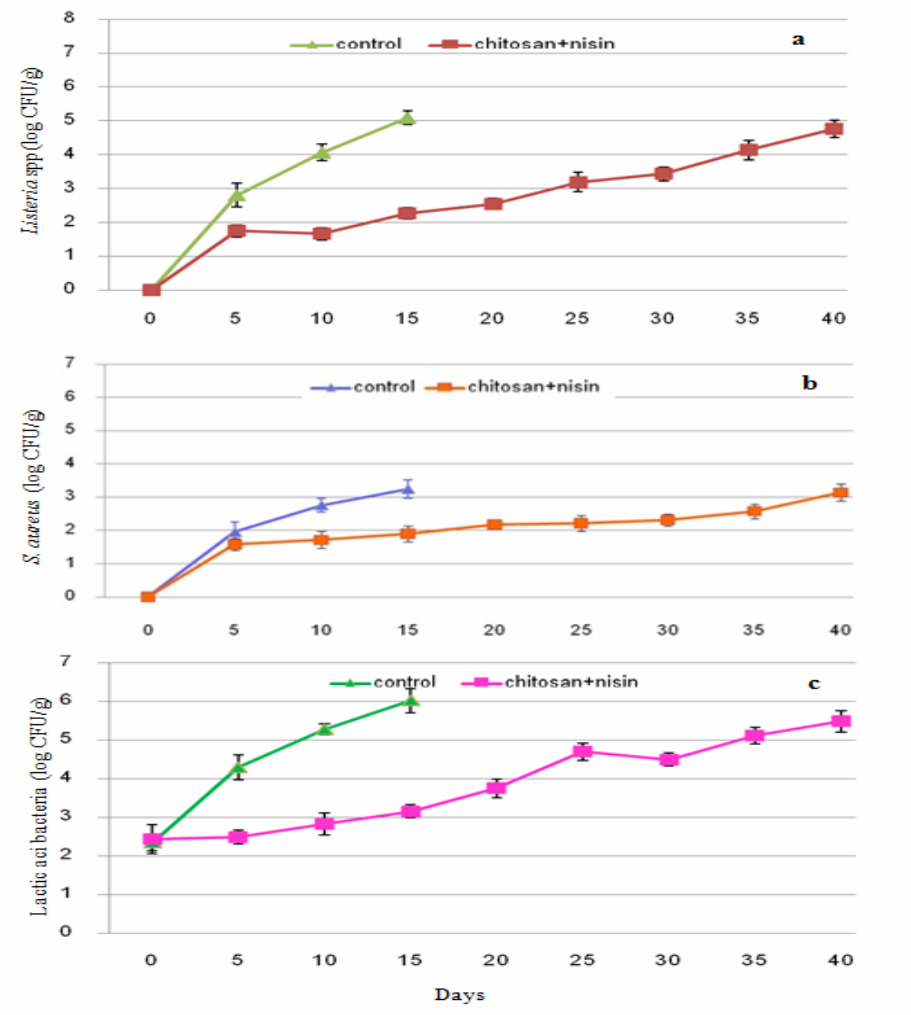

Figure 4 Number of (a) *Listeria* spp. (b) *S. aureus* (c) lactic acid bacteria in control smoked giant perch and treatment sample wrapped with CNF in LDPE bag stored at 4 ± 1 °C

In Figure 4a-4c it can be seen that CNF can inhibit the growth of all tested microorganisms i.e. *Listeria* spp, *S. aureus* and lactic acid bacteria over storage time of 40 days while control sample have shelf-life only 15 days. Therefore, CNF has proven to be used effectively in smoked giant perch product from Thailand. *Listeria* spp., *S. aureus* and lactic acid bacteria are natural spoilage microorganisms that can be found on the fish. After processing and cold smoking of fish, number of spoilage microorganisms was decreased due to dead or injured cell. Therefore, on the beginning of storage the total number of *Listeria* spp. and *S. aureus* are lower than 30 colonies per gram sample which can be reported as zero log CFU/g. However, when storage time is increased, *Listeria* spp. and *S. aureus* cell can recover from injured and starting to increase in number of population when they are in appropriate environment.

4. CONCLUSION

Edible films added with antimicrobial agents are able to extend shelf-life of smoked giant perch product from Thailand when stored in LPDE bag at 4 ± 1 °C. The best performance film is chitosan film in-cooperated with nisin. It can prolong shelf-life of smoked giant perch for 40 days followed by carragenan film in-cooperated with cinnamon (30 days) and pectin film in-cooperated with Thai herb (25 days). Chitosan film in-cooperated with nisin can retard the growth of total plate count, Lactic acid bacteria, *Listeria* spp. and *Staphylococcus aureus* during storage for 40 days.

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SWEET SORGHUM USED AS FEEDSTOCK FOR BIOETHANOL PRODUCTION

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ABSTRACT

In recent years, research and development efforts directed toward commercial production of ethanol as the most promising biofuel from renewable resources have increased. In many countries in Europe the use of bioethanol as an alternative fuel or a gasoline supplement in amounts up to 15% is highly recommended. Fuel ethanol production has increased remarkably because many countries look for reducing oil imports, boosting rural economies and improving air quality. The world ethyl alcohol production has reached about 51,000 million litters, being the USA and Brazil the first producers. On average, 73% of produced ethanol world-wide corresponds to fuel ethanol, 17% to beverage ethanol and 10% to industrial ethanol. The sweet sorghum contains sufficient quantities of sucrose, glucose, hemicellulose and fermentable sugars and can serve as an ethanol feedstock. Fresh juice extracted by pressure from 9 sweet sorghum varieties as well as whole stalks was fermented by *Saccharomyces cerevisiae* var. *bayanus* for bioethanol production. The juice was used directly and whole stalks after previous acidic hydrolysis. The extracted juice varied from 38.7 to 55.4% of fresh stalks of sweet sorghum and contained 8.9% to 16.4% fermentable sugars. The results showed that the highest ethanol production was 9.57 gL^{-1} and 14.35 gL^{-1} ethanol from the fermentation of juice and whole stalks, respectively. The ethanol yield was high and corresponds up to 84% of the maximum theoretical yield for the fermentation of juice and 93% for the whole stalks.

Keywords*:* bioethanol; sweet sorghum; *Saccharomyces cerevisiae*; fermentation.

1. INTRODUCTION

In recent years, research and development efforts directed toward commercial production of ethanol as the most promising biofuel from renewable resources have increased. In many countries in Europe the use of bioethanol as an alternative fuel or a gasoline supplement in amounts up to 15% is highly recommended (Mojovic, Nikolic, Rakin and Vukasinovic, 2006). If EUregulations are adopted in light of the Kyoto agreement, the mandatory blending of bioethanol with traditional gasoline in amounts up to 10% will result in requirements of large quantities of bioethanol. Many countries have implemented or are implementing programs for addition of ethanol to gasoline. Fuel ethanol production has increased remarkably because many countries look for reducing oil imports, boosting rural economies and improving air quality. The world ethyl alcohol production has reached about 51,000 million liters, with the USA and Brazil being the first producers. In average, 73% of produced ethanol world-wide corresponds to fuel ethanol, 17% to beverage ethanol and 10% to industrial ethanol (Sanchez, and Cardona, 2008).

The EU directive for bio-ethanol requires from member states to establish legislation about utilization of fuel from renewable resources. In 2005, this utilization should cover 2% of the total fuel consumption. This quota is expected to increase to 5.75% in 2010 and furthermore (Berna, 1998). In EU the annual bio-ethanol production was 6×10^6 m^3 at 2006 and should be 11.8×10^6 m^3 at 2010.

Among the bioenergy crops used for fuel ethanol production, sugarcane is the main feedstock utilized in tropical countries like Brazil and India. In North America and Europe, fuel ethanol is mainly obtained from starchy materials, especially corn. It is estimated that the feedstock (in the case of corn) alone is already almost half of the total production costs (46%). This is based on total estimated cost of USD 0.40/L of ethanol produced. On the other hand, intensive research on the utilization of lignocellulosic biomass as feedstock is going on during the last years. Any further increases in ethanol production will have to come from feedstocks other than corn grain because of limitations in supply. These feedstocks are typically grouped under the heading of “biomass” and include agricultural residues, wood, municipal solid waste and dedicated energy crops.

The varied raw materials used in the production of ethanol via fermentation are conveniently classified into the three main types of raw materials: sugars, starches, and cellulose materials. Sugars (from sugarcane, sugar beets, sweet sorghum, molasses and fruits) can be converted into ethanol directly (Liu and Shen, 2008). Starches (from corn, cassava, potatoes, and root corps) must firstly be hydrolyzed to fermentable sugars by the action of enzymes from malt and molds. Cellulose (from wood, agricultural residues, waste sulfite liquor from pulp, and paper mills) must likewise be converted into sugars, generally by the action of mineral acids. Once simple sugars are formed, enzymes from microorganisms can readily ferment them to ethanol (Lin and Tanaka, 2006). As for materials, one of the prime sources being investigated for ethanol is sweet sorghum.

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a high biomass and sugar-yielding crop (Bryan, 1990). In the meantime, the stalk of sweet sorghum contains quite a few quantities of soluble (glucose and sucrose) and insoluble carbohydrates (cellulose and hemicelluloses) and has been considered as an important source for the production of fuel ethanol (Jasberg et al., 1983; Mamma, Christakopoulos, Koullas, Kekos, Makris and Koukios, 1995). The sugar content varies with different years, soil conditions and sweet sorghum varieties. Therefore, of many crops currently being investigated for energy and industry, sweet sorghum is one of the most promising, particularly for ethanol production (Gnansounou, Dauriat and Wyman, 2005).

Sweet sorghum can be cultivated from Northern to Southern Greece in any type of soil. The sugar ratio range to 9-13.2 % of net weight and the yield comes up to 1.2 tonnes/1000 sq. meters. In recent years there is an increase of cultivable area with sorghum (+15.6% from 2005 to 2006) with production 400 000 tonnes in this year.

In this paper extracted juice as well as whole stalk of eight varieties of sweet sorghum (*Sorghum bicolor* (L.) Moench) were fermented by *Saccharomyces cerevisae var. bayanus* to determine fermentability and ethanol production.

2. MATERIALS AND METHODS

Seven Sweet sorghum cultivars, harvested in November 2007, were kindly provided by the Agricultural Research Station of Palamas, Carditsa - Greece. Leaves were stripped from the

fresh stalks by hand and stored in the freezer at -20°C. Following thawing at room temperature, the stalks were milled to small particles (approx. 0.5 cm long) in a laboratory mill and subjected to 70 bar pressure in a hydraulic press to extract the juice.

2.1 Microorganism

Saccharomyces cerevisae var. *bayanus* was used for the fermentation of sorghum juice and hydrolyzed stalks. It was maintained on a malt agar slant at 4°C. The agar slant consisted of malt extract (3 gL^{-1}), yeast extract (3 gL^{-1}), peptone (5 gL^{-1}), agar (20 gL^{-1}) and distilled water (up to 1 L). For the inoculums, the culture was grown aerobically in 250 mL flasks in a shaking bath at 32^{o}C for 48 h. The pH was adjusted to 5.0. The liquid media was made of yeast extract (3 gL^{-1}), peptone (3.5 gL^{-1}), KH_2PO_4 (2 gL^{-1}), $MgSO_4$ $7H_2O$ (1 gL^{-1}), $(NH_2)_2SO_4$ (1 gL^{-1}), glucose (10 gL^{-1}) and distilled water. Six per cent of inoculums were used for the fermentation.

2.2 Fermentation of extracted juice

In a 250 mL Erlenmeyer flask with fermentation trap (Figure 1) we added 25 ml of extracted juice and 125 mL H_2O, 0.3% (w/v) NH_4NO_3 and 0.2% (w/v) Peptone. The mixture was sterilized at 121^{o} C for 15 min. After sterilization, the mixture was inoculated with *Saccharomyces cereviciae* var. *bayanus* under anaerobic conditions (pH 5.0, 100 rpm, 32^{0}C) and the fermentation was carried out for three days.

2.3 Acidic hydrolysis of stalks

In an 250 mL Erlenmeyer with fermentation trap (Figure 1) we added 5 g dry milled stalks of sweet sorghum, 0.3% (w/v) NH_4NO_3, 0.2% (w/v) Peptone and 120 mL HCl 0.5M. The mixture was sterilized at 121^{o}C for 15 min. During sterilization the carbohydrates, due to acid hydrolysis, were transformed to fermentable sugars. After sterilisation the pH was corrected to 4.15 with NaOH (1 M). Carbohydrate hydrolyzates obtained by the acidic hydrolysis were subjected to ethanol fermentation with inoculums a 48 hours old culture 6% (v/v) of *Saccharomyces cereviciae* var. *bayanus* under anaerobic conditions (32^{0}C, pH 5.0, 100 rpm). The fermentation was carried out for three days.

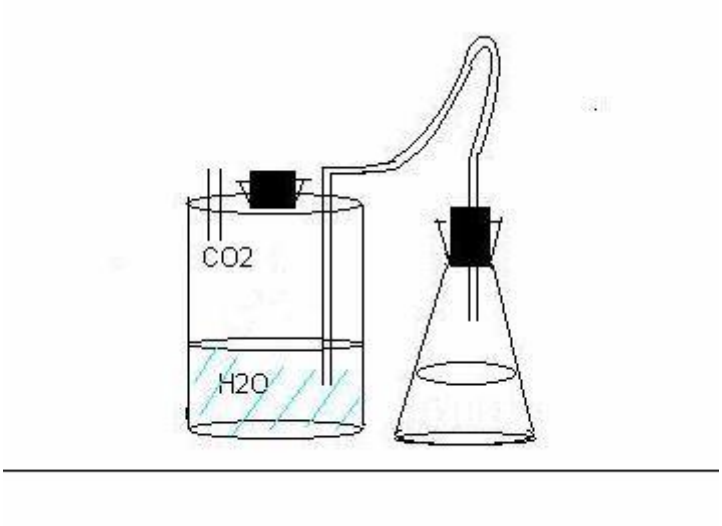

Figure 1: Laboratory set up for sweet sorghum ethanol fermentation with a trap.

2.4 Methods of analysis.

Ethanol was measured with the Böehringer Mannheim/R-Biopharm, Kit. Cat. no 10 176 290 035. The degree of polysaccharide degradation was estimated by quantifying the amount of total sugars consumed during fermentation. Total sugars were determined as glucose, by the method described by Dubois et al., 1956 at an optical density of 485 nm.

Two experiments were run under the same conditions and mean values of three replicates have been calculated.

3. RESULTS AND DISCUSSION

Table 1 shows the moisture of eight varieties of sweet sorghum as well as the percentage of extracted juice and total sugar of extracted juice. The moisture ranged from 63% (H133) to 88% (No3) and the per cent of extracted juice from 38.7% (H133) to 55.3% (No 3 and 5). The total sugar content of extracted juice differed according to the varieties. Among the varieties, No5 showed the lower (8.9%) and H133 the highest (16.4%) total sugar content. It is clear that if a variety has low moisture (H133), we extract lower quantity of juice but with high content of sugar.

Table 1. Moisture, extracted juice as per cent of whole fresh material and total sugar of juice, of eight tested sweet sorghum varieties.

Varieties	Moisture %	Extracted Juice (% v/w)	Total Sugar of Juice % (g/v)
No 1	86.32	45.85	11.35
No 2	69.20	46.60	12.54
No 3	88.08	55.33	12.96
No 4	69.90	52.63	14.70
No 5	74.26	55.36	8.89
No 6	69.95	47.15	13.56
No 7	70.66	42.64	8.93
H133	63.76	38.72	16.39

3.1 Fermentation of extracted juice.

The fermentation parameters of sweet sorghum juice with *S. cerevisiae* var. *bayanus* for ethanol production are given in Table 2.

Table 2. Fermentation parameters of sweet sorghum juice with *S. cerevisiae* var. *bayanus* for ethanol production.

Varieties	Total Sugar Initial gL^{-1}	Total Sugar Final gL^{-1}	Total Sugar Consumption (TSC) gL^{-1}	Ethanol Produced gL^{-1}	Ethanol Yield g EtOH / g TSC	% of max theoretical yield
No 1	18.923	1.084	17.839	5.295	0.297	59.36
No 2	20.893	1.690	19.203	8.143	0.424	84.81
No 3	21.596	1.707	19.889	7.288	0.366	73.29
No 4	24.500	1.601	22.899	8.739	0.382	76.33
No 5	14.821	0.850	13.971	5.627	0.403	80.55
No 6	22.604	2.246	20.358	7.180	0.353	70.54
No 7	14.881	1.276	13.605	4.611	0.339	67.79
H133	27.316	2.642	24.674	9.566	0.388	77.54

The initial total sugar concentration of juices for the fermentation with *S. cerevisiae* var. *bayanus* varied from 14.8 to 27.3 gL^{-1} (No5, 7 and H133, respectively). At the end of fermentation the total sugar concentration was very low (about 1-2 gL^{-1}) and the total sugar consumption (TSC) during fermentation was very high.

The production of ethanol ranged from 4.6 gL^{-1} (No7), to 9.6 gL^{-1} (H133). The respective ethanol yield $Y_{p/S}$ (g of product / g of sugar consumed) ranged from 0.297 (or 29.7%) for No1, to the highest 0.424 (or 42.4%) for No2. These yields correspond to a 59.4% of the max theoretical yield for No1 and 84.8 for No2. These results show that fermentation of sweet sorghum juice with yeast *S. cerevisiae* was efficient, but it could be improved (higher conversion of sugar to bioethanol).

3.2 Fermentation of sweet sorghum stalk.

The sorghum stalk contained both soluble sugars and polysaccharides. The fermentation of both carbohydrates to ethanol was affected by an acidic hydrolysis. The results at Table 3 show sugar consumption during fermentation as well as ethanol production and yield. After initial chemical hydrolysis the total sugars ranged from 24.2 gL^{-1} (No2) to 33.6 gL^{-1} (H133).

Table 3. Fermentation parameters of sweet sorghum stalk with *S. cerevisiae* var. *bayanus* for ethanol production.

Varieties	Total Sugar Entered	Total Sugar Final	Total Sugar Consumption (TSC)	Ethanol	Ethanol Yield	% of max theoretical yield
	gL^{-1}	gL^{-1}	gL^{-1}	gL^{-1}	g EtOH / g TSC	
No 1	28.267	1.388	26.879	8.925	0.332	66.41
No 2	30.973	1.850	29.124	12.215	0.419	83.88
No 3	24.236	1.476	22.760	10.632	0.467	93.43
No 4	30.932	1.701	29.230	13.109	0.448	89.69
No 5	26.432	1.928	24.504	9.405	0.384	76.76
No 6	30.273	2.051	28.222	10.770	0.382	76.32
No 7	29.894	1.364	28.530	8.565	0.300	60.04
H133	33.647	2.513	31.134	14.349	0.461	92.18

After three days of fermentation practically all total sugars were fermented (final concentration about 2 gL^{-1}). The lowest ethanol production was 8.6 gL^{-1} for No7 and the higher was 14.35 gL^{-1} for H133. The respective ethanol yield $Y_{p/S}$ (g of product / g of sugar consumed) ranged from 0.3 (or 30%) for No7, up to 0.46 (or 46%) for No3 and H133. These yields correspond to a range of the max theoretical yield from 60% for No7 to 93.4% for No3.

4. CONCLUSIONS

The fermentation of extracted juice and hydrolyzed stalk from eight sweet sorghum varieties by *Saccharomyces cerevisae* var. *bayanus* for ethanol production was studied. There was great variation in ethanol production among different varieties. Ethanol production was at high levels in most of the varieties. Whole stalks produced more ethanol than juice.

Variety No H133 showed the highest ethanol production and yield from stalk hydrolyzates, compared to the rest. Additionally it showed the highest ethanol production, and one of the highest ethanol yields, from extracted juice. This variety had low ratio juice per stalk and the highest total sugar concentration. On the other hand, the variety with the minimum ethanol yield was No1 in both juice and stalk fermentation.

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POSSIBILITIES FOR CHARACTERIZATION OF HONEYS ANTIOXIDATIVE PROPERTIES

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ABSTRACT

The purpose of the research was to describe antioxidative characteristics of the honey following by total content of phenolic compounds, antiradical scavenging activity and intensity of honey's colour.

The total content of phenolic compounds in the analyzed samples of the honey was determined spectrophotometrically. The total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

The antiradical scavenging activity of the honey was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl). The results of the honeys antiradical scavenging activity were figured as the broken down quantity of DPPH on 100 grams of the honey.

The intensity of the honey's colour samples was determined spectrophotometrically, following by Pfund scale. Also, colour of the honey samples was evaluated by measuring CIE L*, a*, and b* parameters by means of ''ColorTec–PCM/PSM''.

The total content of phenols in the samples of the honey against the equivalent of gallic acid ranges from 35 to 115 mg GAE/ 100g of honey. Antiradical scavenging activity of the honey was found to be 120 μmol on 100 g of the honey. The intensity of the honey's colour, following by Pfund scale, ranges from 11-160 mm. Also, there has been ascertained the correlation between content of the honey's total phenolic compounds and intensity of honey's color.

Keywords: Honey, colour, phenolic coumponds.

1. INTRODUCTION

Honey is one of the oldest and widely used food products. It contains approximately 400 compounds. The main ingredients of honey are sugars (fructose ≈ 38 %, glucose ≈31 % and

sucrose not more than 5 %), water amount is less than 20 %, while the acids are approximately 0,08 %, and the content of mineral substances are approximately 0,18 %.

Honey also contains a wide variety of other substances in small concentrations. There include various phenol acids, flavonoids, aminoacids, enzymes, proteins, etc. Content of honey components varies depending on many factors, such as pollen in honey and also from climate, environment and honey processing (Belitz et al., 2009; Kaškoniene et al., 2009; Ouchemoukh et al., 2007).

Antioxidants are known for their ability to control many functions in human body. Antioxidants participate in regulation of oxidative processes of lipids, proteins and nucleic acids.

As a result of oxidative process free radicals are formed in human body. These radicals are important in process of respiration and metabolism. They eliminate foreign bacteria as well.

Nevertheless if antioxidative properties of body are low, the free radicals can be produced in excessive amount and create some medical problems. But antioxidants can control the formation of free radicals in organism (Кочикян et al., 2009; Макарова and Лиманова, 2009).

By the colour of honey, partly we can estimate what antioxidative properties and many other connections it contains. The colour of honey, beside flavour and aroma, is one of the characteristics that serve to indicate the plant source. It ranges from very pale yellow through amber and dark reddish amber to nearly black. It is related to the content of minerals, pollen and phenolics, and is characteristic of floral origin. Darkening of honey during storage may occur because of Maillard reactions, fructose caramelization and reactions of polyphenols. The degree of darkening depends on the temperature and/or time of storage. However, heating treatments darken honeys, modifying the initial colour. Minerals are among the components related to honey colour, and phenolic compounds, associated to its antioxidant activity, were also found to be positively correlated to colour (Caivano and Buera, 2012; Bertoncelj et al., 2007).

The purpose of the research was to describe antioxidative characteristics of the honey following by total content of phenolic compounds, antiradical scavenging activity and intensity of honey's colour. Also, has been determined individually existing polyphenols in the content of the honey: 4 – hydroxybenzoic acid, rutin, gallic acid, catechin, caffeic acid, syringic acid, chlorogenic acid, epicatechin, coumaric acid, sinapic acid and ferulic acid.

2. MATERIALS AND METHODS

2.1. Chemicals and instruments

All of the chemicals and reagents used were of analytical grade. DPPH 2,2-diphenyl-1-picrylhydrazyl), gallic acid were purchased from Sigma, Folin-Ciocalteu's reaġent, Na_2CO_3, CH_3OH, CH_3COOH were purchased from Merck.

UV–Vis spectrophotometer (JENWAY 6405 UV/VIS) was used for absorbance measurements and a Minolta Color of the honey samples was evaluated by measuring CIE L*, a*, and b* parameters by means of ''Color Tec–PCM/PSM''.

2.2. Samples

It has been analyzed more than 20 honey samples, collected at area of Daugmale farmers farm "Dorites". The honey was harvested in the season of 2012. The honey samples were taken from different places of mentioned area (Daugmale).

The honey samples were classified into 12 groups, phacelia (Phacelia tanacetifolia Benth), buckwheat *(Fagopyrum esculentum)*, lime *(Tilia cordata)*, wild, fire wead *(Chamaenerion angustifolium)*, heather *(Calluna vulgaris)*, gray willow *(Salix cinerea L.)*, various flower, spring flower, rape *(Brassica napus L.)*, hogweed *(Heracleum sosnowsky)*, red raspberry *(Rubus idaeus)*.

Honey samples were stored at 4 °C in the dark until analysed.

2.3. Methods

2.3.1. Total phenolic content

The total phenolic compounds were determined by using the Folin – Ciocalteu method. Each honey sample was dilute with distilled water and filtered. This solution was than mixed with 0,2 N Folin – Ciocalteu reagent for 5 min and then solution of sodium carbonate was added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against methanol blank. Gallic acid was used as standart to produce the calibration curve. The total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey (Kaškoniene et al., 2009).

2.3.2. Antiradical scavenging activity

The antiradical scavenging activity of honey was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl). Honey sample was dissolved in methanol and fixed volume of each honey sample solution was mixed with fixed volume of DPPH solution in methanol. The mixtures were left for 15 min at room temperature and then the absorbance was measured at 517 nm.
As mentioned before, make test analysis using methanol instead of honey solution.
Knowing the DPPH solution and sample solution concentration and use of analysis sample and reagent volumes, as well as light absorption readings calculated to break down DPPH μmol amount per 100 g of honey, considering the control solution readings.

2.3.3. Colour analysis

Honey samples were heated up to 40 °C to dissolve sugar crystals, and the colour was determined by spectrophotometric measurement of the absorbance of a 50 % honey solution at 635 nm. The honeys were classified according to the Pfund scale after conversion of the absorbance (A) values: mmPfund = -38.70 + 371.39 X A.
Colour of the honey samples was evaluated also by measuring CIE L*, a*, and b* parameters by means of "Color Tec–PCM/PSM". L*, a*, and b* indicate whiteness/darkness, redness/greenness, and blueness/yellowness values, respectively. The maximum value for L* is 100, which would be a perfect reflecting diffuser. The minimum for L* would be zero, which would be black. The values of a* and b* axes have no specific numerical limits. Positive a* is red and negative a* is green. Positive b* is yellow and negative b* is blue.

2.4. Statistical analysis

All analyses were carried out in triplicate and the data were expressed as means ± standard deviations (SD). Correlation analysis between parameters of honey colour and total content of phenols was carried out following by SPSS 12.0 and MS Excel offered options. The statistical analysis of data was carried out following by 0.05 criterion of significance level.

3. RESULTS AND DISCUSSION

When analyzing honey samples, the total phenolic compound concentrations were found to be 115 mgGAE/100g, but minimum: 35 mgGAE/100g of honey. The highest phenolic compounds observed to heath-bell honey samples, which range from 105 to 110 mgGAE/100g. A rather high phenolic compound exists in wild flower honey. It ranges

from 85 to 90 mgGAE/100g. Honey samples of rapeseed flowers have the lowest phenolic compounds, which is from 45 to 48 mgGAE/100g of honey. Very broad range is for lime honey samples: 36 - 98 mgGAE/100g. The figures are average for all other honey samples.

By Pfund scale honey colour ranges from 12 to 160mm.

While statistical analysis of data was carried out, observed relatively good correlation between total phenolic compounds in the honey and honey's colour.

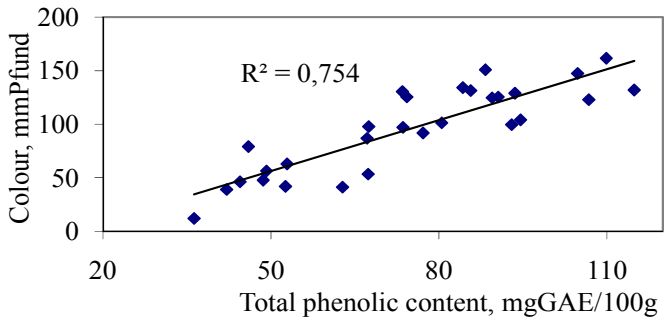

Fig. 1. Correlation between total phenolic content and colour of honey.

As can be seen from the Figure 1, honey darkens when total phenol content increases in the honey.

Colour characteristics were assessed also by the CIE L*a*b* method where lightness L* and two colour coordinates, a* and b*. Trend for corellation has been observed between honey brightness parameter L.

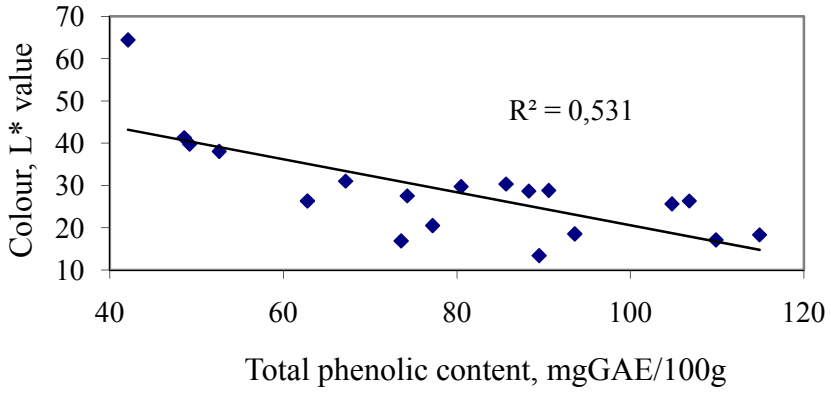

Fig. 2. Correlation between total phenolic content and parameter L* of honey colour.

As can be seen from the Figure 2: as honey gets lighter, as less phenolic compounds it contains.

There also were analyzed several honey samples of one type, which were taken from the same region, but different collection areas. Therefore a* and b* usability option of colour parametres has been evaluated to characterize biological (floral) origin of honey.

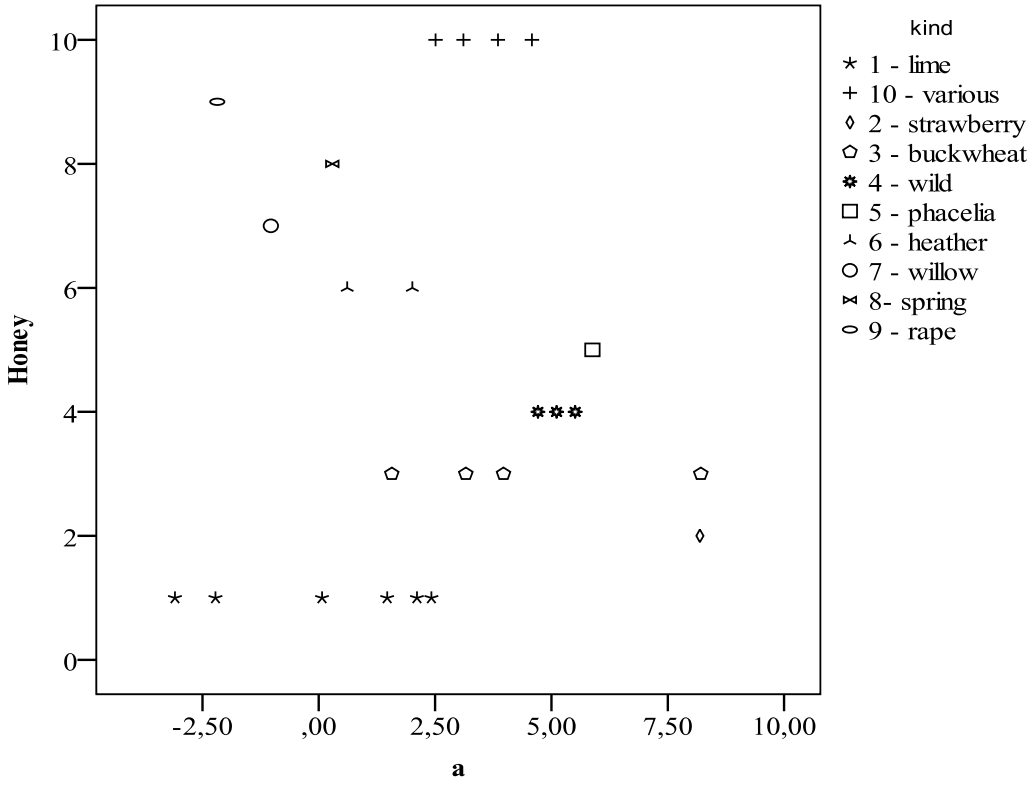

Fig. 3. Parameter a*display of honey colour.

As can be seen from the Figure 3, both red and green tones are typical to all honey samples (negative a* parameter display). Completely different display of color a* parameter are to lime flower and wild flower honey samples. Whereas buckwheat flowers honey samples parameter a* display mostly vary from lime flower honey, this display of parameter partly overlaps parameter a*display of lime flower honey.

Data dispersal of parameter a*display of honey colour shows that it is not practically possible to collect 100 % pure honey of same sort biological origin. Certain types of honey always will be slightly blended with other types of honey, therefore observed higher or lower data dispersion. Likewise, there are several varieties of honey, which shade is quite similar.

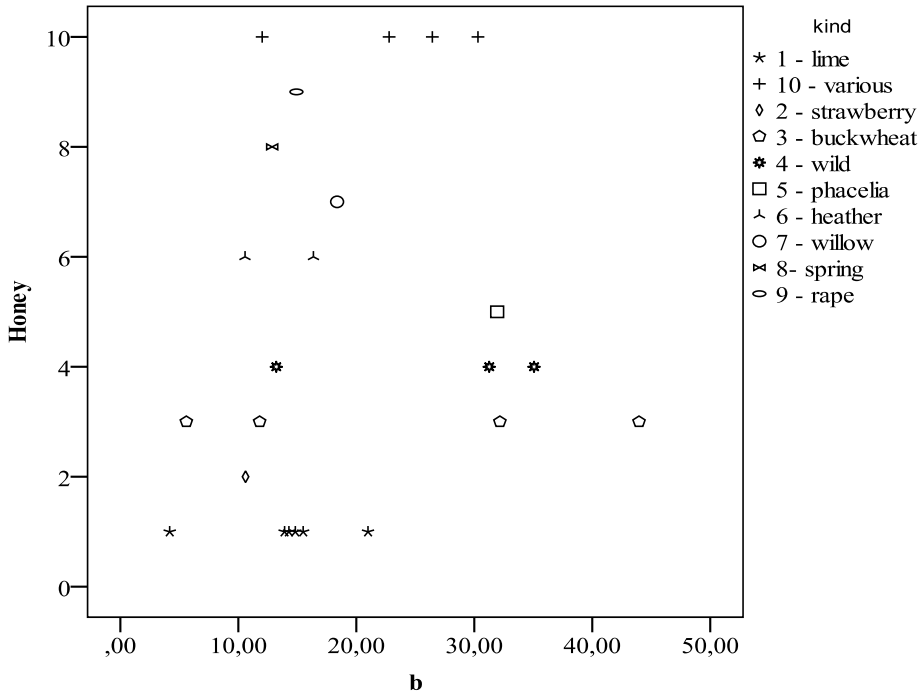

Fig. 4. Parameter b*display of honey colour.

Data of parameter b* shows, that honey also is characterized by the colour yellow tones.

Following by assessment of data of parameter b* display of honey colour, it is not possible to identify some specific type of honey , whose data of parameter b* would be different in comparison with data of other types of honey.

However, due to data of parameter a* of honey colour, it is possible to make an assumption, that parameter a* of honey color for some honey varieties are significantly different. This was also proved by K-Means Cluster analysis.

As it can be seen from Table 1 of cluster analysis, then some honey varieties can be dividend into groups. Mainly single in the cluster is lime flower honey. Wild and buckwheat honey samples are divided between clusters 2 and 3. Wild flower honey primarly is in the cluster 2, but buckwheat flower honey belongs to both: 2 and 3. In turn, as various flower honey is made from different varieties of honey, then these samples can be found in the all cluster groups.

TABLE 1 K Means Cluster analysis of honey samples

Nr.	Honey	Cluster	Interval	Nr.	Honey	Cluster	Interval
1	lime	1	3.429	13	buckwheat	2	5.424
2	strawberry	2	3.624	14	heather	2	4.158
3	buckwheat	2	2.209	15	lime (4)	1	2.281
4	wild	3	2.186	16	rape	1	2.723
5	phacelia	3	2.231	17	buckwheat	3	1.970
6	heather	1	1.959	18	lime (5)	1	3.836
7	wild	3	2.116	19	lime (6)	2	5.973
8	wild	2	3.598	20	buckwheat	3	2.512
9	willow	1	2.181	21	various	1	6.996
10	lime (2)	1	2.180	22	various	3	3.058
11	spring	1	3.625	23	various	3	6.609
12	lime (3)	1	4.513	24	various	2	2.447

Final cluster centers

	Cluster		
	1	2	3
a*	0.05	4.68	3.93
b*	16.49	9.72	33.03

For the analyzed honey samples have also defined antiradical activity.

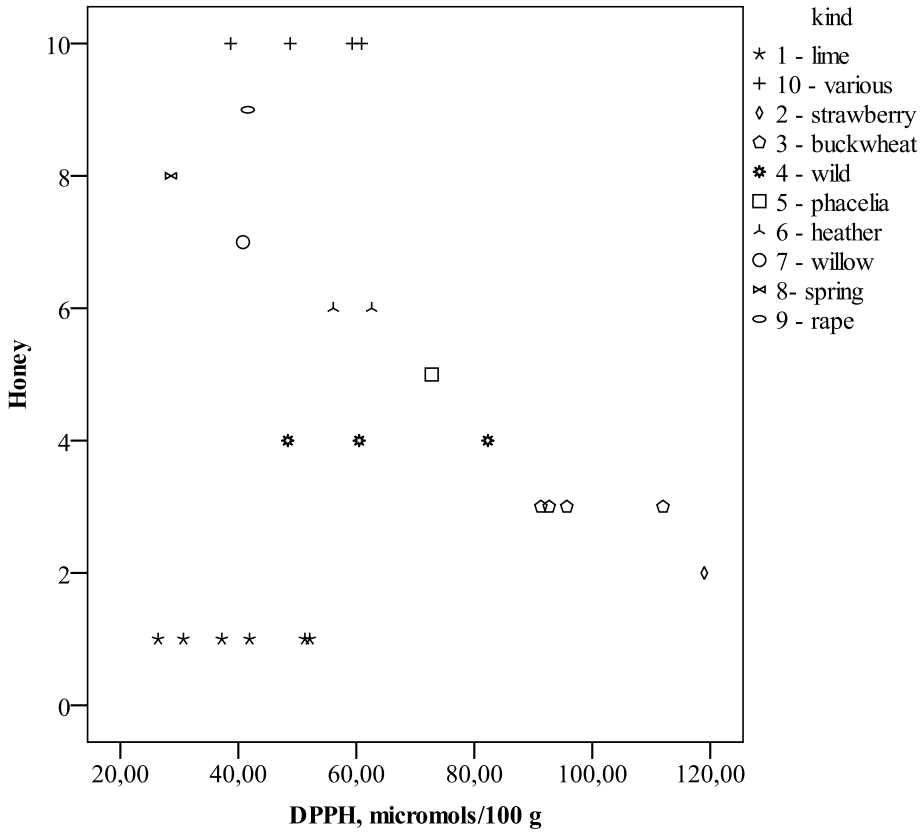

Fig. 5. Broken down DPPH of honey samples.

As can be seen from the data in the Figure 5, antiradical activity of honey after broken down DPPH (2,2-diphenyl-1-picrylhydrazyl) quantity is 120 μmol/100g of honey.

The figure is different for some specific sorts of honey. For example, in separate groups are eliminable lime and buckwheat flower honey. Antiradical activity is significantly higher for buckwheat flower honey. Partially in separate groups are eliminable heather and wild flower honey samples. In order to determine wheather floral (botanical) origin play a role with regards to honey antiradical activity, the K-Means Cluster analysis has been completed.

TABLE 2

K Means Cluster analysis of honey samples

Nr.	Honey	Cluster	Interval	Nr.	Honey	Cluster	Interval
1	lime	1	14.142	13	buckwheat	2	9.860
2	strawberry	2	16.860	14	heather	3	2.329
3	buckwheat	2	9.440	15	lime	1	11.558
4	wild	3	17.371	16	rape	1	1.058
5	phacelia	3	7.871	17	buckwheat	2	6.440
6	heather	3	8.829	18	lime	1	9.842
7	wild	3	4.429	19	lime	1	3.342
8	wild	1	7.858	20	buckwheat	2	10.840
9	willow	1	1.258	21	various	1	1.842
10	lime	1	1.358	22	various	1	8.258
11	spring	1	11.942	23	various	3	4.029
12	lime	1	10.758	24	various	3	5.629

Final cluster centers

	Cluster		
	1	2	3
DPPH	40.54	102.14	64.93

Table 2 Cluster analysis data confirms, that it is possible to separate in groups antiradical activity after broken down quantity of DPPH for the following honey samples: lime flower, buckwheat and wild flower. Thus lime flower honey is in the cluster 1, buckwheat flower honey is the cluster 2 and wild flower honey mostly is in the cluster 3, where also is heather flower honey. Therefore it may hypothesise, that it is possible to judge the potential varieties of honey samples by parameter a* of honey colour and broken down quantity of DPPH.

4. CONLUCIONS

From the results and their analysis showed, that there is a correlation between the colour of honey and total phenolic compound content in the honey. The higher phenolic compound

content, the darker the honey shade. There is evidence that honey colour assessment parameter a* values are partly dependant on floral (botanical) origin. The antiradical activity of honey is dependant on floral (botanical) activity partially. Increased phenolic compound content is typical for heather honey and wild flower honey.

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GASSY OZONE APPLICATION FOR HULL-LESS BARLEY AND TRITICALE GRAIN TREATMENT

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ABSTRACT:

Ozone as an oxidant has numerous potential applications in the food industry because of its advantages over traditional food preservation techniques. Aim of the present research was to investigate the effect of gassy ozone on microbiological, chemical and biological parameters of hull-less barley and triticale grains. Grain quality parameters using standard methods were evaluated: protein, starch, β-glycan, moisture, yeasts, moulds, mycotoxins, germinating power, and starch granules volume. In present experiment was found, that there are no significant differences in albumen and starch content in analysed non-treated hull-less barley and triticale grains. The microflora of non-treated triticale grains was significantly different from non-treated hull-less barley grain microflora: yeasts were by 97% and moulds by 83% less. Optimal grain processing parameters with ozone were developed for triticale grains – 3.92 mg m^{-3} 8 min, for hull-les barley grains – 11.76 mg m^{-3} 6min. During present research it was detected, that ozone concentration and treatment time not significantly influence changes in grain protein, moisture, starch and β-glycan content. Ozone concentration and treatment time significantly influence grain germinating power: the germination power of processed triticale grains increased 15% after three days germination and 7% after five days; the processed hull-less barley grains increased 11% after three days of germination and 9%after five days. Ozone significantly influence starch granules volume changes – its increase. The amount of mycotoxins before and after grain treatment with gassy ozone was under detection limit.

Keywords: gassy ozone, hull-less barley, triticale, treatment.

1. INTRODUCTION

Cereal-based foods have been staples for humans for millennia. Cereal grains contain the macronutrients (protein, fat and carbohydrate) required by humans for growth and maintenance. They also supply important minerals, vitamins and other micronutrients essential for optimal health. However, it is becoming apparent that cereals in general have the potential for health enhancement beyond the simple provision of these nutrients and that their consumption can lower the risk of significant diet-related diseases quite substantially (Topping, 2007). Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop produced in the world. Barley grain is an excellent source of soluble and insoluble dietary fibre and other bioactive constituents, such as vitamin E (including tocotrienols), B-complex vitamins, minerals, and phenolic compounds. β-glucans, the major fibre constituents of barley, have been implicated in lowering plasma cholesterol, improving lipid metabolism, and reducing glycaemic index (Izydorczyk and Dexter, 2008). In food industry, hull-less barley is considered as more valuable and more economical compared to flaky barley. The hull-less barley flour has a little darker colour, because compared to flour from soft wheat it has a higher ash value, and a higher protein and β-glucans content. Soluble dietary fiber, mainly β-glucans, provides a promoted viscosity. As a result, digestion, cholesterol and fat absorption are decreased. Compared to wheat and rye grain, the highest content of natural antioxidants (tocotrienols) and of vitamin E were established in barley grain. Protein content in hull-less barley grain is from 9 to 20% from total dry matter (Rakcejeva et al, 2007). Triticale (*Triticosecale*) is a man-made hybrid of wheat and rye that carries the A and B genome of durum wheat and the R genome of rye. Triticale is intended to combine the high yield potential and good grain quality of wheat with the disease resistance and environmental tolerance of rye, including winter hardiness. It can be grown in poor soils, giving high yield where wheat does not perform well. Triticale is mainly used as an ingredient in animal feed, but also on a smaller scale as a food ingredient, for example in bread or as a replacement for soft wheat in biscuits, cakes, and cookies (Rakha et al, 2011). Main grain processing product is flour. Flour is generally regarded as a microbiologically safe product as it is a low water activity commodity. Although the growth of pathogenic bacteria may not be supported under such conditions, pathogens that contaminate flour may survive for extended periods. There are few reported incidents of food poisoning resulting from contaminated flour. Australian, European and US studies indicate that *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* and

spoilage microorganisms are present in wheat and flour at low levels (Berghofer, 2003). Ozone (O_3) is a strong oxidant recognized since 1997 as a generally recognized as safe substance and used in a number of applications in the food industry for destruction or detoxification of chemicals or microorganisms. These applications include the surface decontamination, storage and preservation of perishable foods as well as water or manufacturing equipment and packaging sterilization (Desvignes et al., 2008). Ozone in gaseous or aqueous form is reported to reduce levels of the natural microflora, as well as bacterial, fungal and mould contamination in cereals and cereal products, including spores of *Bacillus, Alcaligenes, Serratia, Aspergillus and Penicillium*. Ozone is not universally beneficial and in some cases may promote oxidation degradation of chemical constituents present in the grains. Surface oxidation, discoloration or development of undesirable odours may occur from excessive use of ozone. However, higher ozone concentrations (>50 ppm) cause considerable oxidative damage to cereal grain flours (Tiwari et al., 2010). In a three-year study carried out at a rural site in Switzerland, spring wheat was exposed to different levels of ozone (O_3) in open-top-field chambers from the two-leaf stage until harvest. Grain recovered from the different treatments was analyzed for minerals, starch, protein, amino acids and α-tocopherol, in order to investigate the effect of O_3 on grain composition. As a result no effect of O_3 on the content of α-tocopherol and on the essential amino acid index of the protein was observed. It is concluded that compositional changes in wheat grain in response to O_3 are minor, and that ambient O_3 is not likely to cause important changes (Fuhrer et al., 1990).

After analysing of results from scientific literature the aim of the present research was developed as follow to investigate the effect of gassy ozone on microbiological, chemical and biological parameters of hull-less barley and triticale grains.

2. MATERIALS AND METHODS

Triticale and hull-less barley grains from State Priekuli Breeding Institute (Latvia) harvested in 2012 were used in the experiments.

In current research, a gassy ozone "OZ-15G" ozonizer (China) was used for grain treatment and "IKG-6M" (Russia) measure for the ozone concentration measurement. For grain treatment several gassy ozone concentrations was adopted as 3.92, 7.84, 11.76 and 15.68 mg m^{-3}; treatment time – 4, 6 and 8 minutes.

For measurement of protein, starch, moisture and β-glycan content "Infratec™ 1241 Grain

Analyser" (Denmark) corresponding to ISO 12099 was used.

Grain germinating power test after three and five days germination was realised. Grains (2x100) were placed on Whatman no. 3 filter paper in a 90mm diameter Petri-dish saturated with 5.5 mL of distilled water. The Petri-dishes were covered with a polyethylene bag and kept at 25 °C for 3 and 5 days (Manickavasagan et al., 2007).

Yeasts and moulds content were determined regarding the ISO 21257-2:2008.

Grain microstructure was studied under the triocular microscope Axioskop 40 ('Zeiss'). The pictures of the microstructure were taken with digital camera using the 20 x 40 magnification of the microscope. Starch granules were measured using software Axiovision Le Rel 4.5. Ten measurements were done for starch granule volume. The average values are presented.

Mycotoxins as deoxynivalenol (DON), zeralenone (ZEN) and T2 were analysed using HPLC-MS-MS method.

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (ANOVA) was calculated. Experiments were carried out in fivefold.

3. RESULTS AND DISCUSSION

3.1. Grain chemical parameters

In the present experiments was established, that ozone concentration and treatment time not significantly influence grain chemical parameters changes – protein ($p=0.114$), starch ($p=0.558$), moisture content ($p=0.119$) and β-glycan ($p=0.124$) in analysed grains in general. However, results from scientific literature (Naito, 1989) indicate that ozone have negative influence on grain quality if concentration exceed 98 mg m^{-3}. During research it was found, that in hull-less barley grains albumen content decreased by 4%, moisture by 2% and β-glycan by 12%, what is not significant. However in triticale grains albumen content decrease only by 2%, moisture – by 3%, starch – by 2%, what is not substantially too. For example Desvignes et al. (2008) indicate, that significant differences out between mechanical properties of the aleurone layer isolated from ozone-treated grains compared to untreated grains but could not be detected in the outer layers from which only the outer pericarp was removed. An apparent reduction of the aleurone layer extensibility and an increase in tissue rigidity were observed as shown by the decrease of maximum strain to rupture and the increase of tensile modulus, respectively.

In the present research very similar results were obtained as follow –grain pericarp was

changed during grain processing with ozone (example of triticale grain is shown on Fig. 1).

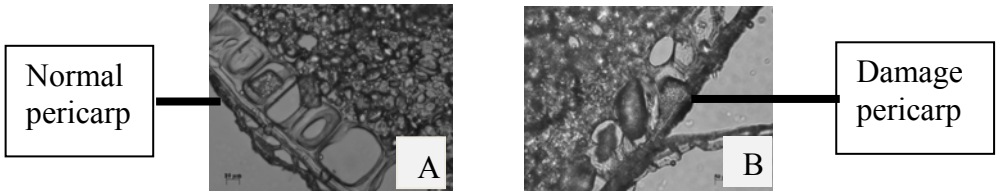

Figure 1 Pericarp of triticale grains
(A – control and B – 15.68 mg m^{-3} 8 min)

The high ozone concentration and the time of treatment negatively influenced the quality of the grain pericarp, what is not acceptable for grain processors. Not significant decreases of starch granules volume was detected if gassy ozone concentration was not higher than 7.84 mg m^{-3} and processing time 4–6 min. Starch granules form become lenticular (Fig. 2). However, if gassy ozone concentration increase from 7.84 to 15.68 mg m^{-3} and grain treatment time is 8 min – starch granules swell up and band together, split and its arrangement become chaotic (Fig. 2).

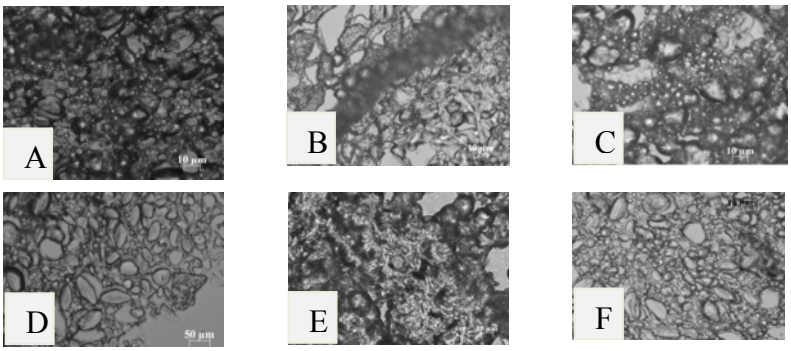

Figure 2 Starch granules of triticale grains
A *– control,* ***B*** *– 3.92 mg* m^{-3} *4 min,* ***C*** *– 11.76 mg* m^{-3} *4 min* ***D*** *– 3.92 mg* m^{-3} *6 min,*
E *– 7.84 mg* m^{-3} *6 min* ***F*** *– 7.84 mg* m^{-3} *8 min*

Main differences were found in grain starch granules volume too (Fig. 2). As a result the high ozone concentration and prolong treatment time negatively influence the quality parameters of grains in general.

3.2. Grain biological parameters

3.2.1. Grain germinating power after three days germination

Non-treated with gassy ozone triticale grain germinating power after three days germination was 67.5±0.5%.

TABLE 1: Grain germination power changes

Gassy ozone concentration, mg m^{-3}	Treatment time, min	Grain germination power, %			
		After 3 days germination		After 5 days germination	
		Triticale	Hull-less barley	Triticale	Hull-less barley
0.00	0	67.5±0.5	73.0±0.5	85.0±0.5	90.5±0.5
3.92	4	72.0±0.5	73.5±0.1	90.0±0.5	92.0±0.5
	6	76.0±0.5	75.5±0.5	90.5±0.1	93.5±0.5
	8	78.0±0.2	70.5±0.4	93.5±0.4	93.0±0.5
7.84	4	65.5±0.4	72.0±0.5	85.0±0.5	94.0±0.5
	6	60.5±0.5	71.5±0.2	88.0±0.8	94.5±0.5
	8	60.5±0.5	70.0±0.5	86.0±0.5	91.0±0.1
11.76	4	62.5±0.4	68.0±0.4	87.0±0.4	93.0±0.1
	6	61.5±0.3	67.0±0.5	86.0±0.5	92.0±0.1
	8	60.5±0.5	66.5±0.1	84.0±0.7	90.0±0.5
15.68	4	62.0±0.5	67.0±0.5	86.0±0.5	91.0±0.5
	6	60.0±0.1	65.0±0.5	86.0±0.5	90.0±0.5
	8	60.0±0.1	62.0±0.5	82.0±0.4	85.0±0.5

Increase of grain germinating power (Table 1) after three days germination was detected if grains were treated with ozone using low concentration (3.92 mg m^{-3}) and short time (4 min) till 72%; treated at mentioned concentration for 8 min – till 78%. Opposite results was obtained for grains treated with higher (from 7.84 till 15.68 mg m^{-3}) ozone concentrations (Table 1). Lower grain germinating power after three days germination was detected in triticale grains treated with 15.68 mg m^{-3} ozone. What mainly could be explained with possible negative chemical reactions in grains influenced by high zone concentration, as a result the grains become lifeless. Non-treated with gassy ozone hull-less barley grain germinating power after three days germination was 73.0±0.5%. It was detected in experiments that high ozone

concentration and longer treatment time negatively influenced grain germination power. Similarly as triticale grain results – lower hull-less barley grain germinating power after three days germination was detected if grains were treated with 15.68 mg m^{-3} ozone concentration (Table 1).

3.2.2. Grain germinating power after five days germination

Hull-less barley grain germinating power after five days germination was 90.5±0.5% what is very similar with results indicated in scientific literature (Legzdina and Gaile, 2008). It was proved, that prolong treatment time (more than 6 min) and elevated ozone concentrations negatively influence grain germinating power after five days germination (Table 1). During grain treatment with duration longer than 4 min, if ozone concentration was 3.92 mg m^{-3} as a result grain germinating power was 92%, if concentration was 7.84 mg m^{-3} – 94%, if concentration was 11.76 mg m^{-3} – 93% and if concentration was 15.68 mg m^{-3} – 91%. Triticale grain germinating power after five days germination was 85.0±0.5% that is very similarly too with results indicated in EC directive (Commission Directive 1999/8/EC, 1999) – 80%. Higher grain germinating power was detected if ozone concentration was 3.92 mg m^{-3}: after 4 min processing – 90.0%, 6 min – 90.5% and 8 min – 93.5% (Table 1). However, if ozone concentration was higher than mentioned – grain germination power decrease.

3.3. Grain microbiological parameters

3.3.1. Yeasts

Close interconnection was found between grain treatment time with gassy ozone, ozone concentration and yeasts amount on grain surface.

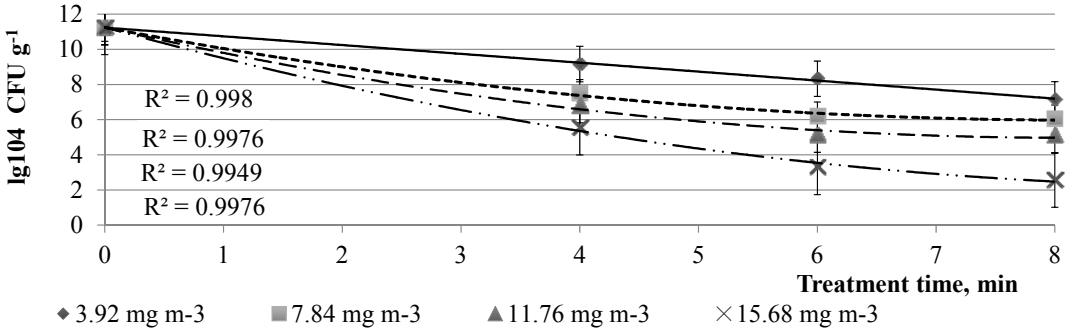

Figure 3 Yeasts content changes in triticale grains during ozonising

The amount of yeasts decreased as did the ozone concentration and the treatment time increased. For maximal yeasts content decrease in triticale (Fig. 3) and hull-less barley grains, the optimal ozone concentration is 15.68 mg m^{-3} and treatment time recommended is 8 min .

3.3.2. Moulds

In literature is mentioned, that the effect of ventilation duration and ozone impact was evaluated according to the changes in grain contamination with micromycetes propagules, and alternation of micromycetes species on the grain surface. At drying grains by active ventilation with an ozone-air mixture, at O_3 concentration of 700 ppb, the contamination was reduced by up to 2.2–3.0 times. At the same time, the composition of micromycetes species on the grain surface changed significantly: in non-ventilated grain there were detected micromycetes of 26 species, and in ventilated grain – of 11 species. Efficient ozone impact was established (Raila et al., 2006). Positive results were acquired in present research too. For the triticale and hull-less barley grains very similar results was reached as follow: grain treatment for 8min using elevated ozone concentration give positive result as mould destroying (example is shown on Fig. 4). Mould content of triticale grains before treatment was 1675 CFU g^{-1}. If the concentration was 3.92 mg m^{-3} the mould amount decreases 88%, if concentration was 7.84 mg m^{-3} –90%, if concentration was 11.76 mg m^{-3} –95% and if concentration was 15.68 mg m^{-3} –99.99%, what is significantly (p=0.004). If grains were treated using ozone with low concentration (3.92 mg m^{-3}) after 4 treatment minutes mould amount decrease by 72%, but after 2 treatment minutes only by 42% what is not plenty.

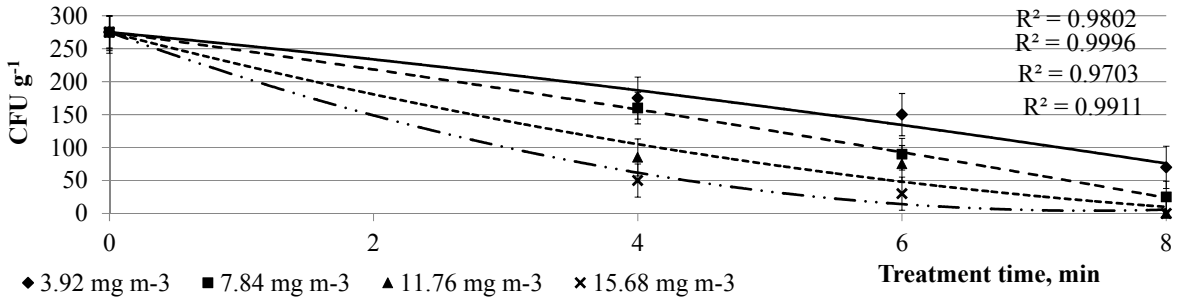

Figure 4 Mould content changes in hull-less barley grains during ozonising

Similar results were detected in analysis of hull-less barley grain microflora. It was possible to decrease mould amount on grains surface after 8 min treatment if concentration was 3.92 mg m^{-3} by 75%, if concentration was 7.84 mg m^{-3} by 91%, if concentration was

11.76 mg m^{-3} and 15.68 mg m^{-3} by 99.99%.

3.3.3. Mycotoxins

Fusarium species are the most important group of mycotoxigenic moulds. They are generally encountered as contaminants of cereal grains and produce trichotecenes: deoxynivalenol, zearalenone, T-2 and others. The production of mycotoxins in grain is often influenced by moisture, temperature and other factors and mycotoxin levels can vary significantly from field to field (Suproniene et al., 2006). The amount of mycotoxins before and after grain treatment with gassy ozone was under detection layer as follow DON < 50 μg kg^{-1}, ZEN < 25 μg kg^{-1}, and T-2 < 5 μg kg^{-1}.

CONCLUSIONS

1. Optimal grain processing parameters with ozone for maximal microbiological safety and optimal germinating power were for triticale grains – 3.92 mg m^{-3} 8 min, for hull-les barley grain – 11.76 mg m^{-3} 6 min.
2. Ozone concentration and treatment time not significantly influence changes in grain protein, moisture, starch and β-glycan content.
3. Ozone concentration and treatment time significantly influence grain germinating power: germinating power of processed triticale grains increase after three days germination by 15% and after five days germination by 7%; of processed hull-less barley grains – after three days germination by 11% and after five days germination by 9%.
4. Ozone significantly influences grain starch microstructure. As a result starch granules swell up, its volume increases.
5. The amount of mycotoxins before and after grain treatment with gassy ozone was under detection layer.

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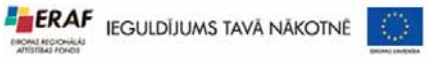

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HYDROGEN PEROXIDE EFFECT ON THE QUALITY OF SHREDDED CARROTS DURING STORAGE

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ABSTRACT

Minimally processed carrots are very common in developed countries and are gaining popularity due to their convenience and freshness. Therefore new directions for carrots processing was searched mainly for shelf-life extension. Hydrogen peroxide (H_2O_2) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity. The main purpose of the research was to determine shelf-life of shredded carrots treated by H_2O_2 and packaged in several packaging materials. ′Nante′ cultivar carrot hybrid ′Nante/Forto′ was used for the experiments. Shredded carrots were treated with 1.5% H_2O_2 water solution for 30±1s and packaged in DuniForm PP trays (138x114x53 mm) and hermetically sealed by breathing polymer film BOPP Propafilm^{TM} P2GAF, cellulose based biodegradable NatureFlex NVS. Polilactid BIO-PLA containers were used as well. Samples were stored at temperature +4±1 °C for 8 days. As a control samples, non-processed carrots packaged in mentioned packaging materials were tested. Main quality parameters using standard methods were analysed: carotenoid and β-carotene, soluble solids, mass loses, moisture, pH, environment composition in package - CO_2 and O_2 and microbiological parameters. Results of presented experiment demonstrate that treatment carrots by H_2O_2 do not prolong their shelf-life comparing wit not treated carrots. The shelf-life of shredded carrots treated by H_2O_2 and packed in different packaging materials was 8 days.

Keywords: carrot, hydrogen peroxide, shelf-life, packaging materials.

1. INTRODUCTION

Carrot (*Daucus carota* L.) is considered one of the vegetables whose consumption, both fresh and processed, has increased over the past years due not only to the nutritional and health benefits this vegetable provides, but also to the introduction of new carrot-derived products (Gamboa-Santos et al., 2012). A global processing and storage design to achieve high-quality minimally processed foods requires a combination of different strategies and technologies to help reduce spoilage processes in fresh-cut vegetables (Martín-Belloso et al., 2011). Minimally processed fruit and vegetables are often washed in water or water containing chemicals (Watson et al., 2007). One of the new approaches is the use of ‘‘generally recognized as safe’’ (GRAS) compounds due to minimal concerns about their environmental impact and low residues in the treated commodity. The US Food and Drug Administration (FDA) have published lists of GRAS compounds that can be used in many food processing applications where they have been declared safe by expert panels. Regarding the FDA list as a reference, among the chemicals used in this study, chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), citric acid ($C_6H_8O_7$), and ethanol (EtOH) are listed as GRAS (Generally Recognized As Safe) substances (Loredo et al., 2013). Hydrogen peroxide (H_2O_2) is also a well-studied oxidant agent (Sahin et al., 2012; Rodrigues et al., 2012; Yildiz et al., 2009), directly toxic to pathogens. It was both bacteriostatic and bactericidal activity, due to its capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals (Alexandre et al., 2012; Delgado et al., 2012; Ruelas et al., 2007; Tornuk et al., 2011). Hydrogen peroxide (oxygenated water) is characterized by containing a pair of oxygen atoms (-O-O), which are highly oxidative with the release of O_2 in aqueous solutions and thus create antimicrobial activity, mainly for Gram-positive and Gram-negative bacteria (Rodrigues et al., 2012; Demirkol et al., 2008). Hydrogen peroxide is a strong oxidizing agent proposed as an alternative to decontaminate fruits and vegetables due to its low toxicity and safe decomposition products. It is effective against a wide spectrum of organisms such as: bacteria, yeast, moulds, viruses and spore-forming organisms (Loredo et al., 2013). H_2O_2 provides a host of benefits by cleansing water of harmful substances such as spores, dead organic material and disease-causing organisms while preventing new infections from occurring (Khandaker et al., 2012). Hydrogen peroxide has been shown to inactivate a wide variety of infective biological agents ranging from the vegetative cells and spores of bacteria and fungi, protozoa and their cysts, viruses and even prions (Malik et al., 2012). Hydrogen

peroxide has a considerably low toxicity and it is usually recognized as friendly due to its low impact on environment. Hydrogen peroxide present in food after treatment can be removed by action of endogenous catalase or by rinsing water immediately after treatment to avoid reactions with food constituents (Alexandre et al., 2012). The bactericidal activity of hydrogen peroxide is well known. Hydrogen peroxide decomposes rapidly in water and oxygen through the action of catalase, an enzyme commonly found in plants, leaving no residual toxicity (Ruelas et al., 2007). Microbial action can also degrade it to water and oxygen. Hydrogen peroxide possesses many properties that render it particularly useful as sterility and disinfectant; it is colourless and odourless and ultimately decomposes to water and oxygen. Hydrogen peroxide has been shown to inactivate a wide variety of infective biological agents ranging from the vegetative cells and spores of bacteria and fungi, protozoa and their cysts, viruses and even prions (Malik et al., 2013). Hydrogen peroxide is a stable, partially reduced form of oxygen, and its rapid turnover is characteristically mediated by enzyme action (Khandaker et al., 2012). H_2O_2 is a major reactive oxygen species (ROS) generated in plants, which is scavenged by a network of low molecular weight antioxidants and antioxidant enzymes (Hung et al., 2008). Further studies on the effectiveness of hydrogen peroxide in inactivating pathogens on a wide range of raw vegetables are needed. Therefore, the main purpose of the research was to determine shelf-life of shredded carrots treated by H_2O_2 and packaged in several packaging materials.

2. MATERIALS AND METHODS

Experiments were carried out at the Department of Food Technology of the Latvia University of Agriculture. The object of the research was: carrots *(Daucus carota* L.*)* grown in Latvia and harvested in Zemgale region in the first part of October 2012 and immediately used for experiments. Shredded carrots were treated with 1.5% H_2O_2 water solution for 30±1s. Shredded carrots were packed by 100±5.0 g in DuniForm PP trays (138x114x53 mm) hermetically sealed by breathing polymer film BOPP Propafilm^{TM} P2GAF, cellulose based biodegradable NatureFlex NVS and Polilactid BIO-PLA containers. Samples within 8 days were stored in a Commercial Freezer/Cooler „Elcold" at the temperature of +4.0±0.5 °C. The results were reported as averages of all determinations. Samples were analysed before packaging (day 0), after 3, 5 and 8 days storage; ten measurement repetitions of each sample were performed. Samples were immersed in the following 1.5% H_2O_2 water solution for

30±1s. Hydrogen peroxide solution was prepared by mixing food grade concentrated hydrogen peroxide 30% w/w (Peróxidos do Brasil Ltda, Curitiba, Brazil) with sterile deionized water and used on the same day (Watson et al., 2007). Carotenoids and β-carotene were analysed by spectrophotometric method (used the UV/VIS spectrophotometer Jenway 6300) at 440 nm (Kampuse et al., 2012; Biswas et al., 2011).

The content of soluble solids (^{o}Brix) was determined at the temperature +20±2 °C with a digital refractometer (deviation of measuring instrument face value ± 0.1%) by standard method ISO 2173:2003.

Samples for microbiological testing were prepared by dilution method in conformity with standard LVSENISO6887-1:1999 and 6887-4:2044.TPC (total plate count) - determined in conformity with standard LVSENISO4833:2003A; yeast and mould plate count - determined in conformity with standard LVSISO21527-2:2008. Plate counts evaluated as decimal logarithm of colony forming units (CFU) per gram of a product (log cfu·g^{-1}).

The dynamics of gas composition inside each hermetically sealed package headspace within the storage time was measured as a percentage of oxygen and carbon dioxide by a gas analyser OXYBABY®V O_2/CO_2.

At each sampling time, for each type of carrots, the mass was measured three times on different samples (Mastromatteo et al., 2012).

Moisture content was determined by ISO6496:1999 as accordant to the storage time by verified balance KERN (Germany) with precision ±0.001g; mass loss calculation (%) - were determined by weighing packed samples on the electronic scales, by standard LVS ISO 1442:1997.

pH was measured by JENWAY 3510 pH-meter, standard method LVSISO5542:2010.

The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS, version 16.00. Two-way analyses of variance ($p \leq 0.05$) were used to determine significance of differences between by different samples.

3. REZULTS AND DISCUSSION

3.1. Microbiological parameters

Microbiological safety is the main quality parameter of food. Therefore for the determination of shredded carrots, the shelf-life microbiological parameters was evaluated as main quality

indicator. The results demonstrated that treating by H_2O_2 wasn't effective method to prevent microbial growth during storage and enhancing the shelf-life of shredded carrots till 8 days (Fig. 1). It was found, that packaging materials not significantly ($p=0.114$) influence microbiological parameters changes in carrots during storage too.

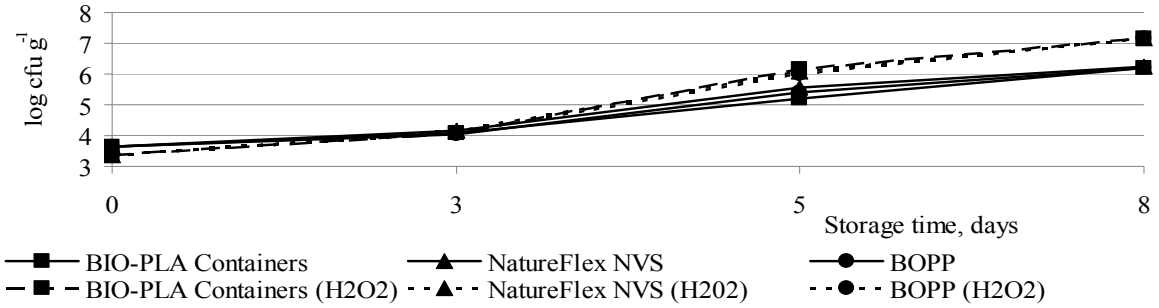

Figure 1. MAFAm dynamics of carrots during storage

Minimally processed carrots were microbiologically spoiled by bacteria rather than yeasts and mould. It was reported that microbial growth increased due to the increase in surface by peeling and cutting, high pH, and the moisture content of minimally processed carrots. It is suggested, that the microbial load should not be more than 7.70 log cfu g^{-1} in order to guarantee 5–6 days of shelf life for minimally processed carrots (Jacxsens et al., 2003). In our study, the total aerobic mesophilic bacteria count was around 7.16 log cfu g^{-1} at 8th storage day. However, there was significant difference ($p<0.05$) observed between treated and non-treated samples on the total mesophilic aerobic bacterial count during storage. The effect of storage time on the total mesophilic aerobic bacteria was statistically significant.

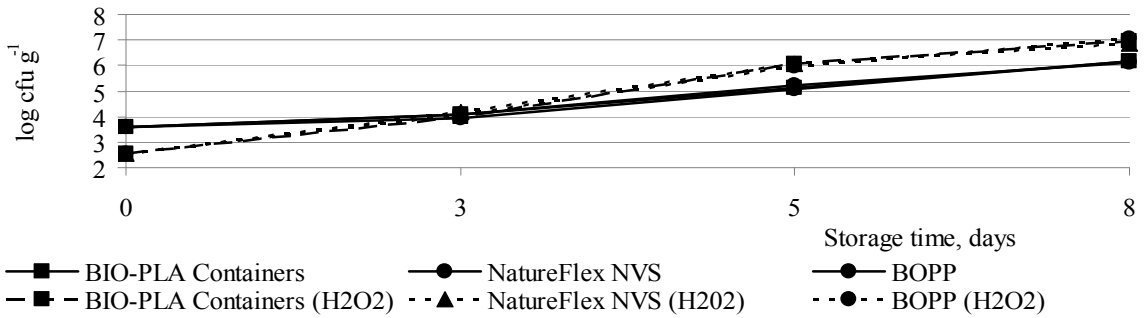

Figure 2. Yeasts and moulds count dynamics in carrots during storage

The initial count of yeasts and moulds was 3.59 log cfu g^{-1} in the non-processed samples and 2.56 log cfu g^{-1} in treated by H_2O_2 carrots (Fig. 2), what was 1.4 times les that initial results.

However, independently form packaging material, total moulds and yeasts count of non-treated carrots increase 1.7 times and of with H_2O_2 treated carrots - 2.5 times during 8 days storage, what is significantly ($p=0.008$). Therefore, H_2O_2 use for shredded carrots shelf-life prediction and microbiological safety is not necessary, mainly because it's non-pronounced effect.

3.2. Physically-chemical parameters

Water migration phenomena and the resulting moisture content change in food products affect their shelf-life through undesirable modifications of their physical, sensory and microbial qualities (Roca et al., 2008). Therefore, it is very important to determine the *moisture content* changes of shredded carrots. However, experimentally it was established that not significant differences in moisture content and *mass losses* values during the 8 days storage of all carrot samples packed in different materials and treated with H_2O_2 were not found ($p=0.448$ and $p=0.225$) comparing with non-storage samples.

Not significant changes of the *pH value* was established during current experiments ($p=0.192$). However, at the beginning of experiments pH value of non-processed carrots was 6.20, but during storage it slightly increased. The pH values after storage for 8 days achieved 6.33 value in average. The pH values of carrot treated with H_2O_2 pH was 6.12 at the beginning, while after storage it slightly decreased till 5.98.

Carotenoids in fresh-cut products are highly susceptible to oxidative deterioration due to the enhanced susceptibility under acute abiotic stress. In the present study it was detected, that with H_2O_2 ($p<0.05$) processed carrots *carotenoids* and *β-carotene* content decreased significantly during storage. Carotenoid content of fresh and treated with H_2O_2 carrots was 196.32 mg 100 g^{-1} and 166.14 mg 100g^{-1} respectively; however, it is necessary to specify carotenoids and β-carotene decreases during storage mainly in non-processed carrots. Significant differences were found in carotenoids ($p=0.007$) and β-carotene ($p=0.001$) content of non-treated carrots - the content decrease by 20% in average, not independently from packaging materials. However, not significant differences were found in carotenoids ($p=0.448$) and β-carotene ($p=0.161$) content with H_2O_2 treated carrots during 8 days storage – it's practically was not changed. Food processing has both positive and negative effects on the levels of carotenoids in food, but in overall it is more evident that processing stage may be beneficial through disruption of matrix (cell walls) which facilitates carotenoids release and solubilisation as free or esterified/glycosylated forms

in appropriate solvents, or after long heating times, leading to chemical changes (Patras et al., 2009). The results of this study indicate the needs for further insights into the carotenoid role of H_2O_2 treatment. Similar results was obtained within β-carotene changes measurement in carrots during storage.

Significant influence (p=0.177) of H_2O_2 on *soluble solids* content in shredded carrots was detected in the present research. As a result, the content of soluble solids decrease by 22% in H_2O_2 processed carrots, compared to non-processed shredded carrots. However, not significant changes of soluble solids content changes in processed and non-processed packaged in several packaging materials shredded carrots was detected - the content of analysed parameter changed in 5% frame.

Another target of the present work was to assess dynamic of *O_2 and CO_2 composition* inside packaging during sample storage. The oxygen content 19.8% decreased during carrots storage as a result of fresh-cut carrots breathing mainly. The decrease of O_2 content and corresponding gas composition in different packaging made from various materials is disparate. In non-hermetically sealed Bio-PLA Containers (PLA) with carrots the decrease of O_2 was significant - at the 3rd, 5th and 8th day storage it was 11.6, 4.3 and 3.6%, respectively (Fig. 3). Very positive results were observed during O_2 content analysing inside packaging with treated shredded carrots. The content of oxygen inside PLA containers after 8 days storage decrease from 19.8 till 14.5%, however in other analysed samples (treated and non-treated carrots) the content of oxygen inside packaging decrease till 4% in average, what is not acceptable for sensory properties of analysed samples.

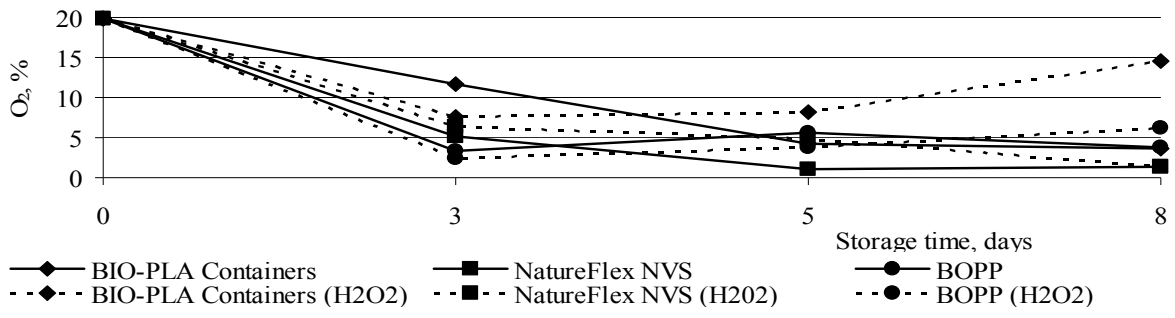

Figure 3. The dynamics of oxygen (O_2) content inside packaging

The CO_2 content increasing inside packaging atmosphere mainly could indicate the beginning of spoilage process. Of course, elevated amounts of CO_2 indicate the non-acceptable quality of packaged product. During present experiments influence of H_2O_2 not only on quality

parameters of shredded carrots, but on CO_2 increases was detected. Inside the (BOPP) packaging with H_2O_2 processed carrots, after 8 days storage, the CO_2 amount increased 15 times, however in same packaging material with non-processed carrots - by 22 times, what is substantially different (Fig. 4). It mainly could be explained with H_2O_2 positive influence on maintenance of carrots quality parameters. The CO_2 content increased by 7 times after 8 days storage in PLA and NatureFlex NVS packaging with carrots processed with H_2O_2.

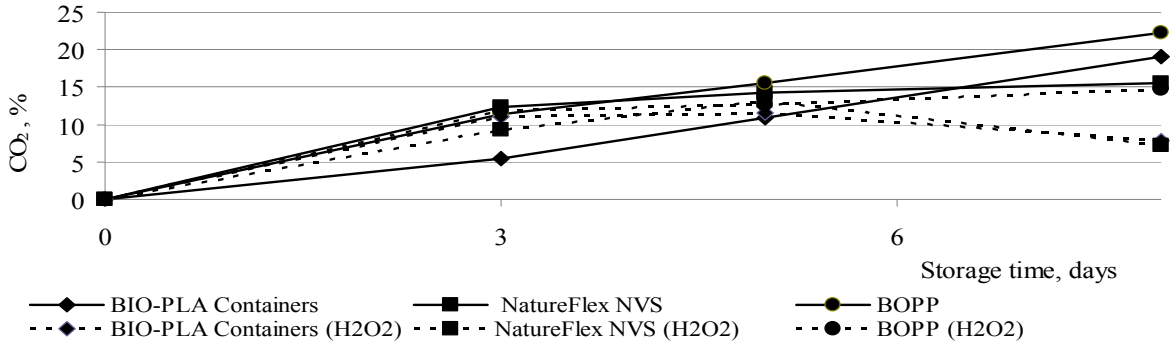

Figure 4. The dynamics of carbon dioxide (CO_2) content inside packaging

However, a CO_2 amount increase in same type of packaging with non-processed carrots was more pronounced - it increased by 19 and 16 times respectively.

CONCLUSIONS

H_2O_2 have significant influence on shredded carrots quality parameters. There are no found substantial differences of with H_2O_2 processed and non-processed shredded carrots packaged in different type of materials carotenoids, β-carotene, soluble solids, mass losses, moisture content and pH value during storage. It is not possible to prolong the shelf-life of shredded carrots by its treatment with 1.5% H_2O_2 water solution - maximal shelf-life was 8 days. Positive influence of H_2O_2 was observed on O_2 and CO_2 gas formation inside packaging during shredded carrots storage - its inverse not so fast.

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THE APPLICATION OF A SIMPLEX CENTROID MIXTURE DESIGN TO THE PRODUCTION OF PROTEASE BY *ASPERGILLUS ORYZAE* UNDER SOLID STATE FERMENTATION

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ABSTRACT:

Wheat bran, soybean meal and cottonseed meal, alone or in combinations, were used as the substrates for protease production by *Aspergillus oryzae* under solid state fermentation. The interactions amongst the three substrates in the production of protease were studied by applying a simplex centroid mixture design. The protease was produced in 500 mL conical flasks containing 40 g medium. The cultivation parameters were: 50% initial moisture, temperature set at 30°C and an inoculum of 10^7 spores.g^{-1}. The protease activity was tested at 24 h intervals during 72 h of fermentation. A synergetic effect was found for two of the binary formulations after 24 h of fermentation. The medium composed of wheat bran (1/2) and soybean meal (1/2) showed increases of 34.4 and 90.7% in protease production as compared to the isolated substrates, respectively. In the medium formulated with wheat bran (1/2) and cottonseed meal (1/2), increases of 22.2 and 136.9% in protease production were observed as compared to the isolated substrates, respectively. However, protease production using the ternary (wheat bran, soybean meal and cottonseed meal) and binary (cottonseed and soybean meals) mixtures in equal proportions, showed antagonistic effectsafter 24h. After 48 h fermentation, a synergetic effect was found for the medium composed of soybean meal (1/2) and cottonseed meal (1/2), showing increases of 2.4 and 86.4% in protease production as compared to the isolated substrates, respectively. After 72 h fermentation, all the mixtures evaluated showed antagonistic effects. The highest protease activities were found using the medium containing wheat bran after 48 and 72 h of fermentation.

Keywords: protease, fermentation, *Aspergillus oryzae*, agroindustrial residues, mixture design

1. INTRODUCTION

Proteases are multifunctional enzymes and are extremely important in the pharmaceutical, medical, food and biotechnology industries, accounting for nearly 60% of the whole enzyme market (Ramakrishna, Rajasekhar and Reddy, 2010). They can be isolated from plants, animals and microorganisms. Of these sources, the microorganisms show great potential for protease production due to their broad biochemical diversity and their susceptibility to genetic manipulation. It has been estimated that microbial proteases represent approximately 40% of the total worldwide enzyme sales (Rao, Tanksale, Ghatge and Deshpande, 1998).

Proteolytic enzymes can be produced under submerged and solid state fermentation. For the growth of fungi, solid state fermentation is the most appropriate method because it resembles the natural habitat of the fungi. Some characteristics make solid state fermentation more attractive than submerged fermentation: simplicity, low cost, high yields and concentrations of the enzymes, and the use of inexpensive and widely available agricultural residues as substrates (Chutmanop, Chuichulcherm and Sirinophakun, 2008).

In the literature, the use of different agroindustrial wastes for the production of protease under solid state fermentation, such as wheat bran, soybean meal and rice bran (Chutmanop, Chuichulcherm and Sirinophakun, 2008; Castro and Sato, 2011) have been reported. However, these studies were all regarding the production of protease using separate substrates,and no investigations using statistical mixture designs were found.

Mixture designs are a special class of response surface design, where the proportions of the components or factors are considered important rather than their magnitude, and they are useful in the design of mixtures. The interactions between the components of a mixture,aimed at maximizing the response have been studied using the mixture design approach. Statistical methods were applied to different engineering problems in order to improve the performance and find the optimum process variables (Rao and Baral, 2011).

In this study a simplex centroid mixture design involving different agroindustrial wastes was applied to the production of protease by *Aspergillus oryzae* under solid state fermentation.

2. MATERIALS AND METHODS

2.1 Microorganism

The strain used in this study was *A. oryzae* LBA 01, previously selected as a proteolytic strain from the culture collection of the Food Biochemistry Laboratory, Faculty of Food Engineering, State University of Campinas, Brazil. The strain was periodically subcultured and maintained on potato dextrose agar slants. To produce fungal spores, the microorganism was inoculated into a medium composed of 10 g wheat bran and 5 mL of a solution containing 1.7% (w/v) $NaHPO_4$ and 2.0% (w/v) $(NH_4)_2SO_4$, and incubated at 30°C for 3 days. The fungal spores were dispensed into a sterile Tween 80 solution (0.3%) to prepare the inoculum for fermentation. The number of spores per milliliter in the spore suspension was determined using a Neubauer cell counting chamber.

2.2 Protease production.

Wheat bran, soybean meal and cottonseed meal were kindly provided by Bunge Foods S/A. These agricultural residues were used to select the most appropriate substrate for the production of protease by *A. oryzae* LBA 01. The protease was produced under solid state fermentation in 500 mL conical flasks containing 40 g medium. The initial cultivation parameters, previously defined as the most appropriate conditions for the production of protease by *A. oryzae* LBA 01, were 50% moisture, temperature set at 30°C and an inoculum level of 10^7 spores.g^{-1}. Protease activity was tested at 24 h intervals during 72 h of fermentation. The crude extract was obtained by adding 150 mL acetate buffer (200 mM, pH 5.0) and leaving at restfor 1 h. The solution was filtered through a filter membrane to obtain an enzyme solution free of any solid material.

2.3 Proximate composition and determination of the water absorption index (WAI) of the agroindustrial wastes

The moisture, protein, lipids and ash contents of the agroindustrial wastes were determined by AOAC methods (AOAC, 2010). The carbohydrate content was determined from the difference between the total value of 100% and the sum of the other components.The tests were carried out in triplicate and the results were expressed as the mean ± standard deviation.

The water absorption indexes (WAI) were determined using the method of Anderson et al. (1969) with slight modifications. Briefly, the sample (1.25 g) was suspended in 15mL of distilled water in a previously tared 50 mL centrifuge tube. The slurry was manually stirred for 1 min at room temperature (25°C) and centrifuged at 8000 x *g* and 25°C for 15 min. The supernatants were discarded, and the WAI calculated from the weight of the remaining gel expressed as g gel/g dry weight.

2.4 Statistical mixture design

The mixture design of the experiment was used to obtain the optimum mixture composition of the agroindustrial wastes for maximum protease production, and to investigate the presence of either synergistic or antagonistic effects in the component blends. A three component augmented simplex-centroid design was employed, in which each component was studied at four levels, namely 0 (0%), 1/3 (33%), 1/2 (50%) and 1 (100%)(TABLE 2).

Quadratic or special cubic regression models were fitted for the variations in all the responses studied as a function of significant ($p< 0.05$) interaction effects between the proportions, obtaining acceptable determination coefficients ($R^2 > 0.80$). Equation 1 represents these models:

$$Yi = \sum \beta iXi + \sum \beta ijXiXj + \sum \beta ijkXiXjXk$$

where Y_i is the predicted response, β_i is the regression coefficients for each linear effect term, and β_{ij} and β_{ijk} are the binary and ternary interaction effect terms.The Statistica® 10.0 software from Statsoft Inc. (Tulsa, Oklahoma, USA) was employed to prepare the experimental design, and for the data analysis and model building.

2.5 Determination of protease activity

The protease activity was determined using azocasein as the substrate, according to Charney and Tomarelli (1947), with modifications. The reaction mixture containing 0.5 mL 0.5% (w/v) azocasein (Sigma) pH 5.0, and 0.5 mL of the enzyme solution was incubated for 40 min. The reaction was stopped by adding 0.5 mL 10% TCA and the test tubes centrifuged at 17,000 x *g* for 15 min at 25°C. A 1.0 mL aliquot of each supernatant was neutralized with

1.0 mL 5 M KOH. One unit of enzyme activity (U) was defined as the amount of enzyme required to increase the absorbance at 428 nm by 0.01 under the assay conditions described.

3. RESULTS AND DISCUSSION

Enzyme production under solid state fermentation can be affected by the lipid, protein and carbohydrate compositions of the substrates and consequently manipulated by various cultivation factors. With regard to protease production, the presence of protein sources can induce secretion of the enzyme by the microorganism, and thus substrates with high protein contents are desirable. On the other hand, in aerobic processes the substrate must have a suitable carbon to nitrogen ratio (C:N) for fermentation. Therefore, statistical mixture designs have high scientific value, since they make it possible to manipulate the composition of the fermentation medium,using different combinations of the substrates in order to produce the most suitable medium for the production of protease. The proximate compositions of the agroindustrial wastes used as fermentation substrates for the production of protease by *A. oryzae* LBA 01 under solid state fermentationare shown in TABLE 1.

Chemical components	Wheatbran	Soybeanmeal	Cottonseedmeal
Moisture (%)	12.78 ± 0.08	11.93 ± 0.02	6.42 ± 0.01
Protein (%)	14.74 ± 0.51	49.24 ± 0.07	25.91 ± 0.60
Carbohydrates (%)	63.04	31.53	55.80
Lipids (%)	4.47 ± 0.22	1.40 ± 0.02	7.83 ± 0.01
Ash (%)	4.99 ± 0.06	5.90 ± 0.04	4.04 ± 0.01

TABLE 1– Average values of the proximate composition (%) of the agroindustrial wastes used for protease production by *Aspergillus oryzae* LBA 01 under solid state fermentation.

The WAI indicates the quantity of water that can be absorbed by the support. Materials with high WAI are preferred for solid state fermentation since their moisture contents can be modified during solid state culturing (Robledo et al., 2008). Wheat bran and the formulations containing it showed higher WAI values, whereas the soybean meal and the formulations containing it showed the lowest WAI values (Figure 1). The highest values for protease production were observed in the substrates with higher WAI values, indicating a positive impact of this physicochemical parameter on protease production, probably caused by maintenance of the moisture content throughout the fermentation process. Orzua et al. (2009) studied ten agroindustrial wastes for their suitability as fungus immobilization carriers for

solid-state fermentation, and indicated the materials with high water absorption capacities as the most appropriate for use in SSF.

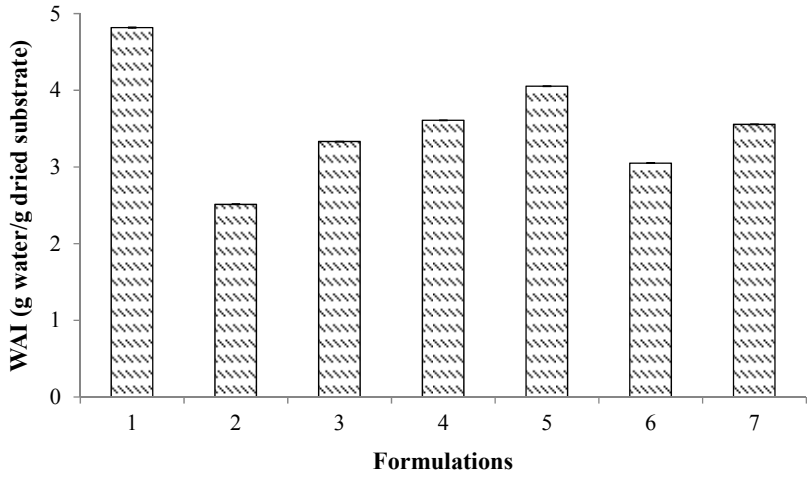

Figure 1: Water absorption index (WAI) of the agro industrial wastes and their mixtures: wheat bran (1), soybean meal (2), cottonseed meal (3), wheat bran (1/2) + soybean meal (1/2) (4), wheat bran (1/2) + cottonseed meal (1/2) (5), soybean meal (1/2) + cottonseed meal (1/2) (6) and wheat bran (1/3) + soybean meal (1/3) + cottonseed meal (1/3) (7).

The interactions amongst the three substrates in the production of protease were studied in the 7 assays using a simplex centroid mixture design (TABLE 2). The highest protease activities were found using the medium containing wheat bran after 48 and 72 h of fermentation. Some studies have described the use of wheat bran as a potent substrate for the production of proteases by *A. oryzae* (Sandhya et al., 2005; Vishwanatha, Rao and Singh, 2010).

A synergetic effect was found for two binary formulations after 24 h fermentation. The medium composed of wheat bran (1/2) and soybean meal (1/2) showed increases of 34.4 and 90.7% in protease production as compared to the isolated substrates, respectively. In the medium formulated with wheat bran (1/2) and cottonseed meal (1/2), increases of 22.2 and 136.9% in protease production were observed, as compared to the isolated substrates, respectively. However, protease production using the ternary (wheat bran, soybean meal and cottonseed meal) and binary (cottonseed and soybean meals) mixtures in equal proportions, showed antagonistic effects after 24h. After 48 h of fermentation, a synergetic effect was found for the medium composed of soybean meal (1/2) and cottonseed meal (1/2), showing increases of 2.4 and 86.4% in protease production as compared to the isolated substrates,

respectively. After 72 h of fermentation, all the mixtures evaluated showed antagonistic effects (TABLE 2).

TABLE 2 - Matrix of the simple centroid mixture design for the optimization of protease production by *Aspergillus oryzae* LBA 01 under solid state fermentation, and the results for protease activity after 24, 48 and 72h fermentation.

Run	Independentvariables			Protease activity ($U.g^{-1}$)		
	Wheatbran	Soybeanmeal	Cottonseedmeal			
	x_1	x_2	x_3	24h	48h	72h
1	1	0	0	18.30± 1.13	59.87± 1.30	43.27± 3.44
2	0	1	0	12.91± 1.09	15.66± 3.05	14.79± 0.75
3	0	0	1	9.44± 1.05	8.60± 1.27	14.57± 0.42
4	1/2	1/2	0	21.76± 1.24	22.90± 0.99	14.46± 3.02
5	1/2	0	1/2	19.46± 1.70	29.54± 0.87	10.64± 1.75
6	0	1/2	1/2	9.22± 2.70	16.03± 1.37	13.76± 1.37
7	1/3	1/3	1/3	12.63± 1.30	19.72± 1.37	15.89± 1.77

TABLE 3 shows the models, correspondingR^2 andadjusted R^2values of the regression equations for the responses, and also the corresponding*F*-ratio and *p*-value for each term in the predicted regression equations. The high coefficients of determination (R^2), which were above 0.90 (TABLE 3), indicate that all the response functions adequately fitted the experimental data, and that the models could therefore be used for predictive purposes in the production of protease by *A. oryzae* LBA 01 under solid state fermentation using different substrate mixtures.

TABLE 3 – Models, R^2, adjusted R^2 and probability values for the final reduced models (component proportions) for protease activity.

Response	Anova					
Protease activity ($U.g^{-1}$)	**Model**	***F*calculated**	***F*tabulated**	**R^2**	**adjusted R^2**	***p*-value**
24h	Special cubic	31.04	2.85	0.93	0.90	<0.001
48h	Quadratic	387.46	2.90	0.99	0.98	<0.001
72h	Special cubic	110.22	2.90	0.97	0.96	<0.001

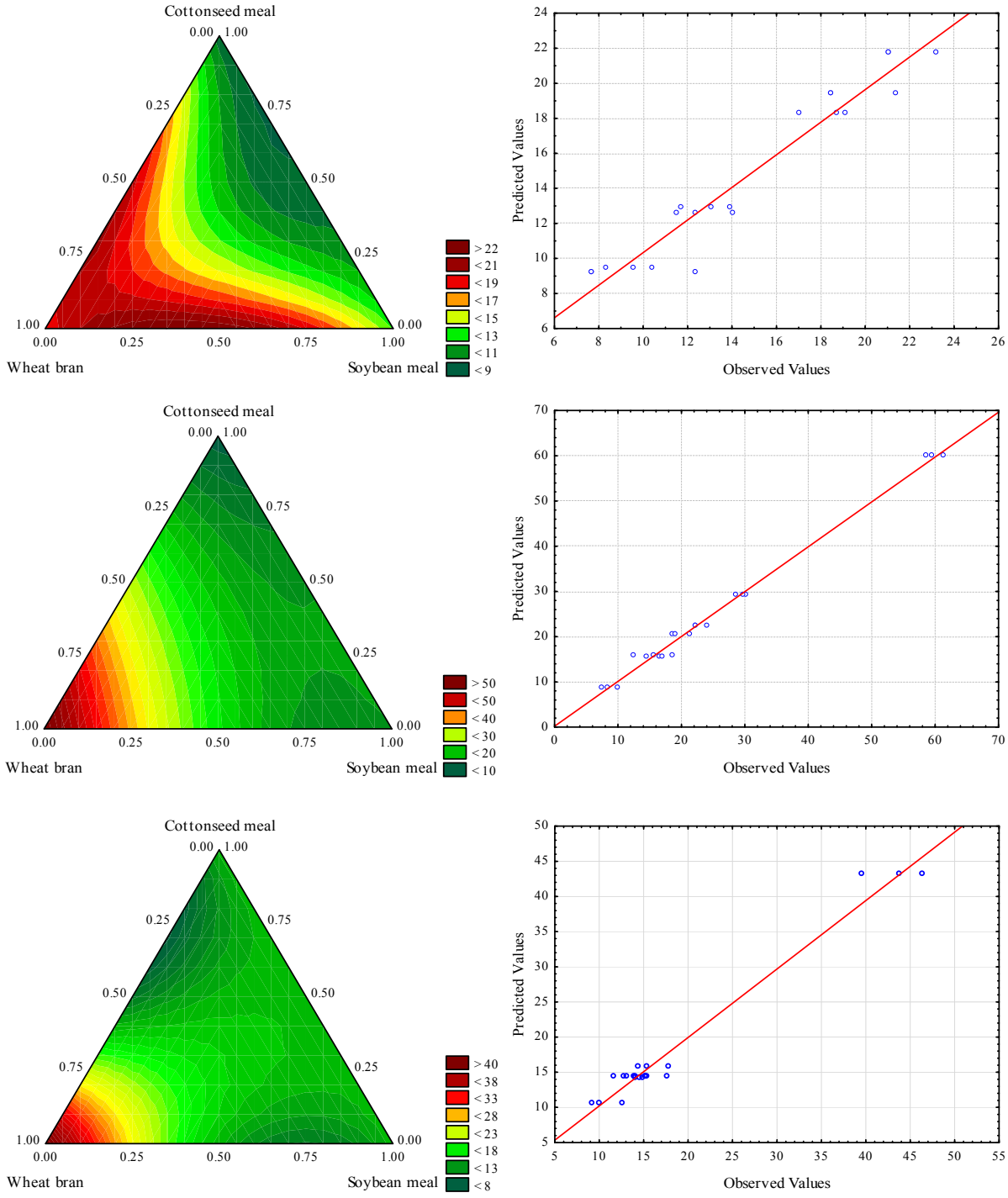

Figure 2 - Mixture contour plots and fitted line plots between the experimental and predicted values for protease production by *Aspergillus oryzae* LBA 01 under solid state fermentation for 72h as a function of the significant ($p< 0.05$) interaction effects between the proportions of the agroindustrial wastes.

The variation in the amounts of protease produced by *A. oryzae* using different proportions of the agroindustrial wastes is also shown using mixture contour plots, in which each factor (pure mixture component) is represented in a corner of an equilateral triangle, each point within the triangle referring to a different proportion of components in the mixture (Figure 2). The maximum percentage of each ingredient considered by the regression is placed at the corresponding corner while the minimum is positioned at the center of the opposite side of the triangle. The center of the whole triangle represents the mixture in equal parts (Martinello, Kaneko, Velasco, Taqueda&Consiglieri, 2006).

The predicted regression equations below represent the models with significant factors for protease production after 24, 48 and 72h of fermentation:

$$Y_{24h} = 18.3x_1 + 12.9x_2 + 9.4x_3 + 24.6x_1x_2 + 22.4x_1x_3 - 7.8x_2x_3 - 142.4x_1x_2x_3$$
$$(\pm 0.90) \quad (\pm 0.90) \quad (\pm 0.90) \quad (\pm 4.41) \quad (\pm 4.41) \quad (\pm 4.41) \quad (\pm 30.99)$$

$$Y_{48h} = 59.9x_1 + 15.8x_2 + 8.7x_3 - 61.3x_1x_2 - 20.6x_1x_3 + 13.7x_2x_3$$
$$(\pm 0.93) \quad (\pm 0.93) \quad (\pm 0.93) \quad (\pm 4.29) \quad (\pm 4.29) \quad (\pm 4.29)$$

$$Y_{72h} = 43.3x_1 + 14.5x_2 + 14.3x_3 - 57.7x_1x_2 - 72.5x_2x_3 + 171.5\ x_1x_2x_3$$
$$(\pm 1.16) \quad (\pm 1.06) \quad (\pm 1.06) \quad (\pm 5.63) \quad (\pm 5.63) \quad (\pm 37.56)$$

CONCLUSION

The results suggest that the application of statistical mixture designs for the production of protease by *A. oryzae* LBA 01 using different agroindustrial wastes under solid state fermentation, is an attractive process for improving the performance and for finding the optimum formulations. Maximization of the responses could be observed when the media contained mixtures, as compared to mediacontaining only isolated substrates. The highest protease activities were found using the medium containing wheat bran after 48h fermentation, reaching 59.87 $U.g^{-1}$.

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COMPARISON OF THE FUNCTIONAL PROPERTIES AND ANTIOXIDANT ACTIVITIES OF INTACT PROTEINS AND THEIR HYDROLYSATES USING A STATISTICAL MIXTURE DESIGN

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ABSTRACT:

Soy protein isolate (SPI), bovine whey protein (BWP) and egg white protein (EWP) were used to study the effects of different protein sources and their mixtures on their functional properties (solubility, heat stability, emulsion activity index and foaming capacity) and antioxidant activities (ORAC and DPPH assays). The interactions amongst these three substrates were studied using a simplex centroid mixture design. The hydrolysates were prepared using a commercial protease from *Aspergillus oryzae*. Enzymatic hydrolysis was carried out in conical flasks each containing 50 mL of the determined protein solution (100 $mg.mL^{-1}$) for 2h, under the optimum pH and temperature conditions of the enzyme (pH 5.0 at 50.0°C). Hydrolysis increased protein solubility, except for EWP and the mixtures containing it. However, the hydrolysates exhibited a tendency to decrease their foaming capacities and heat stability. For the emulsion activity index, a synergetic effect was found for two binary formulations and the ternary mixture, with increases of up to 155.2%. In the ORAC assay, the SPI hydrolysates showed an increase in antioxidant activity of up to 400.0%, followed by the EWP (335.2%) and BWP (205.3%) ones, as compared to the intact proteins. The increases were even greater in the mixtures, reaching up to 600.0% for the formulation contained SPI (1/2) and EWP (1/2), as compared to the intact proteins mixture. In the DPPH assay, the SPI hydrolysates showed an increase in antioxidant activity of up to 89.7% followed by the BWP ones (74.1%), as compared to the intact proteins. However, the antioxidant activity (DPPH assay) of the EWP hydrolysates and the intact proteins showed no statistically significant difference ($p < 0.05$).

Keywords: enzymatic hydrolysis, protein, functional properties, antioxidant activities, mixture design.

1. INTRODUCTION

Processes involving protein hydrolysis have been studied for bioactive peptide production. Bioactive peptides can be defined as specific amino acid sequences that promote beneficial biological activities. Bioactive peptides can be produced by enzymatic hydrolysis using digestive, microbial and plants enzymes. The limited and controlled proteolysis unfolds the protein chains, reducing the incidence of allergenic factors, thus resulting in the formation of small peptides with biological activities (Korhonen, 2009).

In the last decade, the enzymatic hydrolysis of proteins from animal and plant sources for the production of bioactive peptides has attracted much attention. The antioxidant activities of peptides are extensively reported in several studies. The mechanism of action of peptides with antioxidant properties is related to the inactivation of reactive oxygen species (ROS), scavenging of free radicals, chelation of prooxidative transition metals and reduction of hydroperoxides (Zhou et al., 2012).

In addition to their antioxidant activities, protein hydrolysates have been shown interesting functional properties, such as high solubility resulted by an increase in the concentration of free amino and carboxyl groups. Hydrolysis also disrupts the protein tertiary structure and reduces the molecular weight of the protein and, consequently, alters the functional properties of proteins (Liu, Kong, Xiong and Xi, 2010).

In the literature, different protein sources have been used for the enzymatic hydrolysis, such as rice, egg white protein and whey protein (Zhao et al, 2012; Naik, Mann, Bajaj, Sangwan and Sharma, 2013; Hoppe, Jung, Patnaik and Zeece, 2013). However, these researches show many studies on enzymatic hydrolysis using separately substrates; no investigations were found using statistical mixture designs.

Mixture designs are special class of response surface designs where the proportions of the components or factors are considered important rather than their magnitude and are useful in mixture design. The interactions between the components of a mixture for maximizing the response are studied using mixture design approach. Statistical methods were applied to different engineering problems for improving the performance and to find the optimum process variables (Rao and Baral, 2011).

In this work a simplex centroid mixture design was used for production of hydrolysates of different protein sources by enzymatic hydrolysis and study of the effects on their functional properties and antioxidant activities.

2. MATERIALS AND METHODS

2.1 Statistical mixture design

The mixture design of experiment was used to obtain the optimum mixture composition of the different protein sources for maximum antioxidant activities and to investigate the presence of either synergistic or antagonistic effect in a blend of components. A three component augmented simplex-centroid design has been employed in which each components is studied in four levels, namely 0 (0%), 1/3 (33%), 1/2 (50%) and 1 (100%) (TABLE 1). Quadratic or special cubic regression models were fitted for variations of all studied responses as function of significant ($p < 0.05$) interaction effects between the proportions, with acceptable determination coefficients ($R^2 > 0.80$). Equation 1 represents these models:

$$Y_i = \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ijk} X_i X_j X_k$$

where Y_i is the predicted response, β_i is the regression coefficients for each linear effect terms, β_{ij} and β_{ijk} are binary and ternary interaction effect terms. The Statistica® 10.0 software from Statsoft Inc. (Tulsa, Oklahoma, USA) was employed for experimental design, data analysis, and model building.

2.2 Determination of antioxidant activities

The ORAC method, which uses fluorescein (FL) as the ‘‘fluorescent probe’’, described by Dávalos, Gómez-Cordovés and Bartolomé (2004) was used to estimate the antioxidant activities of the hydrolysates. A Novo Star Microplate reader (BMG LABTECH, Germany) with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 520 nm was used for the measurements, and Trolox was used as the standard. The reaction was carried out in a Costar 96 plate by mixing a 120 µL aliquot of FL solution with either 20 µL of sample, blank or standard (Trolox solutions) and 60 µL of AAPH (2,2′-Azobis (2-methylpropionamidine) dihydrochloride) (108 $mg.mL^{-1}$). The measurements were taken in triplicate at 37°C. ORAC values were calculated using the difference between the area under the FL decay curve and the blank (net AUC) (Cao, Sofic and Prior, 1996). The ORAC values were expressed as µMol of Trolox equivalent.g^{-1} of protein hydrolysate.

The DPPH radical-scavenging activity of the hydrolysates was determined as described by Bougatef, Hajji, Balti, Lassoued, Triki-Ellouz and Nasri (2009). A 500 µL

aliquot of each sample at 5mg.mL^{-1} was mixed with 500 μL of 99.5% ethanol and 125 μL of 0.02% DPPH in 99.5% ethanol. The mixtures were then maintained at room temperature in the dark for 60 min, and the reduction in DPPH radical measured at 517 nm using a UV-Visible spectrophotometer. The control experiments were conducted in the same manner, except that distilled water was used instead of a sample. The DPPH radical-scavenging activity was calculated as follows:

Radical scavenging (%) = [(Absorbance of control - Absorbance of sample) / (Absorbance of control)]*100

2.3 Determination of the functional properties

2.3.1 Solubility

The protein solubility was determined according to Li, Luo, Shen and You (2012), with slight modifications. Solutions containing 100 mg of the protein hydrolysates were dispersed in 10 mL of distilled water (pH 6.5 ± 0.3) at room temperature and centrifuged at 17000 × g for 10 min. Total protein content in the samples was determined after dissolving them in 0.5 mol.L^{-1} NaOH. The protein content was determined using the Biuret method. Protein solubility was calculated as solubility (%) = (protein content in supernatant / total protein content in sample) × 100.

2.3.2 Heat stability

To determine the heat stability of the hydrolysates, 100 mg protein samples were dispersed in 10 mL of distilled water (pH 6.5 ± 0.3). After heating at 93 °C for 1 min, the solution was cooled in ice-water bath. The heat stability, expressed as solubility, was calculated as the ratio of the total protein after the heat treatment and total protein before the heat treatment.

2.3.3 Emulsifying property

The emulsifying property was measured with a modified version of the method described by Pearce and Kinsella (1979). Vegetable oil (0.5 mL) and 1.5 mL of 10 mg.mL^{-1} protein solutions were mixed. The mixture was homogenized for 1 min. A 50 μL aliquot of the emulsion was pipetted from the bottom of the container at 0 min after homogenization and mixed with 4.95 mL of 1 mg.mL^{-1} sodium dodecyl sulfate solution. The absorbance was measured at 500 nm. The emulsifying activity index (EAI) was calculated as EAI ($m^2.g^{-1}$) = (2 × 2.303 × A_{500}) / (0.25 × protein weight (g)).

2.3.4 Foaming capacity

Foaming capacity was measured with a modified version of the method described by Klompong, Benjukal, Kantachote and Shahidi (2007). Protein solutions (10 mL, 10 $mg.mL^{-1}$) were homogenized for 1 min. Foaming capacity, expressed as a percentage, was calculated as the ratio of the volume after whipping (mL) and the volume before whipping (mL).

3. RESULTS AND DISCUSSION

The interactions amongst the three substrates in the functional properties were studied in the 7 assays using a simplex centroid mixture design (TABLE 1). Selective enzymatic hydrolysis under controlled conditions has been used to improve the solubility, heat stability, emulsifying and foaming properties of proteins. Hydrolysis also disrupts the protein tertiary structure, reduces the molecular weight and alters the functional properties of proteins (Liu, Kong, Xiong and Xi, 2010). In our study, it was observed that the enzymatic hydrolysis increased protein solubility, except for EWP and the mixtures containing it. Some studies showed that the enzymatic hydrolysis increased the solubility, this may be due there is decrease the molecular size of protein by creating small peptides and unfolding the protein molecule, leading to more polar and ionisable groups being exposed on the protein surface, which could improve the protein molecule's ability to form hydrogen bonds with water and thereby augment solubility. However, other studies reported decrease of the solubility after hydrolysis; this probably occurs because the protein molecule exposes more hydrophobic groups. The hydrolysates exhibited a tendency to decrease their foaming capacities and heat stability. For the emulsion activity index, a synergetic effect was found for two binary formulations and the ternary mixture, with increases of up to 155.2%. In the ORAC assay, the SPI hydrolysates showed an increase in antioxidant activity of up to 400.0%, followed by the EWP (335.2%) and BWP (205.3%) ones, as compared to the intact proteins. The increases were even greater in the mixtures, reaching up to 600.0% for the formulation contained SPI (1/2) and EWP (1/2), as compared to the intact proteins mixture. In the DPPH assay, the SPI hydrolysates showed an increase in antioxidant activity of up to 89.7% followed by the BWP ones (74.1%), as compared to the intact proteins. However, the antioxidant activity (DPPH assay) of the EWP hydrolysates and the intact proteins showed no statistically significant difference ($p < 0.05$).

TABLE 1 - Matrix of the simple centroid mixture design for study of the functional properties and antioxidant activities of different proteins sources and their hydrolysates obtained by enzymatic hydrolysis.

Run	Independent variables			Functional properties: Solubility (%)		Functional properties: Heat stability (%)	
	x_1	x_2	x_3	Control	Hydrolysates	Control	Hydrolysates
1	1	0	0	11.19 ± 0.62^{a}	99.17 ± 1.67^{a}	100.44 ± 2.30^{a}	100.49 ± 4.24^{a}
2	0	1	0	21.09 ± 1.02^{b}	86.52 ± 4.00^{b}	101.90 ± 1.39^{a}	$88.02 \pm 1.64^{b, c}$
3	0	0	1	82.14 ± 2.55^{c}	26.92 ± 0.48^{c}	91.68 ± 1.15^{d}	86.56 ± 0.92^{c}
4	1/2	1/2	0	41.44 ± 1.58^{d}	$88.16 \pm 3,50^{b}$	95.01 ± 1.53^{d}	$90.76 \pm 4.13^{b, c}$
5	1/2	0	1/2	89.53 ± 2.29^{e}	39.27 ± 0.61^{d}	$99.72 \pm 1.66^{a, b}$	$94.78 \pm 1.37^{a, b}$
6	0	1/2	1/2	94.78 ± 1.84^{f}	36.09 ± 2.01^{d}	81.41 ± 0.69^{e}	99.23 ± 1.90^{a}
7	1/3	1/3	1/3	$86.99 \pm 1.67^{c, e}$	49.46 ± 5.25^{e}	$96.15 \pm 0.70^{b, c}$	99.70 ± 2.59^{a}
				Emulsion activity index ($m^2.g^{-1}$)		Foaming capacity (%)	
1	1	0	0	30.67 ± 0.09^{a}	4.26 ± 0.22^{a}	$20.00 \pm 5.00^{b, c}$	22.50 ± 2.50^{a}
2	0	1	0	15.31 ± 0.09^{b}	13.53 ± 0.07^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
3	0	0	1	26.55 ± 0.09^{c}	27.41 ± 0.07^{c}	52.33 ± 2.52^{a}	37.50 ± 2.50^{c}
4	1/2	1/2	0	14.62 ± 0.12^{d}	13.86 ± 0.09^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
5	1/2	0	1/2	22.95 ± 0.07^{e}	37.05 ± 0.15^{d}	28.33 ± 2.89^{b}	27.50 ± 2.50^{a}
6	0	1/2	1/2	23.95 ± 0.06^{f}	49.58 ± 0.07^{e}	45.00 ± 5.00^{a}	5.83 ± 1.44^{d}
7	1/3	1/3	1/3	22.19 ± 0.20^{g}	56.62 ± 0.06^{f}	12.33 ± 2.52^{c}	8.33 ± 1.44^{d}

Antioxidant activities

Run	Independent variables			ORAC (µmol Trolox EQ.g^{-1})		DPPH radical scavenging (%)	
	x_1	x_2	x_3	Control	Hydrolysates	Control	Hydrolysates
1	1	0	0	229.54 ± 8.04^{a}	1157.18 ± 134.66^{a}	27.18 ± 0.15^{a}	51.55 ± 0.56^{a}
2	0	1	0	52.64 ± 0.69^{e}	160.72 ± 26.26^{d}	17.13 ± 2.33^{b}	29.81 ± 0.48^{b}
3	0	0	1	125.56 ± 4.39^{b}	$546.45 \pm 55.75^{b, c}$	33.39 ± 0.26^{c}	31.50 ± 0.24^{b}
4	1/2	1/2	0	90.71 ± 3.45^{c}	$530.02 \pm 48.12^{b, c}$	21.06 ± 0.10^{d}	38.53 ± 0.63^{c}
5	1/2	0	1/2	$79.17 \pm 1.63^{c, d}$	595.88 ± 80.74^{b}	45.19 ± 1.86^{e}	47.84 ± 0.59^{d}
6	0	1/2	1/2	71.41 ± 0.41^{d}	$347.16 \pm 64.81^{c, d}$	29.41 ± 1.40^{a}	43.26 ± 0.94^{e}
7	1/3	1/3	1/3	$82.96 \pm 14.47^{c, d}$	$403.33 \pm 86.51^{b, c}$	36.60 ± 1.12^{c}	35.42 ± 0.61^{f}

[a, b, c...] Results are presented as the mean (n = 3) ± SD, and those with different letters are significantly different, with $p < 0.05$. x_1 – soy protein isolate (SPI); x_2 – bovine whey protein (BWP); x_3 – egg white protein (EWP).

The results observed in our study are in according with other researches. Intarasirisawat, Benjakul, Visessanguan and Wu (2012) studied the antioxidative and functional properties of protein hydrolysates from defatted skipjack (*Katsuwonous pelamis*) roe, hydrolyzed by Alcalase 2.4L using different assays (DPPH, ABTS, superoxide anion radical scavenging activities, reducing power and chelating capacity). The results showed that the antioxidant activities increased after enzymatic hydrolysis. For the functional properties, the enzymatic hydrolysis could increase protein solubility to above 80.0%.

The variation of the functional properties and antioxidant activities of the hydrolysates obtained from SPI, BWP and EWP is also shown using mixture contour plots, in which, each protein source is represented in a corner of an equilateral triangle; each point within this triangle refers to a different proportion of components in the mixture (Figure 1).

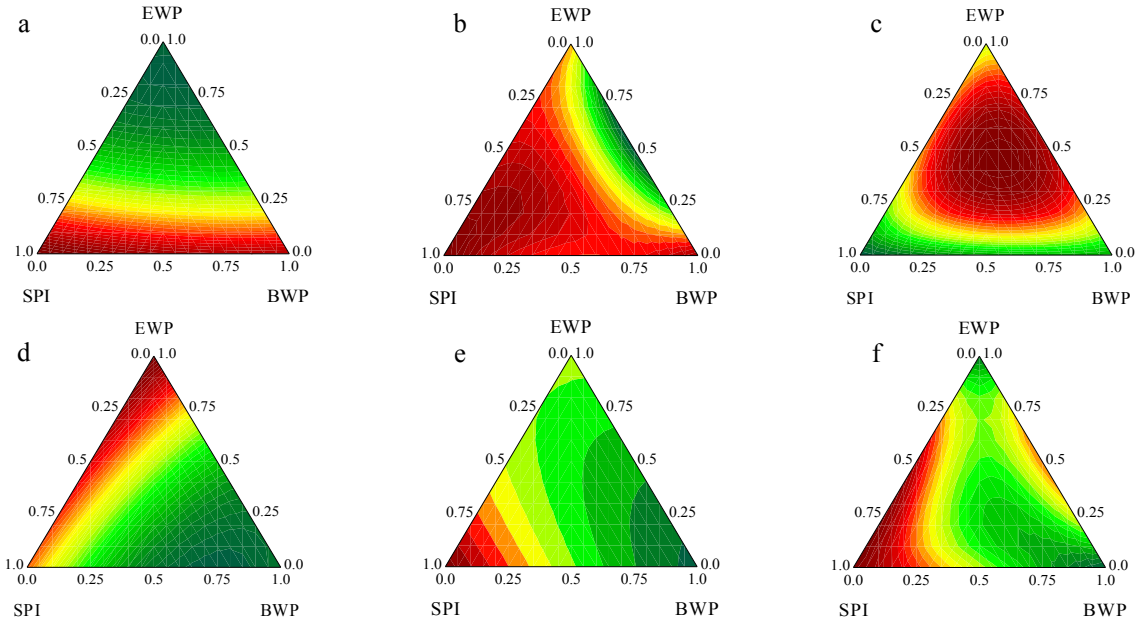

Figure 1 - Mixture contour plots for functional properties (a: solubility; b: heat stability; c: emulsion activity index; d: foaming capacity) and antioxidant activities (e: ORAC; f: DPPH) of the protein hydrolysates.

TABLE 2 contains the models, corresponding R^2 and adjusted R^2 of the regression equations for the responses as well as the corresponding *F*-ratio and *p*-values for each term in the predicted regression equations. The high coefficients of determination (R^2), which were above 0.80 (TABLE 2), indicate that all response functions adequately fit the experimental data, and the models can be used for predictive purposes in the determination of the functional properties and antioxidant activities using different proteins sources and their mixtures.

TABLE 3 – Models, R^2, adjusted R^2 and probability values for the final reduced models for functional properties and antioxidant activities.

Responses	Models	Equations	*F*-test	R^2	*p*-value
		Controls			
Solubility (%)	Quadratic	$Y = 11.23x_1 + 21.13x_2 + 82.18x_3 + 100.35x_1x_2 + 170.58x_1x_3 + 171.80x_2x_3$ (±0.99) (±0.99) (±0.99) (±4.55) (±4.55) (±4.55)	526.61	0.99	<0.001
Heat stability (%)	Special cubic	$Y = 101.66x_1 + 98.88x_2 + 92.90x_3 - 21.05x_1x_2 - 57.94x_2x_3 + 191.96x_1x_2x_3$ (±1.60) (±1.76) (±1.60) (±8.49) (±8.49) (±56.66)	5.75	0.85	<0.001
Emulsion activity index ($m^2.g^{-1}$)	Special cubic	$Y = 30.67x_1 + 15.31x_2 + 26.55x_3 - 33.49x_1x_2 - 22.64x_1x_3 + 12.08x_2x_3 + 78.55x_1x_2x_3$ (±0.07) (±0.07) (±0.07) (±0.32) (±0.32) (±0.32) (±2.25)	2,686.65	0.99	<0.001
Foaming capacity (%)	Special cubic	$Y = 20.00x_1 + 52.33x_3 - 40.00x_1x_2 - 31.33x_1x_3 + 75.33x_2x_3 - 330.00x_1x_2x_3$ (±1.84) (±1.84) (±9.00) (±9.00) (±9.00) (±63.38)	44.15	0.98	<0.001
ORAC (µmol Trolox $EQ.g^{-1}$)	Special cubic	$Y = 229.54x_1 + 52.64x_2 + 125.56x_3 - 201.52x_1x_2 - 393.52x_1x_3 - 70.75x_2x_3 + 567.71\ x_1x_2x_3$ (±3.83) (±3.83) (±3.83) (±18.77) (±18.87) (±18.87) (±132.06)	84.29	0.99	<0.001
DPPH radical scavenging (%)	Special cubic	$Y = 26.81x_1 + 16.76x_2 + 33.40x_3 + 60.35x_1x_3 + 17.33x_2x_3 + 62.34x_1x_2x_3$ (±0.70) (±0.70) (±0.77) (±3.73) (±3.73) (±24.87)	62.32	0.98	<0.001
		Hydrolysates			
Solubility (%)	Quadratic	$Y = 99.14x_1 + 86.49x_2 + 26.89x_3 - 18.16x_1x_2 - 94.53x_1x_3 - 81.98x_2x_3$ (±1.67) (±1.67) (±1.67) (±7.68) (±7.68) (±7.68)	101.66	0.99	<0.001
Heat stability (%)	Quadratic	$Y = 100.33x_1 + 86.86x_2 + 87.30x_3 + 52.75x_2x_3$ (±1.43) (±1.58) (±1.58) (±7.94)	6.42	0,80	<0.001
Emulsion activity index ($m^2.g^{-1}$)	Special cubic	$Y = 4.26x_1 + 13.53x_2 + 27.41x_3 + 19.86x_1x_2 + 84.87x_1x_3 + 116.44x_2x_3 + 458.51x_1x_2x_3$ (±0.07) (±0.07) (±0.07) (±0.33) (±0.33) (±0.33) (±2.36)	29,255.89	0.99	<0.001
Foaming capacity (%)	Quadratic	$Y = 21.63x_1 + 36.63x_3 - 44.29x_1x_2 - 50.96x_2x_3$ (±1.43) (±1.58) (±1.58) (±7.94)	86.52	0.98	<0.001
ORAC (µmol Trolox $EQ.g^{-1}$)	Quadratic	$Y = 1,160.81x_1 + 156.15x_2 + 541.89x_3 - 572.01x_1x_2 - 1,080.05x_1x_3$ (±43.01) (±39.34) (±44.18) (±197.59) (±197.59)	20.42	0.95	<0.001
DPPH radical scavenging (%)	Special cubic	$Y = 51.55x_1 + 29.81x_2 + 31.49x_3 - 8.62x_1x_2 + 25.27x_1x_3 + 50.42x_2x_3 - 260.64x_1x_2x_3$ (±0.35) (±0.35) (±0.35) (±1.72) (±1.72) (±1.72) (±12.09)	191.68	0.99	<0.001

CONCLUSION

The results suggest that the application of the statistical mixture designs for enzymatic hydrolysis using different protein sources is an attractive process for improving the performance and to find the optimum formulations. It was possible to observe the maximization of responses when the mixtures were used compared to the isolated substrates. The functional properties of the hydrolysates, as the high solubility, suggest that they could have wider applications in the formulated food systems. The maximum increases in antioxidant activities were observed in the formulation contained SPI (1/2) and EWP (1/2), reaching up to 600.0%.

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EFFECT OF BLANCHING CONDITIONS ON SULFORAPHANE CONTENT IN BROCCOLI

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ABSTRACT

Functional foods should be consumed as part of a normal diet, and they are composed of naturally occurring components, sometimes in increased concentration or present in foods that would not usually supply those. Since *Brassicaceae* such as broccoli have demonstrated to reduce cancer incidence and to improve the overall health status, they exhibit a high potential as functional foods. Sulforaphane, an isothiocyanate that comes from the hydrolysis of glucoraphanin (the main glucosinolate of broccoli) has been considered responsible for the cancer preventive effect of broccoli intake. The hydrolysis proceeds through the action of myrosinase, which is physically segregated from its substrate in the plant tissue. Then, in the intact broccoli no reaction occurs, and therefore the natural content of sulforaphane in broccoli is negligible. Sulforaphane synthesis is stimulated by plant tissue disruption, situation that can be triggered by processing. Besides, myrosinase activity can be enhanced by an adequate blanching step that exploits the optimal temperature of myrosinase and also inactivates the enzymes that compete with it for glucoraphanin.

A blanching step was optimized through surface response methodology (central composite design with 4 axial points and 5 central points), where the factors were water temperature (50 and 70°C) and immersion time (5 and 15 min). Sulforaphane measurements were made in triplicate for each run.

The statistical analyses show that the optimal blanching step consists of water immersion at 57°C during 14 min, resulting in maximum sulforaphane content in broccoli of 65 μg sulforaphane per g broccoli (dry weight).

Keywords: broccoli, sulforaphane, blanching, functional food

1. INTRODUCTION

Broccoli offers many heath-promoting properties owing to its high content of ascorbic acid, vitamin K, dietary fiber and carotenoids. Besides, it exhibits a high content of glucosinolates (GSL), a group of secondary metabolites that share a common basic structure comprising a β-D thioglucose group, a sulphonated oxime moiety and a variable side chain derived from amino acids (Jia et al., 2009). In recent years GSL have become popular due to the chemoprotective properties exerted by some of their hydrolysis products: isothiocyanates. Epidemiological studies have shown that consumption of a broccoli rich diet reduces the risk of developing some types of cancer. This anti-cancer effect has been related to glucoraphanin, a GSL whose hydrolysis results in sulforaphane [4-(methylsulfinyl) butyl isothiocyanate] (Manchali et al, 2012). Sulforaphane has been recognized as the most powerful anti-cancer compound derived from foodstuff (Matusheski et al., 2004). Besides, sulforaphane has been associated with the prevention of cardio vascular diseases (Wu et al., 2004) and arteriosclerosis (Kim et al., 2012).

In intact vegetal tissues, sulforaphane is absent, since its synthesis proceeds through the hydrolysis of glucoraphanin by the action of the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147), and this enzyme is differently compartmentalized in specific myrosin cells. When the vegetal tissue is broken by mastication, harvesting or processing, myrosin enters in contact with glucoraphanin and then the hydrolysis proceeds (Fahey et al., 2001). Nevertheless, depending on the chemical conditions, a competition reaction occurs through the action of the epithiospecifier protein (ESP), which results in sulforaphane nitrile, a non bioactive and potentially toxic compound (Mithen et al., 2003). At neutral pH the spontaneous conversion to sulforaphane proceeds, while as at acidic pH or in the presence of Fe^{2+}, the production of sulforaphane nitrile by the action of ESP is favored (Mahn and Reyes, 2012). Figure 1 shows a scheme of glucoraphanin hydrolysis. ESP is more thermo labile than myrosinase, and hence an adequate blanching step should inactivate it. The effect of hydrothermal treatment conditions of broccoli on glucosinolates content has been studied before (Sarvan et al., 2012), however, no study about the effect of blanching conditions on sulforaphane synthesis or content in broccoli has been published so far.

In this work, the effect of different blanching conditions on the sulforaphane content of broccoli (*Brassica oleracea L*. var. *italica)* was studied.

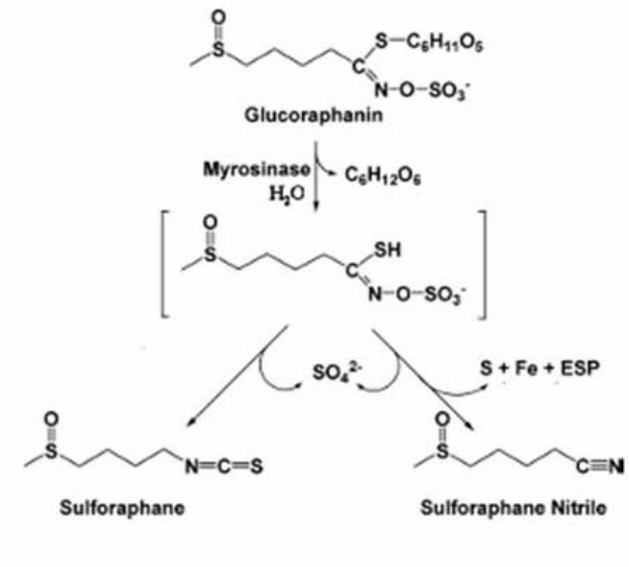

Figure 1. Mechanism of the hydrolysis of glucoraphanin (adapted from Matusheski et al. 2006).

2. MATERIAL AND METHODS

2.1. Chemicals

Sulforaphane standard, acetonitrile (HPLC grade), anhydrous sodium sulfate were purchased form Sigma-Aldrich (Schnelldorf, Germany) and methylene chloride was purchased from J.T. Baker (USA). HPLC - grade water was produced in the laboratory using an ultra pure water system (Barnstead, Thermo Scientific, Waltham, Massachusetts, USA).

2.2. Vegetable material

Broccoli heads were purchased at the local market (Santiago, Chile). All vegetables had three days from harvesting. Leaves and stems were discarded and the inflorescences were cut in 5 – 7 cm pieces (vertical).

2.3. Experimental design

Surface response methodology was used to study the effect of blanching conditions on sulforaphane content in broccoli, and also to determine the optimal conditions. A centered composite design was used, with two factors, four axial points and five central points. The experimental factors were water temperature (50 and 70°C) and immersion time (5 and 15 min). 300 g of vegetable were used in each run. Blanching was performed by immersion in

distilled water using a thermostatic bath (RE300, Stuart). After blanching, samples were immediately put in an ice bath, and were immediately analyzed.

2.4. Sulforaphane content

Sulforaphane content was assessed by reverse phase HPLC, using the method proposed by Liang et al. (2006) with some minor modifications. Fresh and blanched vegetable samples were pulverized with liquid nitrogen in a mortar, until obtaining a homogeneous meal. 5 g of the meal were left to autolyze at room temperature for 30 min. After that, the meal was extracted two times with 50 mL methylene chloride. Extracts were combined and salted with 2.5 g sodium sulfate anhydrous. The methylene chloride fractions were dried at 30 ºC under vacuum on a rotary evaporator (RE300, Stuart). The residue was dissolved in acetonitrile and was then filtered through a 0.22 μm membrane filter prior to injection into HPLC. The equipment was a HPLC-DAD (Agilent mod. 1110), and a reversed-phase C_{18} column (15.5 × 4.6 mm - i.d. 5 μm) was used. The solvent system consisted of 20% acetonitrile in water; this solution was then changed linearly over 10 min to 60% acetonitrile, and maintained at 100% acetonitrile for 2 min to purge the column. The column oven temperature was set at 30 °C. The flow rate was 1 mL/min, and 10 mL portions were injected into the column. Sulforaphane was detected by absorbance at 254 nm. Quantification was carried out by comparison with a sulforaphane standard curve.

2.5. Statistical analyses

The statistical analyses, including calculation of the standardized effects on sulforaphane content, were carried out according to standard procedures (Box et al., 1989), using the software Statgraphics Plus 5.1 (Statistical Graphics Corp., USA, 2000). A 95% confidence interval was considered.

3. RESULTS AND DISCUSSION

The results of the experimental runs and the control material (fresh broccoli) are given in Table 1. The highest sulforaphane concentrations were achieved at 60°C, independently of the immersion time. The lowest sulforaphane content were found at 70°C or higher temperature. This behavior is attributed to thermal inactivation of ESP at temperature below 60°C, and thermal inactivation of myrosinase at 70°C or higher temperature. These results agree with literature (Matusheski and Jeffery, 2001). From the results it is clear that blanching at temperature above 50°C and below 70°C results in a significant increase in sulforaphane

content, in comparison with the fresh vegetable. On the other hand, temperature above 70°C results in sulforaphane concentration significantly lower than that found in fresh broccoli.

TABLE 1. Sulforaphane concentration in blanched broccoli florets after the different treatments. Dw means dry weight.

Temperature (°C)	Time (min)	Sulforaphane (μmol/g dw)
Fresh broccoli		1.181
60	10	3.603
74	10	0.047
60	10	3.815
60	10	4.075
50	15	3.300
60	3	3.116
50	5	2.926
46	10	1.311
70	15	0.096
60	10	3.323
70	5	0.415
60	17	4.132
60	10	4.217

Figure 2 shows the Pareto diagram for sulforaphane concentration. The factor temperature, as well as the binary interaction with itself, had statistically significant effect on the response. This effect is negative, meaning that when temperature moves from the lowest to the highest level, sulforaphane concentration diminishes significantly. Besides, the significance of the binary interaction implies that there is an optimum value for this factor.

Immersion time had no significant effect on sulforaphane content, and therefore this factor would not be relevant for optimization of a blanching step.

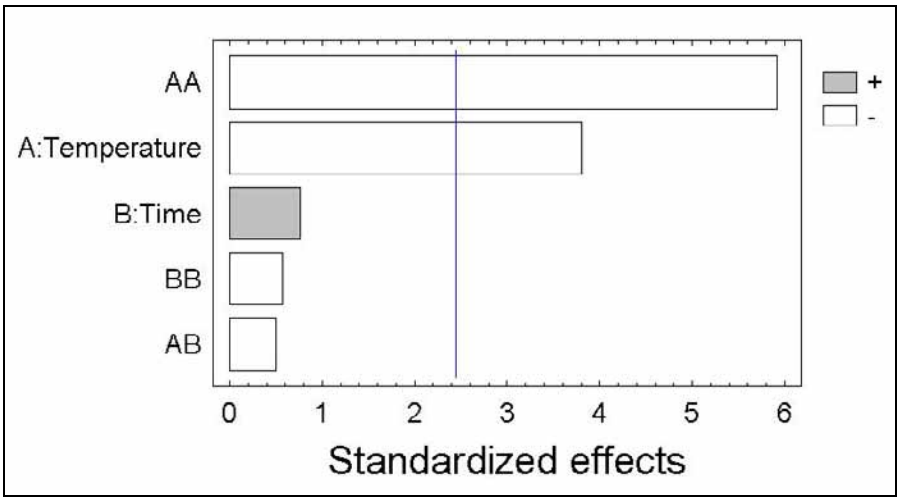

Figure 2. Pareto chart for sulforaphane concentration.

Figure 3 shows the response surface obtained from the statistical analysis for sulforaphane content. An optimum point can be distinguished, corresponding to 57°C and 14 min. The sulforaphane concentration in broccoli subjected to blanching in these conditions was 65 µg sulforaphane per g broccoli (dry weight).

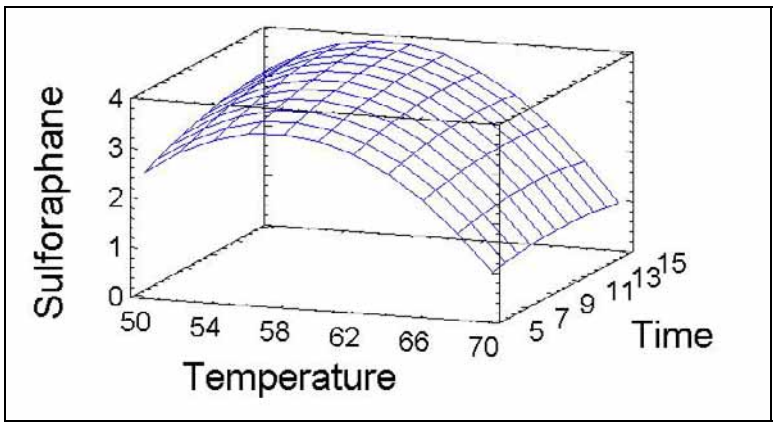

Figure 3. Response surface for sulforaphane concentration.

4. CONCLUSION

Water temperature significantly affected sulforaphane concentration in blanched broccoli. Immersion time had no statistically significant effect on the response. The optimal blanching step consists of water immersion at 57°C during 14 min, resulting in maximum sulforaphane content in broccoli of 65 µg sulforaphane per g broccoli (dry weight).

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THE INFLUENCE OF COPPER ON THE RYE MALT QUALITY

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ABSTRACT

Rye malt is the dried product of rye germinated under controlled conditions. It is widely used in the production of bread, food flavoring, as ingredient for bakery products and as color additives in the preparation of caramel. The purpose of the research was to investigate the influence of different copper concentrations on the content of malt extract, cooper and total phenols, as well as diastase activity in rye malt. Rye grain of 96% viability were soaked and germinated at temperature +6 ± 2 °C for 4 days, using Copper(II) sulfate pent hydrate $CuS0_4.5H_2O$ solutions (Cu concentration 10 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}), dried in the oven for 24 hours at temperature of +73 - 108 °C. The obtained results showed that the increasing of copper concentration in solution changes the content of malt extract (from 72 to 78%), the content of cooper in malt (from 13.1 to 126.3 mg kg^{-1}), the content of total phenol (from 2.47 to 3.12 mg GAE g^{-1}) and diastase activity (from 327 to 338 Gothe units g^{-1} of malt) in rye malt comparing with control sample.

Keywords: copper**,** rye, malt extract, diastase activity, total phenols

INTRODUCTION

Malt is a natural food product produced by germinating grains. The three steps of malting, steeping, germinating, and kilning, can be altered to produce different types of malt (Edney and Izydorczyk, 2003). Rye malt is the dried product of rye germinated under controlled conditions and is widely used in the production of bread, food flavoring, as ingredient for bakery products and as color additives in the preparation of caramel.

The quality of malt depends on the chemical composition of grain and of the processing parameters. The chemical composition of native grains varies significantly depending on the growing environment and the genotype. Variations in the composition may also arise from

differences in harvesting and post-harvesting conditions or from the handling of the grain prior to its use (Heiniö et al., 2008).

Copper is found in all living organisms in the oxidized Cu(II) and reduced Cu(I) states. It is required for survival and serves as an important catalytic cofactor in red ox chemistry for proteins that carry out fundamental biological functions that are required for growth and development (Tapiero, 2003).

Copper (Cu) is an essential micronutrient for plants. Present at a high concentration in soil, copper is also regarded as a major toxicant to plant cells due to its potential inhibitory effects against many physiological and biochemical processes (Weinstein et al., 2011; Vinit-Dunand et al., 2002). In plants, copper interacts with a wide range of physiological and biochemical processes in cells, and elevated copper concentration inhibits the normal growth and development (Ahsan, 2007). It is known that metal sensitivity and toxicity to plants are influenced by not only the concentration and the toxicant types, but are also dependent to several developmental stages of the plants. Seed germination is one of the most highly sensitive physiological processes in plants and is regulated by several hormonal interaction and environmental factors. In addition, seed germination is more sensitive to metal pollution because of a lack of some defense mechanisms (Ahsan, 2007).

The average intakes of copper by human adults vary from 0.6 to 1.6 mg/d and the main sources are seeds, grains, nuts, and beans (concentrated in the germ and bran), shellfish and liver (Tapiero, 2003). Copper in organisms, particularly plants, participates in protein synthesis, membrane activities, and photosynthesis (Weinstein et al., 2011; Vinit-Dunand et al., 2002).

There is match investigation about metal(loid) homeostasis, detoxification and tolerance in relation to seed metabolism and performance (Kranner and Colville, 2011; Vinit-Dunand et al., 2002), the delivery of metals from the mother plant into seeds and their implications on seed development (Weinstein et al., 2011) their uptake upon seed imbibition and subsequent effects on seed germination (Ahsan, 2007; Kranner and Colville, 2011), but information about Cu influence on the rye malt quality is scarce.

The aim of this research was to investigate the influence of different cupper concentrations on the quality of rye malt – content of malt extract, diastase activity, total phenols and copper accumulation degree in rye malt.

MATERIALS AND METHODS

Plant material

The research object was rye grain (variety ´Kaupo´) from Ltd. ´Naukšēni´, harvested in 2011. 3 kg of rye grain were soaked for 48 h in 10 L Copper(II) sulfate pentahydrate $CuS0_4.5H_2O$ solutions and germinated for 48 h at temperature of +6 ± 2 °C. The concentration of copper was 10, 50, and 100 mg L^{-1}. The germination of grain with deionized water served as a control. After germination, all sprouts were dried for 24 h at a temperature of +73 - 108 °C; then they were ground. Moisture of malt samples ranged from 8.9% till 10.2%.

Determination of Cu

ICP-MS measurement method. Analyses were performed with a Perkin-Elmer Elan 6000 ICP-MS. The ICP-MS apparatus is equipped with cross flow nebulizer and a quartz torch. The instrumental operating parameters are listed in Table 1. The instrument with Ni sampler and skimmer cones has been optimized daily.

TEBLE 1. Instrumental parameters for ICP-MS operation

Parameters	Numerical value
Plasma gas flow rate, L/min	**15**
Auxiliary gas flow rate, L/min	1.2
Nebulizer gas flow rate, L/min	0.99
Dwell time, ms	50
Sweeps per reading	30
Replicates	5
RF power, W	1250

Five point calibration graph method was performed for all elements using Perkin Elmer multielement standard solutions by serial dilution method. The regression coefficients for of ^{63}Cu and ^{65}Cu isotopes calibration graph were better than 0.999. In all experiments reagent blanks were taken separately. Mean concentration of isotopes was used for the expression of analysis result

Determination content of malt extract

Content of unfermented malt extract was determined by hot extraction method.

200 ml of 47 ^{0}C warm distilled water was added to 50 g ground malt, hold for 30 minutes at 45 ^{0}C water bath regularly stirring. Then heat up to 70 ^{0}C and add 100 ml heated distilled

water up to 70 ^{0}C. Stirring constantly, hold for 1 hour, distilled water was added till 450 ml, mixed and filtered. The density of filtrate was determined and the mass of extract was found. Content of malt extract was calculated on the air-dried substance E_1 %, as follows:

$E_1 = \frac{e(W+800)}{100-e}$, where

e – extract mass of the filtrate, %;

W – malt moisture, %

Determination of diastase activity with Phadebas

The unit of diastase activity, the Gothe unit, is defined as that amount of enzyme which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40 ^{0}C under the conditions of test. Results are expressed in Gothe units per gram of malt.

The diastase activity of samples was measured with the Phadebas method. A tablet of an insoluble blue-dyed, cross-linked starch was used as the substrate for the degradation reaction. After dissolving 1.00 g of malt in the acetate buffer in a volumetric flask, 5.0 mL of the malt solution was transferred to the test tube and incubated in a water bath at 40 ^{0}C for a few minutes. A blank was prepared by adding 5.0 mL of the acetate buffer solution and was treated in the same manner as a sample solution. After placing the Phadebas tablets into both test tubes, a timer was started. The tubes were quickly removed from the water bath, stirred and then returned to the water bath. After 30 min, the reaction was terminated by adding 1.0 mL of 1 M sodium hydroxide solution. The mixture was stirred again and filtered. The absorbance of the sample was measured at 620 nm with deionised water as a reference. The absorbance of the blank was subtracted from that of the sample solution (DA_{620}). The diastase activity, expressed as DN or diastase number, was calculated from the absorbance measurements, respectively:

$DN=28.2\times\Delta A_{620}-2.64$

Diastase activity was referred to as DN in the Schade scale, which corresponds to the Gothe scale number, or g, of starch hydrolysed per hour at 40 ^{0}C per 100 g of malt (Sak-Bosnar and Sakac, 2012).

Determination of total phenol content (TPC)

The total concentration of phenolic compounds was determined as gallic acid equivalents (GAE) using the Folin Ciocalteu spectrophotometric method with some modifications

(Singleton et al., 1999) methanol was used as solvent and the total volume of the reaction was 1mL.

Statistical analysis

The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant value were calculated. *P*-values<0.05 were regarded as significant.

RESULTS

Whole grains contained higher levels of minerals compared to flours due to the presence of the outer kernel layers where minerals are concentrated. Rye appears to be rich in phosphorus P (3620 mg kg^{-1}), potassium K (3570 mg kg^{-1}), magnesium Mg (1328 mg kg^{-1}), iron Fe (43.0 mg kg^{-1}) and manganese Mn (24.4 mg kg^{-1}), while copper Cu content comply 2.9 mg kg^{-1} (Ragaee, 2006). The delivery of metals to seeds depends upon uptake by the mother plant, from the soil and subsequent transport to developing seeds (Kranner and Colville, 2011). Other studies have demonstrated that it is also dependent on many environmental factors: concentrations in the environment, abiotic factors, exposure time, growth form of the plant, and type of absorption mechanism, affinity of trace elements for the adsorption sites, element speciation, and sampling period (Bonanno, 2011).

The obtained results (Fig.1) confirm the values mentioned above, but it is below the quantities showed in literature: 4.99±0.65 mg kg^{-1} (Shtangeevaa et al., 2011).

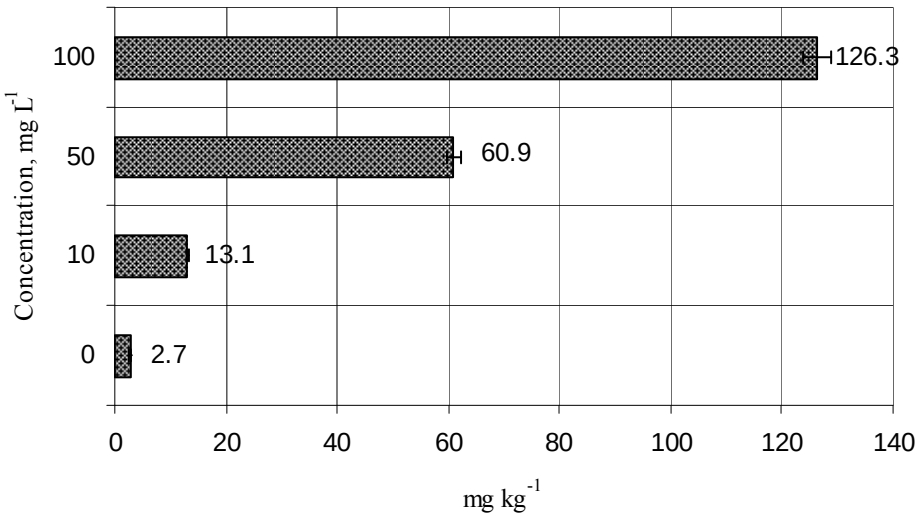

Fig.1. The content of copper in rye malt depending on the concentration of Cu

The obtained results (Fig.1) showed that content of copper in malt depends on the concentration in solution. It increases with increasing of Cu concentration in solution. If concentration of copper in solution is 10 mg L^{-1}, the content of copper in malt increases for 3.85 times, but at concentration 50 mg L^{-1} it increases for 21.6 times compared with control sample. The highest increase for 45.8 times of copper content was observed at concentration 100 mg L^{-1}.

The significant differences of development of sprouts were observed after 48 h of germination. The length of sprouts (8–10- mm) was similar for control sample and sample were concentration of copper in solution 10 mg L^{-1}. But if concentration of copper was 50 mg L^{-1} and 100 mg L^{-1}, the length of sprouts was only 1-3 mm. It gives evidence that concentration of Cu more than 50 mg L^{-1} have an inhibiting effect on the biological processes in grain during germination.

The amount of extractable material is key factor in the suitability of malts production. The main part of the extract comes from starch, which is broken down during malting. The development of extract is a balance of two effects. On the one hand, an increase in the extract content is caused by an improved extractability of compounds, proteins, starch and other poly- saccharides. The breakdown of cell wall structures, which becomes more pronounced with longer germination times, allows access of water to the inner parts of cells. On the other hand, a decrease in the content of extractable substances is caused by the respiration of the embryo. The glucose molecules resulting from starch degradation are used as energy source. Other extractable compounds are used for rootlet growth and become unavailable. These compounds are used for the synthesis of new macromolecules in order to establish structural elements in the growing acrospires and rootlets (Hübner et al., 2010).

The results of quality indices of rye malt are summarized in the Table.

The results of research (Table 1) show evidence that changes of malt extract content depend on the Cu concentration in solution.

TABLE 1. The quality indices of rye malt

Concentration of copper (mg L^{-1})	Content of malt extract (%)	Diastase activity (Gothe units)
0	73,6±0,4	300 ±18
10	78,45±0,3	327±22
50	75,6±0,8	338±27
100	72,4±0,7	332±29

The highest content of malt extract (78.45%) was obtained when copper concentration was 10 mg L^{-1}. As mentioned above the biological processes taking place during germination of rye grain are closely connected with copper concentration in solution and hence on the Cu content in grain. High Cu concentrations 50 mg L^{-1} and 100 mg L^{-1}) prevent the development of sprouts, but didn't influence the content of malt extract significantly.

In 1833, precipitated malt extract was observed to produce a sugar and at that time the mysterious material responsible for the reaction was termed as 'diastase' from the French word meaning 'separation' and then onwards diastase became the generic term for amylases (Muralikrishna and Nirmala, 2005). Cereal α-amylases play a important role in the starch metabolism in developing as well as germinating cereals. The amount of enzyme depends on different factors, such as type of cereal, duration of germination, temperature and environment (Mark et al, 2008; Kádár et al., 2011).

Evaluating the obtained data regarding rye malt diastase activity, it could be concluded that the influence of copper was observed (Table 1). In the sample with copper concentration 10 mg L^{-1} the diastase activity increases for9 %, at concentration 50 mg L^{-1} -for 12,7%, but if Cu concentration was 100 mg L^{-1} the diastase activity increases for 10,1% comparing with control sample. Similar tendencies are can observed to analyze the data regarding total phenols content in rye malt.

Whole-grain cereals are a major source of polyphenols, especially phenolic acids such as ferulic, vanillic, caffeic, syringic, sinapic and p-coumaric acids. All of them have potentially antioxidant properties due to the presence of an aromatic phenolic ring that can stabilize and delocalize the unpaired electron within its aromatic ring. They are believed to act mainly as free-radical scavengers, and/or chelators of transition metals (minerals or trace elements) (Fardet et al., 2008). Some phenolic compounds have flavors which are thought to contribute to the bitter taste of whole meal rye bread (Bondia-Pons et al., 2009).

The total phenols content in rye malt have been little studied. Ragaee (2006) has indicated that the content of total phenols in barley and oat whole grains was found to be higher than that of wheat and rye and lower than that of buckwheat (Ragaee, 2006).

The results of study (Fig.2) show the connections between content of microelement copper and content of total phenols in rye malt.

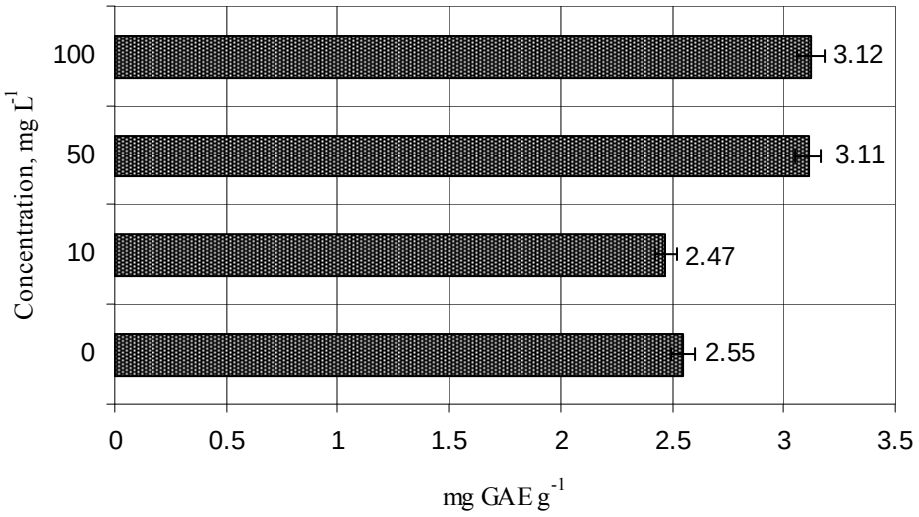

Fig. 2. The content of total phenols in rye malt depending on the concentration of copper

As it seen in Figure 2, all analyzed copper concentrations have an impact on the content of total phenols. The highest increase of total phenols content for 22% was determined at copper concentration in solution 50 and 100 mg L^{-1}.

CONCLUSIONS

The content of copper in rye malt increases in proportion with increase of copper concentration in solution. The changes of sprout development comparing with control sample can`t observed at copper concentration 10 mg L^{-1}. The concentration of Cu in solution 50 and 100 mg L^{-1} starts to inhibit biological processes in grain during germination. The better results of malt quality indices - content of malt extract and diastase activity can be reached at copper concentration 10 and 50 mg L^{-1}. The content of total phenols increases at copper concentration 50 and 100 mg L^{-1}.

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FREEZE-DRYING OF SOYMILK

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ABSTRACT:
Atmospheric freeze drying (AFD) and vacuum freeze drying (VFD) of soymilk was investigated. A factorial design was used in each case. In a VFD the experimental factors were particle diameter (3.6 and 5.5 mm) and pressure (0.6 and 1.2 mmHg), while in AFD the factors were particle diameter (3.6 and 5.5 mm) and temperature profile during the process (the whole process at -5ºC, the first 5 hours at -5ªC and afterwards at 5ºC). The effect of these factors on moisture content, duration of drying process and color were determined.

The frozen spheres of soymilk were obtained by dropping from a burette to a liquid nitrogen tank. The size was adjusted by the diameter of the discharge tube of the burette.

In most runs the final moisture content was lower than 5%, achieved in around 10 hours drying. The VFD-dried product kept the spherical shape, although spheres were fragile. Several AFD-dried particles collapsed. Both VFD and AFD conditions produced a notorious increase in the yellowness of the final product. This effect was more significant in AFD-dried particles.

The freeze-drying curves were adjusted to the Simplified Constant Diffusivity Model (SCDM), whose adjustment parameter is effective diffusivity (D_{eff}). The D_{eff} values obtained were found in the expected range for porous particles in freeze drying process, and were in the order of 10^{-10} to 10^{-13} m^2/s.

Keywords: soy milk, vacuum freeze-drying; atmospheric freeze-drying; effective diffusivity.

1. INTRODUCTION

1.1 Soy milk

Soy milk comes from soy bean (*Glycine max*), which is yellow and exhibits an oval shape with a 3 to 6 mm diameter. Soy milk has high protein content, being a good alternative to supplement conventional protein sources such as cow milk and meat. Besides, since soy milk does not contain lactose, it is suitable for lactose-free diets. Soy bean has also phospholipids,

mainly lecithin, which favors lipids absorption (Cheftel, 1989). Vegetal proteins have shown to reduce cholesterol level in blood. Additionally isoflavones present in soy bean inhibit cancer cells proliferation. As a consequence, the world demand for soy-based foods has increased in the last years (Friedman & Brandon, 2001).

Depending on the obtaining method, soy milk may have off-flavors, due to oils and casein present in the bean. However, through deodorization and the addition of different additives it is possible to improve soy milk taste and aroma, resembling cow milk. A good soy bean extraction process should address the following issues (Riaz, 2006):

- Extract the maximum soluble protein.
- Achieve the desired flavor and texture.
- Inactivate enzymes to avoid side reactions.
- Stabilize the product to prolong shelf-life.
- Guarantee the nutritional value.
- Be economically feasible.

The traditional method for soy milk production considers the following consecutive steps (Riaz, 2006): hydration at room temperature of the soy bean; grinding of hydrated soy bean; cooking and a final filtration step. This allows obtaining a liquid extract (soy milk) and a fiber-rich cake (okara).

In order to preserve the soy milk, water activity must be reduced, then and alternative is dehydration. Several authors have informed the effect of type of dryers and drying conditions on the overall quality of the dehydrated product (Sobral et al., 2011; Hu et al., 2010).

1.2 Freeze Drying

Freeze drying (FD) is usually considered to be the drying method that gives dried products of the best quality, in sensitive substrates that are negatively affected by conventional drying processes, such as pharmaceutical products and medicinal and/or aromatic herbs (Ratti, 2001; Reyes et al., 2011).

Freeze drying consists of (1) freezing of a wet solid (or liquid) material; (2) a primary drying stage, in which sublimation of frozen free water occurs; and (3) a secondary drying stage, in which bound water is eliminated by desorption.

1.2.1 Freezing stage. The product must be cooled down (frozen) in order to minimize the changes that it will undergo in the following sublimation step. Usually the aim is for the frozen product to have a solid structure minimizing any interstices with residual liquid, so that

later drying takes place by sublimation. The freezing stage has a great influence on the overall efficiency of the lyophilization process because it sets the structure of the ice crystals (shape and size) which affects the heat and mass transfer rates, and therefore the subsequent sublimation (drying) stages.

1.2.2 Primary Drying Stage. Initially, due to the energy supply, the sublimation rate increases rapidly until it reaches a maximum. The time to complete this stage is relatively short, with most of the water being removed (65–90%), with heat transfer occurring predominantly by conduction through the porous layer. Strictly, sublimation takes place only in this stage.

1.2.3 Secondary Drying Stage. This stage involves the removal of the unfrozen water (bound water) and it begins when the ice has already been removed by sublimation. The bound water (10–35% of the total moisture content) appears in two forms: physically adsorbed and crystallized. The process of bound water removal is slower and it markedly affects the overall drying time.

1.2.4 Types of lyophilization processes. To remove moisture at a temperature below 0ºC, freeze drying requires high vacuum to increase the mass transfer gradient between the sublimation front and drying medium. However, the process is slow and it involves high investment and operating costs due to the need to produce and maintain a high vacuum (Ratti, 2001). To reduce costs, two alternatives have been studied: (1) inclusion of an additional energy source (such as infrared radiation or microwave) and (2) atmospheric freeze drying (AFD) (Claussen et al., 2007; Kudra and Mujumdar, 2009).

1.3 Fitting Drying Curves

The usual approach to modeling mass transfer in foods employs the concept of effective diffusivity (D_{eff}), which allows describing the diffusion of moisture by Fick's second law.

$$\frac{\partial \dot{X}}{\partial t} = \nabla \cdot (D_{eff} \nabla \dot{X}) \qquad (1)$$

Equation (1) can be integrated for different geometries, boundary/initial conditions, and special physical considerations (Crank, 1975), giving origin to different mathematical models for describing the drying process. In this work, it is assumed that during the drying process the effective diffusivity (D_{eff}) remains constant, as well as the conditions of the drying air, and

the solid to be symmetrical with respect to a unidirectional diffusion coordinate. The frozen soymilk particles are spherical. Equation (1) was solved with the following initial and boundary conditions:

$$I.C. \qquad \dot{X} = X_o; \quad t = 0; \forall R \le R_o \qquad (2)$$

$$B.C.1 \qquad \frac{\partial \dot{X}}{\partial R} = 0; \quad R = 0; \quad \forall\, t > 0 \qquad (3)$$

$$B.C.2 \qquad \dot{X} = X^* \quad ; R = R_o; \quad t > 0 \qquad (4)$$

The solution of Eq. (1) under conditions 2, 3, and 4, in terms of the average moisture content (X) instead of the local moisture content, is given by Eq. (5) (Reyes, 2012):

$$\frac{X(t) - X^*}{X_o - X^*} = 6\sum_{n=1}^{\infty} \frac{1}{(n\pi)^2} \exp\left[-(n\pi)^2 D_{eff} \cdot t / R_o^2\right] \qquad (5)$$

By omitting the factor $6/\pi^2$, and considering only the first term of the series, the Simplified Constant Diffusivity Model (SCDM) is obtained, which is given by Eq. (6). The SCDM allows a semi empirical estimation of D_{eff} in which the external resistance to mass transfer is negligible.

$$\frac{X(t) - X^*}{X_o - X^*} = \exp\left[-\pi^2 \frac{D_{eff}}{R_o^2} \cdot t\right] \qquad (6)$$

2. EQUIPMENT, MATERIALS AND METHODS

2.1 Equipment

Figure 1 shows a scheme of the experimental freeze-dryer where air was circulated by a centrifugal blower of 2HP (A), its temperature was adjusted by a cooling system (B), and air as dried in a fixed bed of silica gel (C). Then, the air stream passed through an air plate distributor (a perforated supporting grid with free cross-sectional area of 23%) (D), and passed through two baskets containing the particles. Figure 2 shows a scheme of the vacuum freeze-dryer (Labconco, USA). The condensation systems achieve a minimum temperature of -48ºC.

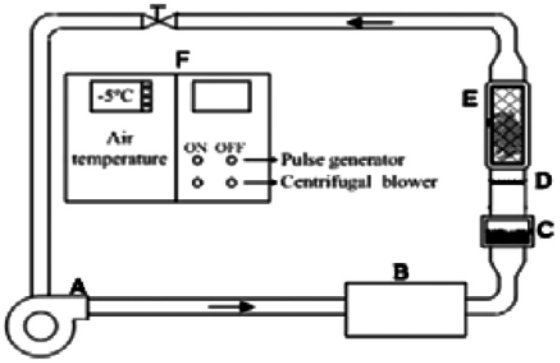

Figure 1. Atmospheric freeze-dryer in a fixed bed. A: centrifugal blower, B: air cooling system, C: silica gel, D: air distributor plate, E: freeze-drying chamber, F: control panel.

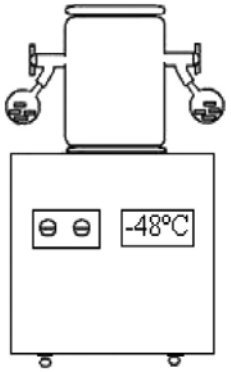

Figure 2. Vacuum freeze-dryer (Lab Conco)

3.1 Experimental procedure

3.1.1 Preparation of soymilk. Green, black and dotted beans, as well as impurities, were discarded. After that, beans were washed. Since husk enzymes trigger undesirable reactions, husk must be removed before processing. This removal was done by manually rubbing the beans while immersed in water. For each run, 0.5 kg soy beans were processed to obtain about 1.8 L soymilk, which was refrigerated at 3°C and it was stored during no more than 2 days. Soymilk production consisted of the following steps:

a) Blanching: the clean, hulled beans were immersed in 1.5 L distilled water at 80°C during 20 min; after that beans were cooled down in water at 15°C.
b) Soaking: soybeans were soaked in distilled water at ambient temperature during 20 hours. Then, the water was discarded and replaced by fresh distilled water.
c) Grinding: The beans-water mixture was crushed in a household homogenizer, obtaining a pulp.
d) Thermal treatment: the pulp coming from the previous step was kept at 55°C during 20 min.
e) Filtration: the pulp passed through a sieve, to separate okara from soymilk.
f) Pasteurization: the soymilk was kept at 70°C during 30 min.

3.1.2 Preparation of soymilk spherical particles. The frozen soymilk spheres were obtained by dropping from a burette to a liquid nitrogen tank. The size was adjusted by the diameter of the discharge tube of the burette to 3.6 ± 0.3 and 5.5± 0.3 mm.

3.1.3 Drying Procedure. In atmospheric freeze-drying (AFD), silica gel (2 kg) was loaded in the corresponding compartment (Fig. 1 [C]), the temperature of -5ºC was set on the cooling system (Fig. 1 [B]), and the air velocity was set on 2 m/s. Once the system reached a steady state and thermal stabilization at ±1ºC, about 20 grams of spherical particles were loaded in two perforated mesh baskets, putting one basket above the other. The initial mass was measured with a balance (Boeco BBL62, Boecktel Co., Germany) with an accuracy of ± 0.01 g. After 0.5 h, the baskets were taken out from the equipment, weighed, and then put back in the drier. The position of the baskets in the drier was interchanged each time this procedure was done. This procedure was repeated every 0.5 hour during 10 hours. Silica gel was replaced every 2 hours to ensure air dryness. The moisture content was determined in a vacuum oven until constant weight was attained, according to AOAC 920.151.

In vacuum freeze-drying (VFD), the refrigeration system was turned on and when the condenser temperature reached -48ºC the vacuum pump was turned on. About 20 g of spherical particles were put inside a glass container and the initial mass was measured. When the pressure in the drying chamber was equal to 0.6 mmHg or 1.2 mmHg (see Table 1), the container was put back in its previous position inside the equipment. After 0.5 hour, the glass container was taken out from the equipment, weighed, and then put back. This procedure was repeated every 0.5 hour during 10 hours.

Table 1. Experimental Design for VFD

Run	Pressure [mmHg]	Particle diameter [mm]
V1	0.6	3.6
V2	1.2	3.6
V3	0.6	5.5
V4	1.2	5.5

3.2 Experimental Design

For each type of drying (AFD and VFD) a factorial 2^2 design in two blocks was used. In VFD (Table 1) the experimental factors were particle diameter (3.6 and 5.5 mm) and pressure (0.6 and 1.2 mmHg), while in AFD (Table 2) the factors were particle diameter (3.6 and 5.5 mm)

and temperature profile during the process (the whole process at -5ºC, the first 5 hours at -5°C and afterwards at 5ºC). The effect of these factors on moisture content, duration of drying process and color were determined.

Table 2. Experimental Design for AFD

Run	Air temperature profile [°C]	Particle diameter [mm]
A1	-5 (constant)	3.6
A2	-5 (first 5 hours); +5 (onwards)	3.6
A3	-5 (constant)	5.5
A4	-5 (first 5 hours); +5 (onwards)	5.5

4. RESULTS

4.1 Drying kinetics

Figure 3 shows the experimental drying curves and predictions made with the SCDM. In most runs, the moisture content was below 0.1 (DW) after 10 hours drying, except in AFD with 5.5 mm diameter particles (Figure 3b). The curves show an increase of drying rate with a decrease of particle diameter, in both AFD and VFD, and a significant increase in drying rate in AFD when drying air temperature increased from -5°C to +5°C. The effect of pressure was slight in VFD.

The statistical analysis (95% confidence) shows that in VFD both pressure and particle diameter had no significant effect on final moisture content. In AFD, since the air temperature profile differed between the runs only form the 5^{th} hour onwards, the statistical analysis considered this time range. In this case, drying air temperature and particle diameter significantly affected final moisture content.

4.2 Effective diffusivity

Table 3 shows effective diffusivity (D_{eff}) estimated in each run. In AFD runs where the temperature profile changed, two D_{eff} were estimated using SCDM by error minimization between experimental and estimated values (Eq. 6).

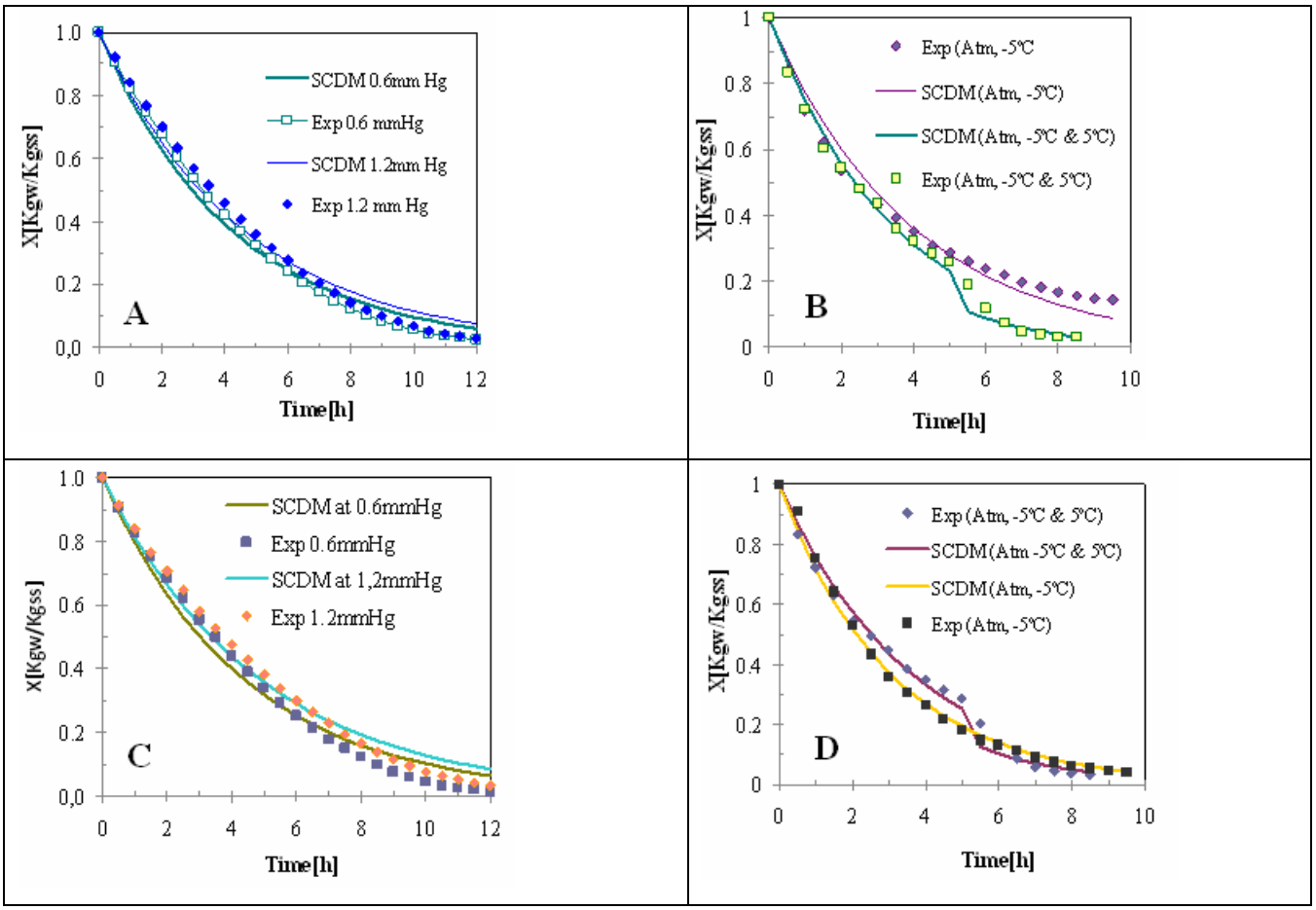

Figure 3. Drying kinetics. A)VFD with Dp =3.6 mm ; B) AFD with Dp = 5.5 mm; C)VFD with Dp= 5.5 mm; D) AFD with Dp= 3.6 mm

Table 4. Effective diffusivity (D_{eff}) and determination coefficient (R^2) for the SCDM.

	Run	Time [h]	D_{eff} [m^2/s] x 10^{11}	R^2
	V1	0 - 10	2.14	0.9949
	V2	0 -10	1.97	0.9932
VFD	V3	0 -10	4.90	0.9912
	V4	0 -10	4.40	0.9925
	A1	0 -10	2.34	0.9913
		5 -10	2.68	0.9957
AFD	A2	0 - 5	6.27	0.9859
	A3	0 -10	7.00	0.9977
		0 - 5	5.87	0.9936
	A4	0 - 5	8.07	0.9677

4.3 Appearance and color

All dehydrated simples resulted fragile, due to their high porosity, especially those subjected to AFD. Most of the AFD particles were broken to give semispherical fragments (Figures 4-c and 4-d).

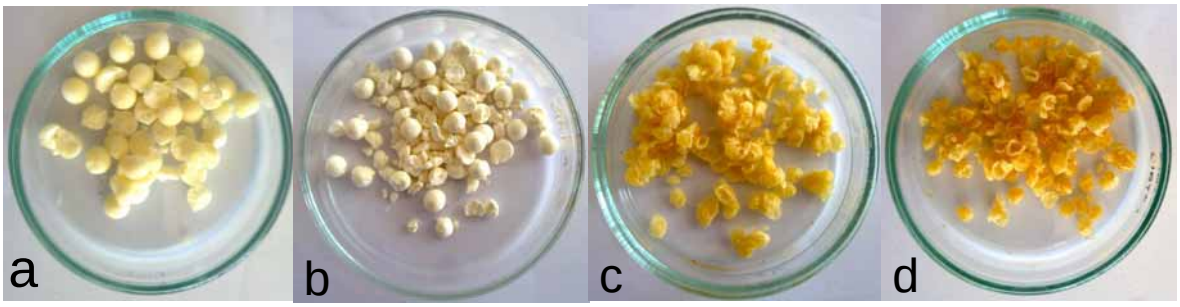

Figure 4. a) frozen particles before drying; b) VFD samples; c) AFD at -5°C samples (run A1); d) AFD at -5 and +5°C samples (run A2).

Considering the color parameters and the classification reported by Chen and Mujumdar (2008), the color difference between frozen and freeze-dried soymilk is considerable. Besides, all treatments significantly affected color parameters, being much important in AFD.

5. CONCLUSIONS

In most runs the final moisture content was lower than 5%, achieved in around 10 hours drying. The VFD-dried product kept the spherical shape, although spheres were fragile. Several AFD-dried particles collapsed.

The experimental factors had no significant effect on final moisture content in VFD, while in AFD temperature and particle diameter significantly affected this response from the 5th hour onwards.

The SCDM gave adequate predictions of drying kinetics in all runs ($R^2 > 0.991$). The estimated D_{eff} values were in the order of 10^{-10} to 10^{-13} m^2/s, agreeing with literature in freeze-drying processes.

Both VFD and AFD conditions produced a notorious increase in the yellowness of the final product. This effect was more significant in AFD-dried particles.

ACKNOWLEDGMENTS

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STUDY OF ALGINATE IMMOBILIZED *BURKHOLDERIACEPACIA* LIPASE IN ESTERIFICATION REACTION TO ISOAMYL ACETATE PRODUCTION

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ABSTRACT

Isoamyl acetate (banana flavor) production was investigated using immobilized *Burkholderiacepacia* lipase in sodium alginate by esterification of acetic acid/isoamyl alcohol in solvent system. Various solvents were tested: acetone, chloroform, toluene, hexane and heptane. On 5thdays, heptane was the solvent which maintained activity in the immobilized enzyme. For the experiments, the enzymatic extract was lyophilized and immobilized in solution of sodium alginate. The effect of acid to alcohol molar ratio (0.1, 0.3 and 0.5 M) and the lipase concentration (10, 20 and 30% w/v) on the response of esterification reaction was detected on the response of ester production yield. Aliquots were taken 0, 3, 6, 9, 12, 24, 48, 72 and 120 hours and titrated with 0.02 M KOH with fenoftalein used as indicator. Higher conversion of isoamyl acetate (92%) was achieved using a molar ratio 0.5M andlipase concentration of 30% w/v.

Keywords: lipase, *Burkholderiacepacia*, esterification, isoamyl acetate, alginate.

1. INTRODUCTION

Esters are one of the most important classes of organic compounds and can are synthesized by reaction between alcohols and carboxylic acids. Short-chain esters are compounds with a great application mainly in industries due to their characteristic fragrance and flavour. The products of short chain (2-8 carbon atoms) acids and alcohols are important flavor and fragrance components in food, beverage, cosmetic and pharmaceutical industries (Malcata et al, 1990). Reactions of ester formation are generally slow and require theuse of suitable

catalyst so that they become economically viable.The traditional catalytic processes are involving acids and bases as acceleratorsreaction (Morrison and Boyd, 1996). However, these catalysts have gradually beensubstituted with enzymes, as these terms have the following benefitsmild operation, specificity and fewer losses in reaction (Gabelan, 1994).Most of the esters are obtained by traditional methods of chemical synthesis or extraction from natural sources. Commercial esters can be directly obtained by extraction from plant materials, but the high cost and low quantities ofthe obtained productmake this technique inadequate for industrial applications. The interest in obtainingnatural esters boosted the flavor industry to search for routesalternatives. In the last decade biotechnology has been considered for the productionesters, since the aroma obtained can be considered as "natural". Thebiotechnological processes offer many advantages and are shown as acompetitive alternative to chemical methods due to the high efficiency catalytic, conditionsoperational mild and selectivity of natural catalysts (Welsh et al, 1989, Romero et al,2007).

The use of biotechnology appears to bequite attractive in a variety of ester preparations under milderconditions and the product may obtain the “natural” label.Isoamyl acetate is one of the most employed esters in foodindustry because of its banana flavour property. In theUSA alone, more than 74 tons of this ester is used in food industry (Welsh et al, 1989). Biotechnological production of flavor ester with lipases is important and lipase-catalyzed reactions have recently received greater consideration relative to the traditional chemical synthetic methods. This facility using lipases is due to the region and stereospecificity expressed by most lipases, the mild operation conditions, the degree purity of the products and their acceptability in the food industries. Moreover, obtaining natural esters by microbial fermentation can be produced in the time short, during all year with substrates at low cost (Rocha et al, 1999, Güvenç, et al, 2002).

The development of immobilization methods hasallowed the reuse of enzymes, facilitated the separation of products and led to enhanced stability in organic solvents. Immobilization of lipases has proved to be a useful technique mainly for improving enzyme activity in organic solvents,however, a large number of methods for its immobilization have been described in the literature in recent years. The method of choice is largely determined by characteristics of solubility of the active components and materials carried, with the aim of enhancing the catalytic activity and stability of lipase. The main purpose of immobilizing an enzyme is to obtain a biocatalyst with activity and stability that are unaffected during the process, which does not occur in its free form (DallaVechia et al, 2004). The number of studies on lipase immobilized from various source lipases in organic solvents to production of isoamyl acetate

is found in the literature. Welsh et al (1989) found that isoamyl acetate conversion was 100% (17g/l) in hexane using immobilized *Candida cylindraccea* lipase at an alcohol-acid molar ratio of 0.52 and an enzyme concentration of 20 g/l at 30°C in 48 h. Vija et al (1997) investigated isoamyl acetate production in supercritical CO_2 and hexane by immobilized *Candida antarctica*and *Mucormiehei* with maximum conversion of 95% using *Candida antarctica.*HariKrishna et al (2001) studied the enzymatic synthesis of isoamyl acetate using immobilized lipase from *Rhizomucormiehei* with heptane solvent. Yield above 80% were achieved with substrate concentration as high as 1.5M and more than 150 g/l in low enzyme concentration of 10 g/l. However, no research has addressed encapsulation of *Burkholderiacepacia* lipase through ionic gelation using alginate as the wall material to esterification reactions. The purpose of this studywas to obtain esters of low molecular weight as isoamyl acetatefrom *Bukholderiacepacia* lipase. Since the mechanisms of obtaining flavor esters using *Bukholderiacepacia* lipase are not well known, the synthesis of esters were studied in systems that contain organic solvent.

2. MATERIAL AND METHODS

2.1 Material

The strain of *Burkholderiacepacia*was obtained from the André Tosello Foundation (Brazil) and kept in test tubes at 4° C on nutrient agar. Acetone, ethyl alcohol, chloroform, toluene, heptane, hexane, monobasic sodium phosphate, dibasic sodium phosphate, monobasic potassium phosphate, dibasic potassium phosphate, magnesium sulfate, sodium and phosphate hydroxide and calcium chloride were obtained from Synth (Diadema, SP, Brazil). Sodium alginate, isoamyl alcohol and acetic acid were provided by Sigma-Aldrich (São Paulo, Brazil). Gum Arabic, agar-agar, yeast extract and bacteriological peptone were obtained from Oxoid (London, UK). Soybean oil (Liza) and olive oil (Gallo) were obtained from the local market, with a maximal acidity of 0.3% and 0.5%, respectively.

2.2 Methods

2.2.1 Inoculums preparation and fermentative system

Inoculums was transferred from test tubes to Erlenmeyer flasks of 125mL, containing yeastextract (3g/L), peptone (3g/L), KH_2PO_4 (4g/L), $MgSO_4$ (0.2 g/L) and 3% of soybean oil. Microorganism adaptation was performed during 48h at 30°C and 150 rpm. After this, inoculum was transferred to a bioreactor Bioflo III. Air was provided by the

compressor,sterilized by glass wool filter and with flow controlled by rotameters. The culture medium hadthe same concentrations as those of the reagents prepared for the inoculums, but with 6% (v/v) of soybean oil to induce lipase production. Fermentation was performed at 30ºC, pH 7.0, 1.5vvm of aeration and stirring of 150 rpm for 96 h (Macedo et al, 1997; Padilha et al, 2009a and2009b and Pastore et al, 2003).

2.2.2 Making of enzymatic extract

During intervals of 24 h, 15 mL of the fermented medium were collected and centrifuged for 10 min at 2016g. Supernatants were used as crude enzymatic extract (Macedo et al. 1997;Padilha et al. 2009a and 2009b).

2.2.3 Determination of the lipolytic activity

Lipolytic activity was determined according to the methodology by Kamimura et al. (1999), Macedo (1997) and Pastore et al. (2003). Lipase assay was performed with olive oil emulsion, which was prepared by mixing 25 mL of olive oil and 75 mL arabic-gum 7% solution in a homogenizer for 4 min. The reaction mixture containing 5.0 mL of olive oil emulsion, 2.0 mL of 100 mM phosphate buffer (pH 8.0) and 1.0 mL of the culture was incubated at 37 °C for 30 min with orbital shaking at 150 rpm. The reaction was immediately stopped after incubation by the addition of 15 mL acetone:ethanol mixture (1:1, v/v), and the liberated free fatty acids were titrated with 50 mMNaOH. One unit (U) of lipase activity was defined as the amount which liberated 1 μmol of fatty acid per min.

2.2.4 Production of microcapsules by gelation: From Padilha's studies (2012c) the polymeric solution (4% m/v) was shaken in a magnetic shaker at 70º C, with slow agitation until the complete dissolution of the sodium alginate. At room temperature, calcium chloride was dissolved in deionized water to obtain concentrations of 4% (w/v). After cooling to 25º C, the sodium alginate solution was blended with the crude extract at a proportion of 1:1. The mixture was homogenized in Turrax at 10.000 rpm for two minutes. The solution was atomized in the $CaCl_2$ solution at 25º C with a two-fluid atomizer with 0.90 Kgf/cm^2 air pressure in constant slow agitation. The height between the atomizer and the bath was 12 cm. Following atomization, the microcapsules were kept in an ionic bath for 30 minutes, separated in sieves (25 μm) and washed with 700 mL of distilled water. The microcapsules were dried in an oven at 30º C during 24 hours so that the enzyme activity was maintained.

2.2.5 Effect of solvents:Enzyme activity and stability were studied with the solvents: acetone, chloroform, toluene, hexane and heptane. The solvents were added to the tubes containing the immobilized enzyme. These solvents have log P values - 0.23, 2.0, 2.5, 3.5 and 4.0, respectively. The tubes were stirred in a homogenizer at room temperature. In one of the tubes only the immobilized enzyme was used as control considering 100% activity. The free enzyme was kept in the other tube in order to evaluate its stability during the incubation time. The enzymatic activity was observed in the 5 days due the esterification reactions for the synthesis of isoamyl acetatebe during 120 hours of storage. All analyses were determined with the titration method described by Kamimura et al. (1999), Macedo et al (1997)and Pastore et al (2003). All solvents were previously dried over molecular sieve.2.2.6 Isoamyl acetate synthesis: The esterification reactions were performed at 37°C in 50 mL screw-capped flasks in shaken incubator at 150 rpm. The molar ratio effect of acid and alcohol (0.1, 0.3 and 0.5M) and the immobilized enzyme concentration (10, 20 and 30% w/v) using heptane as the solvent were investigated. The flasks were incubated with the substrates for 5 minutes before adding the enzyme. To control and monitor the water level in the reaction media synthesis was carried out in the presence of molecular sieves. Aliquots of the reaction mixture were withdrawing time intervals: 0, 3, 6, 9, 12, 24, 48, 72 and 120 hours, quantifying the fatty acid consumption. The analysis was determined by titration with 0.02M sodium phosphate using phenolphthalein as indicator and 10 mL of acetone:ethanol mixture (1:1, v/v) as quenching agent. The fatty acids percentage was estimated according Equation 1.

$$AGL(\%) = \frac{V_{KOH}.M.PM}{10.m} \qquad (1)$$

Where AGL is the fatty acids percentage, V is the volume of the KOH titration at time t (mL), M is the KOH molarity; PM is the molecular weight of the fatty acid titrated and m is the mass of the aliquot titrated (g).The results were evaluated by calculating the isoamyl acetate conversion ratios, as Equation 2.

$$\%Molar = \frac{(C_0 . - C)}{C_0} * 100 \qquad (2)$$

Where Co = initial concentration of the reactant and C = concentration of reactant at a given time.

3. RESULTS AND DISCUSSION

3.1 Effect of solvents

In order to study the effect of solvents on the synthesis of isoamyl acetate, several solvents ranging from polar to nonpolar were screened: acetone, chloroform, toluene, hexane and heptane.The polarity of the organic solvents employed for the esterification reaction is known to affect the enzyme activity (Laane et al., 1987). The log P value of the solvents is the widely used parameter to describe solvent polarity and their possible effects on enzyme activity, where P is the partition coefficient of a given solvent between water and octanol in a two-phase system. It is generally recommended that the use of solvents with a log P4.0 (nonpolar) result in better esterification (HariKrisna et al, 2001). Table 1 shows the effect of various solvents on the immobilized *Burkholderiacepacia* lipase in the first and5th days.It can be seen that the heptane with log P value 4.0 bettersupported the activity. After five days the enzyme of the control sample had most activity lost than the sample in heptane.On the other hand, other solvents tend to give poor activity relationship control.On the 5th day, the activity of the immobilized lipase treated with the others solvents was significantly lowest.

TABLE 1: Effects of organic solvents activity (U.mL^{-1}) immobilized *Burkholderiacepacia* lipase in the first and 5th day.

		Days	
Solvent	**Log P**	**1**	**5**
Acetone	-0.23	2.13	2.04
Choroform	2.0	3.61	2.87
Toluene	2.5	3.15	3.15
Hexane	3.5	3.15	3.15
Heptane	4.0	3.89	3.24
Control	-	3.52	3.15

The probable explanation of the drop of the immobilized enzyme activity into contact with the solvent can be attributed to the variation of the quantity of water removed in the region of the catalytic site, which is necessary to maintain the dynamic properties of the enzyme, disturbing the microaqueous layer of enzyme.

3.2 Effect of the immobilized lipase amount and acid-alcohol molar ratio

In the literaturethe use of acetic acid as an acyl donor was earlier attempted with little or no success (Razafindralambo et al, 1994; Langrand et al., 1998). Compared with its higher homologues, acetic acid is a potent inhibitor of lipase activity causing dead-end inhibition. Acetic acid at higher concentrations could not be esterified easily probably owing to its role in lowering the microaqueous pH of the enzyme. However, as used lower molar concentration of reactants, there was a gradual increase in molar conversion during 120 hours of reaction. The results demonstrated the production of isoamyl acetate from acetic acid and isoamyl alcohol using *Burkholderiacepacia* immobilized in alginate as the catalyst. Tables 2, 3 and 4 showed different molar ratios between the substrates acetic acid and isoamyl alcohol in different enzyme concentrationsproving these assertions. Under the experimental conditions, esterification conversion yields above 90% can be obtained after 120 h of reaction. Table 2 showed increasing in above 60% process yield after 48 h of reaction with 10, 20 and 30% (w/v) amount of enzyme in 0.1M, achieving maximum conversion (80,9%) at 120 h in 0.5 M and 30% (w/v). In this case, this results corroborating withRazafindralambo et al (1994). The author showed that reducingof the amount of acid and increasing the amount of enzyme in media, the inhibiting effect of acetic acid was strongly reduced. On the other hand, Table 3 and 4, in 48 h of reaction the conversion values observed were not similar to those obtained in Table 2. However, the conversion ester ratio increased linearly with the lipase concentration. The better conversion of isoamyl acetate was with 30% (w/v) of immobilized lipase in 0.1, 0.3 and 0.5M of substrate mixture, with maximum yield in 0.5 M (91.8%).The effect of acetic acid/isoamyl alcohol equimolar ratio (1:1)on the conversion percentage yield was also studied. As shown in Figure 1, when the molar ratio was increased, the isoamyl acetate conversion (%) increased. Using low substrate concentrations (maximum 0.5M),the esterification yield reaction ratioreaches a maximum in 120 h. Tables 2 and 3 showed that 3 to 72 hours, 0.1 M has higher molar conversion to the ester was compared to 0.3 M. Although, higher substrate concentration (0.5M)leads to anincrease in the yield esterification with different amount of immobilized lipase, with the maximum conversion of 91.8% was attempted at a molar ratio of 0.5M and 30% (w/v).The results showed that it is possible to obtain isoamyl acetate in the process conditions studied. These results conflict withLanneet al (1987) reported that polar substrates accumulate in the aqueous microenvironment of the enzyme, reaching a concentration level sufficient can cause protein denaturation. Furthermore, acetic acid was reported to be an inhibitor of lipases during ester synthesis. This

shows that the present study presents quite satisfactory conversions using sodium alginate dried as the immobilizing agent in the isoamyl acetate formation.

TABLE 2: The effect of the amount enzyme on the molar ratio 0.1M at the synthesis of the isoamyl acetate

Time (h)	Fatty acids (mM)	Conversion ester (%)	Fatty acids (mM)	Conversion ester (%)	Fatty acids (mM)	Conversion ester (%)
lipase (w/v)	10%		20%		30%	
0	177.88	0.0	174.80	0.0	170.67	0.0
3	174.52	1.9	173.90	2.9	141.50	17.1
6	172.36	3.1	162.41	7.1	135.92	20.4
9	130.59	26.6	144.39	17.4	122.60	28.2
12	121.79	31.5	141.55	19.0	115.13	32.5
24	83.99	52.8	105.30	39.8	98.27	42.4
48	70.88	60.2	69.96	60.0	66.21	61.2
72	49.90	71.9	55.08	68.5	36.94	78.4
120	44.88	74.8	42.23	75.8	32.58	80.9

TABLE 3: The effect of the amount enzyme on the molar ratio 0.3M at the synthesis of the isoamyl acetate

Time (h)	Fatty acids (mM)	Conversion ester (%)	Fatty acids (mM)	Conversion ester (%)	Fatty acids (mM)	Conversion ester (%)
lipase (w/v)	10%		20%		30%	
0	412.04	0.0	454.01	0.0	413.54	0.0
3	338.14	17.9	383.32	15.6	331.03	20.0
6	280.27	32.0	315.09	30.6	268.53	35.1
9	238.66	42.1	278.93	38.6	190.77	53.9
12	239.69	41.8	244.23	46.2	187.10	54.8
24	216.50	47.5	238.21	47.5	132.11	68.1
48	158.53	61.5	197.84	56.4	95.19	77.0
72	138.69	66.3	165.85	63.5	67.95	83.6
120	110.27	73.2	113.55	75.0	54.65	86.8

TABLE 4: The effect of the amount enzyme on the molar ratio 0.5M at the synthesis of the isoamyl acetate

Time h)	Fatty acids (mM)	Conversion ester (%)	Fatty acids (mM)	Conversion ester (%)	Fatty acids (mM)	Conversion ester (%)
lipase (w/v)	10%		20%		30%	
0	1991.49	0.0	1950.44	0.0	1902.03	0.0
3	1695.40	14.9	1365.12	30.0	1316.71	30.8
6	1373.17	31.0	1026.97	47.3	1192.61	37.3
9	1247.25	37.4	913.66	53.2	546.24	71.3
12	1181.29	40.7	828.61	57.5	482.43	74.6
24	887.07	55.5	578.92	70.3	275.46	85.5
48	623.03	68.7	425.20	78.2	238.48	87.5
72	571.30	71.3	348.59	82.1	189.98	90.0
120	442.62	77.8	248.66	87.3	156.77	91.8

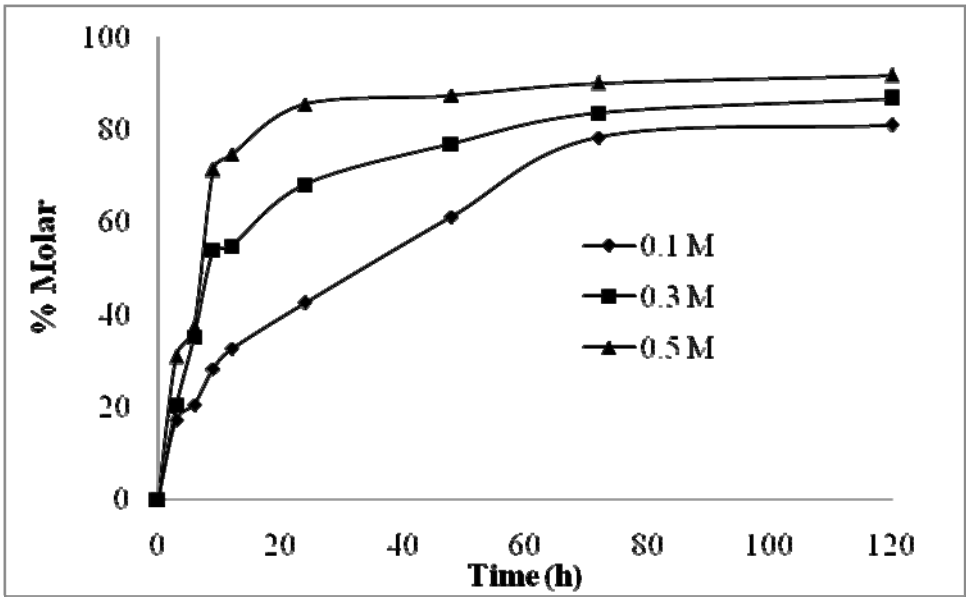

Figure 1: Effect of molar ratio on the isoamyl acetate production for immobilized lipase in the conditions 0.1, 0.3 and 0.5 M in 30% (w/v).

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ALKALINE ESTERASE FROM SORGHUM: IDENTIFICATION, BIOCHEMICAL AND BIOCATALITIC CHARACTERIZATION

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ABSTRACT:

Esterases are enzymes that present good potential in industrial application, and sorghum seed can represent an alternative source for this enzyme. They catalyze the formation or cleavage of ester bonds in water-soluble substrates. The extraction of esterase from the sorghum seeds is an economical alternative to obtain one enzyme of great interesting. Esterases and lipases may improve the quality or accelerate the maturation of cheeses, cured bacon, fermented sausages and resolution of racemic mixture. Recently, seed esterases and lipases have been the focus of much attention as biocatalysts. In some cases, these enzymes present advantages over animal and microbial lipases due to some quite interesting features such as specificity and low cost, being a great alternative for the potential commercial exploitation as industrial enzymes The esterase studied in this work was extracted from sorghum seeds and some of its biochemical properties determined using synthetic substrates (p-nitrophenyl butyrate, caprylate, laurate and palmitate). The enzyme presented optimum activity at pH 8.0 and was stable at all the pH ranges studied. The optimum temperature for its activity was 40ºC but it showed low stability at this temperature (40% relative activity). The values for Km and Vmax were 0.67mM/min and 125 $U.mg^{-1}$, respectively. The enzyme showed an increase in activity when K_2HPO_4 was added to the reaction medium, but the ions Mn^{2+}, CO^{+}, Hg^{+} and Fe^{2+} strongly inhibited enzyme activity. This enzyme showed a preference for the hydrolysis of short chain fatty acids. The characteristics of sorghum esterase are very similar to those of the microbial esterases used in detergent processing.

Keywords: Alkaline, characterization, identification, esterase, sorghum

1. INTRODUCTION

Esterases belong to the group of hydrolases (carboxylester hydrolases; EC. 3.1.1.1). They catalyze the formation or cleavage of ester bonds in water-soluble substrates. Similar to lipase, the acyl-enzyme complex is formed via a serine residue within the active site. Due to their easy availability they are of interest for application in industrial processes. Esterases and lipases may improve the quality or accelerate the maturation of cheeses, cured bacon and fermented sausages. The esterases are also widely used in the resolution of racemic mixtures of compounds in order to produce pure anantomers (Houd, Kademi e Leblanc, 2004; Singh et al., 2006; Fahmy et al., 2008; Er-Zheng , Peng-yong and Dong-zhi, 2010).

Esterases can be of animal (pancreatic, hepatic and gastric), microbial (bacterial, fungal and yeast) or vegetable origin, with variations in their catalytic properties (Mohamed et al., 2000; Barros, Fleuri and Macedo, 2010). The possibility of using enzymes from seed is an industrial economic alternative (Parques and Macedo 2006).

Recently, seed esterases and lipases have been the focus of much attention as biocatalysts. In some cases, these enzymes present advantages over animal and microbial lipases due specificity and low cost, being a great alternative for the potential commercial exploitation as industrial enzymes (Barros, Fleuri and Macedo, 2010; Tuter et al., 2003).

Several studies have been carried out using seed lipases and esterases. A partially purified raw extract of *Nigella sativa* L. was used as a source of lipase for enriching borage oil with gamma-linolenic fatty acid by interesterification (Tuter et al., 2003). Fahmy et al. (2008) studied the biochemical characteristics of the esterase from *Cuncurbita pepo* cv. The authors observed that this enzyme had some interesting characteristics such as high stability, an alkaline pH optimum and high affinity towards short-chain esters, a characteristic they share with some microbial esterases used in the maturation and flavor development of cheeses, such as *Lactobacillus sp.* esterases.

Sorghum (*Sorghum bicolor* L.) is the fifth most produced cereal throughout the world, after wheat, rice, corn and barley. It is widely used as animal feed and has also been studied by some researchers as an alternative to replace barley malt. Nwanguma, Eze and Ezengwa, (1996) detected lipolytic activity in non-germinated sorghum seeds during grain malting and mashing. The authors observed a slight decrease in enzyme activity during steeping for 24 hours, but it increased several fold during the course of germination. Between 24 and 60% of the lipolytic activity was retained after cooking at 48°C, but no activity was found after mashing at 65°C. About 68% of the lipase activity of 72h old malt was detected in the plumule, while 29% and 3% were found in the endosperm and radicle, respectively.

No study has been carried out to characterize the physicochemical properties of the lipolytic enzyme present in sorghum grains, to date only the presence of lipolytic activity was determined. The present study aimed to observe and quantify the presence of esterase activity in non-germinated sorghum seeds, assess the specificity and study the biochemical characteristics of the crude esterase.

2. MATERIALS AND METHODS

2.1 Materials

The sorghum seeds studied were donated by *VITAO Polinutri Industry LTDA*, Brazil, and the commercial lipase used was Lipozyme TLIM obtained from *Thermomyces lanuginosu* (Novozymes®). The concentration of commercial lipase used was 2mg.mL^{-1}. The substrates *p*-nitrophenyl butyrate (pNPB), *p*-nitrophenyl caprylate (pNPC), *p*-nitrophenyl-laurate (pNPL) and *p*-nitrophenyl palmitate (pNPP) were purchased from Sigma Chemical (St Louis, MO). Triacetin and triolein were obtained from Fluka (Swizerland) and the other chemicals were of analytical grade.

2.2 Esterase extraction

The esterase was extracted from the sorghum using a modification of the procedure described by Aizono et al. (1973). Non-germinated and post germination seeds were suspended in 1x10^{-3}M $CaCl_2$ solution and blended for 3 min at 4°C in a waring blender. The sample was transferred to a conical flask, stirred mechanically at 4°C for 30min and then subjected to ultrasound (125W at 200 kHz) for 1 min in an effort to release the bound enzyme. The resulting suspension was centrifuged at 2000x*g* for 45min at 5°C, and the supernatant collected and cooled to 0°C. Solid ammonium sulphate was added with constant stirring to obtain 60% saturation. The resulting precipitate was separated by centrifugation at 2000x*g* for 45 min and then resuspended in a minimum amount of 1 X 10^{-3}M $CaCl_2$ solution. The $CaCl_2$ suspension was dialyzed against the same solution at 4°C until all traces of ammonium sulfate had been removed, and then re-centrifuged to eliminate any insoluble material (Kermasha and van de Voort,1986). The resulting partial purified extract was than assayed for its protein content using the modified Bradford (1976) method.

2.3 Enzyme Assay

Esterase activity was determined spectrophotometer following the hydrolysis at 405nm of *p*-nitrophenyl-butyrate (*p*NPB) as the substrate. An aliquot (0.070 mL) of the purified enzyme

extract was added to 3.43 mL of a reaction mixture with the following composition: 1.12 mM pNPB dissolved in 50mM phosphate buffer, pH 7.0, also containing 0.2% (N/P) Triton X-100 and 0.42 M tetrahydrofuran. The enzymatic activity was determined for a period of 5 min at 37°C, determining the absorption at one minute intervals against a blank solution. One unit of esterase activity was defined as the amount of enzyme required to release one micromole of *p*-nitrophenol per minute under the standard assay conditions (Macedo and Pio, 2005).

The esterase activity was also determined by a titrimetic method using triacetin and triolein as the substrates. One milliliter of the purified enzyme extract was added to a reaction mixture with the following composition: 5mL of 0.1M phosphate buffer, pH 7.0, and 1mL of triacetin. The solution was incubated at 37°C for 6h under shaking at 120 rpm. After incubation, the reaction was stopped by adding 15mL ethanol:acetone 1:1 (v/v), and the fatty acids released were titrated with 0.05 M sodium hydroxide. One unit of esterase activity was defined as the amount of enzyme required to release one μmol of oleic acid per minute per mL under the specified conditions (Paques et al., 2008).

The substrate specificity of the enzyme was determined evaluating its activity in different *p*-nitrophenyl-esters. Several *p*-nitrophenyl-esters were analyzed: *p*-nitrophenyl butyrate (pNPB), *p*-nitrophenyl caprylate (pNPC), *p*-nitrophenyl laurate (pNPL) and *p*-nitrophenyl-palmitate (pNPP). The specificity was also tested using different triacylglycerols (triacetin, tributyrin, tricaproyn, tripalmistin and triolein) by titration with NaOH as previously described.

2.4 Optimum temperature and heat stability

The effect of temperature on the esterase activity was studied by carrying out assays at different temperatures in the range from 30-90°C at pH7.0, obtained using 0.1M phosphate buffer.

The heat stability of the esterase was tested by pre-incubating the enzyme for 1h at different temperatures ranging from 30 to 60°C. After the pre-incubation, the residual activity was measured at 37°C and expressed as a percentage of the relative esterase activity.

2.5 pH optimum and stability

The optimum pH for esterase activity was determined using the following buffers: 0.1M acetate in the range from pH 3.6 to 5.6, 0.1M phosphate from pH 6.0 to 7.0, 0.1M Tris-HCl from pH 7.2 to 9.0 and 0.1M borax-NaOH from pH 9.5 to 10.0.

The pH stability was evaluated by incubating the enzyme with different buffers at pH values ranging from 3.6 to 10.0 for 24h at room temperature. After incubation, the residual

activity was determined (37°C, phosphate buffer pH 7) and expressed as a percentage of the relative esterase activity. All the tests were carried out in duplicate.

2.6 Effect of salts and chemical agents

The effects of different salts ($CaCl_2$, KCl_2, $HgCl_2$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$, K_2HPO_4, $NaNO_3$, $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, $ZnSO4.7H_2O$, $MnSO_4.H_2O$, K_2SO_4, Na_2SO_4, $NaHSO_3$, $HgCl_2$, NaH_2SO_4, $CuSO_4$ and $NaCl_2$) on the esterase activity were studied. The salts were added to the reaction buffer at concentrations of 1 and 10 mM. The relative activity was expressed as a percentage of the relative esterase activity as measured in the reactive medium without the added salts. The effects of different chemical agents (EDTA, urea, sodium lauryl sulphate, sodium bisulphate, glutathione (reduced), ascorbic acid, sodium citrate, cysteine, Tris and sodium persulfate) were also evaluated at concentrations of 1 and 10mM.

2.7 Statistical analysis

The data from the experiments are presented as the mean±standard error (SEM), and were analyzes a one way analysis of variance (ANOVA), with the differences analyzed by the Tukey test at level of 5% probability (SAS version 9.2 – Institute Inc., Cary, NC, USA).

3. Results and Discussion

It was obtained esterase activity only in the non-germinated (dormant) seed of the *Sorghum bicolor.*

3.1 Study of the specificity of the sorghum esterase

The specificity of the sorghum esterase was studied using different substrates and the results are shown in Figure 1.

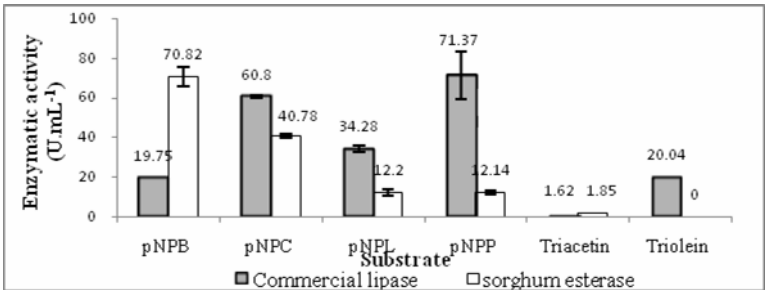

Figure 1: Activity of sorghum esterase and commercial lipase (*Thermomyces lanuginosu*) on different substrates.

The highest value for activity was observed with pNPB (70 $U.mL^{-1}$) after 30 minutes of reaction, with pNPC in second place, the maximum activity being 40.78$U.mL^{-1}$. The preference of the sorghum esterase for the substrates tested was in the following order: pNPB> pNPC>pNPL>pNPP. According to Marten et al. (2006) and Osborn and Akoh (2002), fatty acids with 2 to 6 carbons in their structure are classified as short chain fatty acids, for example butyric acid (4 carbons), and fatty acids containing from 6 to 12 carbons are classified as medium chain fatty acids (e.g. caprylic acid). The sorghum esterase showed an affinity for short chain fatty acids, this being the expected behavior for an esterase.

Kubicka et al. (2000) evaluated the activity of barley esterase and detected greater affinity for short chain fatty acids, especially for acetic acid esters. Liaquat and Apenten (2000) studied the syntheses of different esters by the lipase from corn seeds and observed the preferential hydrolysis of short chain fatty acids by the enzyme in the following order: acetic acid (2C) > butyric acid (4C) > caproic acid (6C), in an organic medium using isopentanol, after 72 hours of reaction.

Pollizeli, Tiera and Bonilla-Rodriguez (2008), studying lipase from the seeds of *P. aquatica* with 90 minutes of reaction time, found the following values for activity – 2.5; 5 and 4.5 $U.mL^{-1}$ in (*p*NPB*)*, pNPC and pNPP respectively, lower values to those found in the present study. In addition the activity of the sorghum esterase was evaluated in triacetin and triolein and presented greater activity in triacetin.

3.2 Effect of temperature and pH on the activity and stability of sorghum esterase

The temperature showed a considerable influence on the reaction velocity of the enzyme due to various factors. It can affect the stability of the enzyme and the velocity with which the enzyme-substrate complex breaks up, which is determined by the temperature of the reaction medium and the affinity between the enzyme and the substrate.

Figure 2 shows the effect of temperature on the activity and stability of the sorghum esterase.

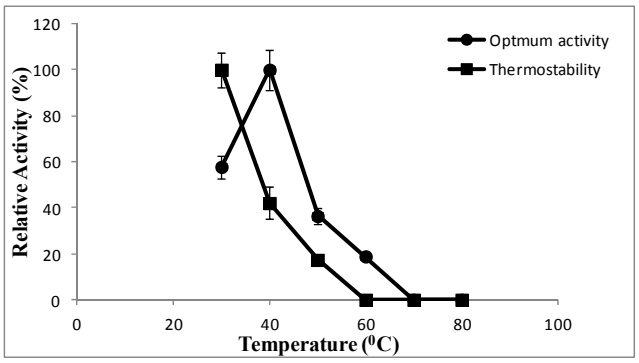

Figure 2: Optimum temperature for the activity of sorghum esterase.

The greatest value for the activity of the sorghum esterase was at a temperature of 40ºC, and at temperatures above 40ºC there was a significant decrease in enzyme activity. Staubmann et al. (1999), studying the esterase from a physic nut (*J. curcas* L.) observed optimal activity at 50ºC in a substrate of (*p*NPB). The lipase from the kapok tree nut (*P. aquatica* Bombacaceae), studied by Polizelli et al (2008), showed optimum activity at 40ºC using pNPP as the substrate, and Isbilir, Ozan and Yagar (2008), studying the lipase from bay seeds (*L. nobilis* L.) found the optimum activity at 50ºC in triolein.

The sorghum esterase was more stable at 30ºC. Although the enzyme showed its greatest activity at 40ºC, at this temperature it only presented 40% of residual activity after one hour of incubation. Figure 3 shows thermostability of the sorghum esterase.

3.3 Effect of pH on the activity and stability of the enzyme

The Figure 3 shows the effect of pH for the activity of sorghum esterase.

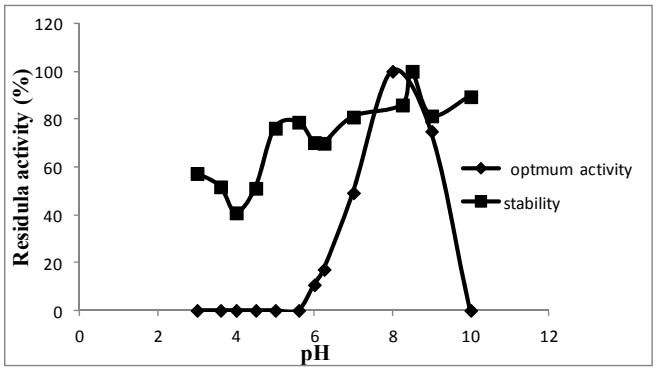

Figure 3: Effect of pH on the activity and stability of the sorghum seed esterase

The sorghum esterase showed its greatest activity at pH8.0, similar to the soybean esterase, and is thus an alkaline enzyme (Barros and Macedo, 2011). This value is similar to that shown by the esterases from physic nut (*J. curcas*) studied by Staubmann et al. (1999) and by Abigor et al. (2002), and by the bay seed lipase (*J. curcas*) studied by Isbilir, Ozcan and Yagar (2008), which showed their maximum activities at pH 7.5.

About to pH stability, the sorghum esterase was stable at all the pH values studied, as shown in Figure 3. The sorghum esterase showed its greatest stability at pH 8.5, but did not show significant difference between the values found at the pH values (Tris-HCl) of 7, 8 and 9. The lowest value for residual activity was found at pH 4, with only 40% of residual activity. The sorghum esterase was shown to be stable at all the pH values studied.

3.4 Effect of salts and some activators and inhibitors on the activity of the freeze-dried enzyme

The effect of salts and of some activators and inhibitors on the activity of sorghum esterase was tested, and the results can be seen in Tables 1 and 2.

TABLE 1: Activity of sorghum esterase in different salts[1, 2]

Salts	Final concentration (1mM)	Final concentration (10mM)
	Relative activity (%)	Relative activity (%)
Control	100±2.42^{bcde}	100±2.42^{bcde}
K_2HPO_4	95.50±5.66 ^{bcdef}	130.77±6.02^{a}
$NaNO_3$	111.29±8.67^{ab}	111.47±9.87^{ab}
Mn Cl_2	84.91±3.79^{def}	109.05±7.90b^{c}
$CaCl_2$	106.32±1.03^{bc}	105.70±3.05^{bcd}
Fe_2SO_4	102.99±6.54^{bcde}	0.0^{h}
Na_2SO_4	88.95±4.07^{cdef}	101.48±11.04^{bcde}
KCl_2	91.45±5.71^{bcdef}	98.71±7.16^{bcde}
K_2SO_4	75.42±2.13^{f}	93.81±8.55^{bcdef}
$MnSO_4$	90.28±7.11^{cdef}	0.0^{h}
$CoCl_2$	83.64±4.18^{ef}	0.0^{h}
$MgSO_4$	83.18±2.98^{ef}	75.33±9.82^{f}
$ZnSO_4$	52.75±10.23^{g}	0.0^{h}
HgCl	0.0h	0.0^{h}

[1]Mean of four repetitions with one determination each ± standard deviation
[2]Means accompanied by the same letters do not differ significantly at p≥0.05.

Of the salts studied in this trial, the only one that favored an increase in enzyme activity was potassium phosphate (K_2HPO_4) at a concentration of 10mM. However, the salts $CoCl_2^+$, HgCl and Fe_2SO_4 strongly inhibited the enzyme activity. The same occurred with the lipase from the kapok tree nut (*Pachira aquatica*) studied by Polizelli et al. (2008).

For the esterase of physic nut (*Jatropha. curcas)* studied by Staubmam et al. (1999) only $BaCO_3$, at a concentration of 10mM showed a positive effect on the enzyme activity. The author mentioned that the ions K^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+} frequently showed a positive effect on esterase activity, but this was not observed with the physic nut esterase. However in the present study potassium did have a positive effect on the enzyme activity.

Isbilir, Ozcan and Yagar(2008), studying the lipase from bay seeds (*Laurus nobilis* L.) observed a significant increase in enzyme activity in the presence of the ions Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+} and Mg^{2+} in the reaction medium, results similar to those observed for the lipase from almond seeds (*Amygdalus communis* L.). The lipase from Kapok seeds (*P. aquatica*) also showed an increase in activity in the presence of the ions Ca^{2+} and Mg^{2+}.

Table 2 shows the effect of some activators and inhibitors on the activity of the esterase from sorghum seeds.

TABLE 2: Activity of sorghum esterase in different activators and inhibitors[1,2]

Chemical agentes	Final concentration (1mM)	Final concentration (10mM)
	Relative activity (%)	Relative activity (%)
Control	100±5.33^{bc}	100±5.33^{bc}
Urea	105.04±11.67^{ab}	120.80±11.46^{a}
Sodium citrate	87.39±3.18^{bcde}	90.67±3.40^{bcd}
EDTA	85.74±9.89^{cde}	69.14±0.35^{efgh}
Reduced glutathione	79.82±4.68^{def}	0.0^{j}
Sodium bisulfate	75.37±5.56^{defg}	0.0^{j}
L-cisteine	69.58±7.68^{efgh}	0.0^{j}
Ammonium persulfate	64.28±3.55^{fgh}	35.09±5.58^{i}
TRIS	63.00±1.60^{fgh}	101.68±9.65^{bc}
Ascorbic acid	60.80±2.33^{gh}	56.36±7.07^{h}

[1]Means of four repetitions with one determination each ± standard deviation
[2]Means accompanied by the same letters do not differ significantly at p≥0.05.

The urea showed a positive effect on the enzyme activity, however the reduced glutathione, sodium bisulfate and L-cysteine at a concentration of 10mM inhibited the enzyme activity. Polizelli et al (2008), studying the effects of some chemical compounds on the activity of the lipase from the kapok tree nut (*Pachira aquatica*) also observed the negative effect of reduced glutathione at a concentration of 10mM.

The values for K_m and V_{max} of the enzyme were be 0.67mM (*p*NPB) and 125 $U.mg^{-1}$, respectively (Figure 4).

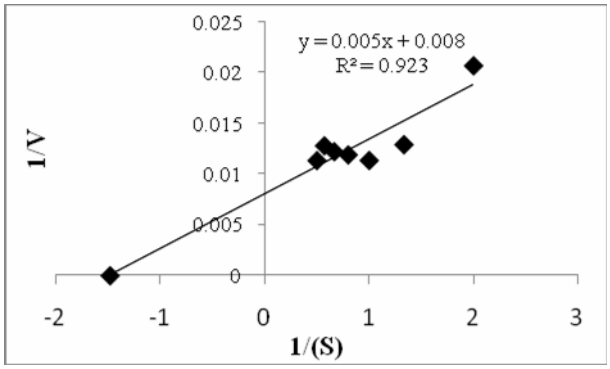

Figure 4: Graphic determination of K_m and V_{max} values of the sorghum esterase, using *p*-nitrophenyl butyrate as substrate, according to the method of Liner Weaver-Burk.

4. Conclusion

The sorghum esterase showed some very interesting characteristics such as specificity, activity and catalytic stability at alkaline pH (pH 8). These characteristics demonstrate the potential for application of this enzyme in various industrial sectors, in the synthesis of low molecular weight esters (aromatic esters), in industrial detergents and in other applications.

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STABILITY OF MULTIPLE EMULSIONS WITH OREGANO ESSENTIAL OIL AND ITS APPLICATION TO *Aspergillus niger* INHIBITION IN MODEL SYSTEMS

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ABSTRACT:

Plant essential oils have demonstrated antimicrobial activity against many deteriorative microorganisms. However, essential oils are susceptible to degradation due to environmental factors, therefore, microencapsulation is suggested to confer protection and good stability. In this study, multiple emulsions (W/O/W) were formulated with oregano essential oil (OEO) and corn oil, for the oil phase (30% w/w) and for the external aqueous phase, arabic gum (AG) (4% w/w) or carboxymethylcellulose (CMC) (0.4%) homogenized by high intensity ultrasound. Dispersion index, zeta potential and creaming were measured in the emulsions prepared with different ultrasound homogenization conditions, 84µm or 108µm of wave amplitude during 10 or 20 min for the preparation of the primary emulsion and a subsequent homogenization at 42µm or 54µm of wave amplitude during 10 min for the secondary emulsification. The antimicrobial activity tests were performed with the more stable emulsions of AG and CMC in model systems adjusted to different a_w (0.95, 0.97) and pH (4.5, 5.5), to evaluate the antifungal effectiveness against *Aspergillus niger* of the multiple emulsions. Results showed that ultrasonic homogenization time was not a factor which affected the stability of the evaluated emulsions ($p > 0.05$), while the stability was increased with a higher intensity applied and using CMC as stabilizer. Finally, it was observed that the slow delivery of OEO in the multiple emulsions inhibited *Aspergillus niger* growth for 72 h with CMC at lower a_w and pH, while with AG the OEO delivery lead to a mold inhibition only for 24 h.

Keywords: multiple emulsions, oregano essential oil, creaming, mold inhibition.

1. INTRODUCTION

Recent studies have demonstrated that plant extracts of herbs and spices have antimicrobial properties against foodborne pathogenic bacteria (Burt, Vlielander, Haagsman and Veldhuizen, 2005) or fungi (Portillo-Ruiz, Viramontes-Ramos, Muñoz-Castellanos, Gastélum-Franco and Nevárez-Moorillón, 2005). In particular, Mexican oregano (*Lippia berlandieri* Schauer) essential oil (OEO) has high content of thymol and important content of carvacrol, which are responsible of the antimicrobial activity of this essential oil (Avila-Sosa et al., 2010). Nevertheless, the intense odor of OEO represents a limited use in food products, for direct application, and its chemical components are susceptible to degradation when exposed to different environmental factors. Therefore, microencapsulation is an alternative to protect its components from undesirable reactions with other components, forming a barrier between them.

Microencapsulation by emulsification has numerous applications in pharmaceutic, cosmetic and food industries due to the advantage of these systems for the controlled release of the active compounds (Schmidts, Dobler, Guldan, Paulus and Runkel, 2010; Dickinson, 2011). Simple emulsions are conventionally prepared by an aqueous and an oil phase with the help of an emulsifier (Mc Clements, 2005; Stoian, Zgherea and Peretz, 2009); in contrast, multiple emulsions are composed of two or more phases. Multiple emulsions are also called double emulsions since these may be prepared by using a simple emulsion within another one. These have demonstrate to have more stability when submitted to different environmental stress factors, with respect to simple emulsions (Aoki, Decker and McClements, 2005).

The common techniques for multiple emulsions preparation is applying high pressure or high intensity ultrasound homogenization. Both techniques led to small and homogeneous droplets for the disperse phase which is a favorable condition to avoid aggregation or coalescence of the droplets that may affect the emulsion separation and therefore promoting instability. Droplet average size for the disperse phase in multiple emulsions is suggested to be around 1 micron to expect a good emulsion stability (Schmidts, Dobler, Guldan, Paulus and Runke, 2010).

Emulsions stability determination may be done by measuring droplet size of the inner phase, zeta potential and creaming separation of the emulsion (Gullapalli and Sheth, 1999; Schmidts, Dobler, Guldan, Paulus and Runke, 2010). Droplet size distribution may be modified by the homogenization technique and its conditions like intensity and time, besides the chemical composition of the emulsion, affected mainly by the aqueous or oil proportions

in the emulsions formulation (Schubert and Engel, 2004; McClements, Decker and Weiss, 2007). The zeta potential values >|40| indicates a high stability of emulsions since is a parameter which indicates the interphase charge, related to the electrostatic interactions among the phases, which reflects the distance between the disperse phase or inner phase of the emulsions (Aoki, Decker and McClements, 2005). During the storage of emulsions, evidently separation of the phases is observed, the less dense phase in the upper part of the emulsion due to the oil droplet coalescence is measured, giving arise to the creaming concept from these observations, being an important parameter to compare emulsion stability (Jiao and Burgess, 2003).

The objective of this study was to determine the stability of multiple emulsions (W/O/W) formulated with oregano essential oil prepared by high intensity ultrasound and evaluate its antifungal effectiveness against *Aspergillus niger* in model systems.

2. MATERIALS AND METHODS

2.1 Materials

Mexican oregano essential oil (OEO) was provided by CiReNa (Natural Resources Research Center of Salaices, Chihuahua, Mexico). Commercial corn oil was obtained in a local supermarket (La Gloria ®, Mexico). The emulsifier used for the primary emulsion was Tween 80 (Sigma-Aldrich, Mexico) while soy lecithin (Gelcpas ®, Mexico) was used as emulsifier for the multiple emulsion. The aqueous solutions for the multiple emulsions were prepared with acacia gum (Freskal, Mexico) or carboxymethylcellulose (CMC) (CNI, France).

2.2 Emulsions preparation

Primary emulsions water in oil (W/O) were prepared (30:70), using Tween 80 as emulsifier (0.3 % w/w) and corn oil for the continuous phase. An ultrasonic homogenizer (CPX-400, Cole Parmer, USA) was used applying 84µm or 108µm of wave amplitude during 10 or 20 min. Then, multiple emulsions were prepared with 20% of the primary emulsion, using soy lecithin as emulsifier (1% w/w) and an aqueous solution of acacia gum (4% w/w) or CMC (0.4 % w/w) for the continuous phase. The ultrasonic homogenization for the multiple emulsions was applied with a 50% less intensity (42µm or 54µm wave amplitude) than for the primary emulsion preparation, during 10 min.

2.3 Emulsions stability measurments

Size distribution of the disperse phase of multiple emulsions as well as the zeta potential were determined with the equipment Nanotrac Wave (Microtrac Inc., USA). Creaming was calculated as the separation volume of the cream phase from the emulsion, as proposed by Jiao and Burgess (2003): creaming (%) = 100 x (volume of initial multiple emulsion – volume of cream phase) / volume of initial multiple emulsion. Therefore, small values of creaming represents a more stable emulsion.

2.4 Antimicrobial activity

Spores of *Aspergillus niger*, from the collection of the Food Microbiology Laboratory of Universidad de las Américas Puebla (Mexico) were cultivated in tubes with potato dextrose agar (PDA) (Merk, Mexico) for 7 days at 25°C. Sterile distilled water with 0.1% of Tween 80 was used to remove the spores from the cultivation tubes and the suspension concentration was determined with a Neubauer chamber and an optic microscope (Zeiss Primo Star, Germany), which was approximately of 10^5 spores/mL. Model systems were prepared in dishes (duplicate) with PDA adjusted at pH 4.5 or 5.5 with HCl 0.1N and a_w 0.95 or 0.97 with NaCl. Once the systems were solidified, 200 µL of the multiple emulsions were spread in sterile conditions and after 5 min of drying at room temperature, 5 µL of the *A. niger* spores suspension were inoculated in 3 different points of each dish; these were stored at 25°C for 4 days. The control system was inoculated with *A. niger* but with no multiple emulsion added.

3. RESULTS AND DISCUSSION

3.1 Stability of multiple emulsions

Size distribution parameters obtained for the different multiple emulsions prepared demonstrated a median diameter (d_{50}) of 2.72 – 5.48 µm when using arabic gum (AG) or CMC as stabilizers in the water phase of the multiple emulsions containing oregano essential oil (OEO) (TABLE 1). Nevertheless, multiple emulsions prepared with AG showed wide size distributions, with d_{90} values of 5.86 – 10.48 µm, which is related to the presence of some agglomerates; compared to the multiple emulsions prepared with CMC presenting d_{90} values between 5.35 – 6.38 µm. Similar values of d_{50}, between 4 and 11 µm, were obtained in a study of Li, Jiang, Liu, Chai, Li and Leng (2011) when using AG and soy protein for multiple emulsions as stabilizers. In TABLE 1, zeta potential values of the multiple emulsions shows an important difference for the multiple emulsions prepared with AG (7.04 – 26.75 mV), with

respect to the ones prepared with CMC (48.45 – 166.65 mV), which clearly demonstrates that CMC is a better emulsion stabilizer, considering the criteria that good stability of emulsions is achieved with zeta potential values above ±40 mV (Aoki, Decker and McClements, 2005). Zeta potential values of multiple emulsions when GA was used as stabilizer were among 1.5 – 31.2 mV (Charoen, Jangchud., Jangchud, Harnsilawat, Naivikul and McClements, 2011; Li, Jiang, Liu, Chai, Li and Leng, 2011), which are lower values than the ones obtained in the present study, confirming the lack of effectiveness of AG as stabilizer. With respect to the homogenization conditions for the preparation of the primary emulsions, it is observed in TABLE 1 that a higher ultrasound wave amplitude and lower homogenization time, produces smaller droplet sizes (d_{50}).

TABLE 1. Size parameters, zeta potential and creaming of multiple emulsions prepared with oregano essential oil.

Stabilizer of multiple emulsion	Homogenization conditions				Multiple emulsion properties				
	Wave amplitude (μm)		Time (min)		Droplet size (μm)			Zeta potential (mV)	Creaming (%)
	Primary emulsion	Multiple emulsion	Primary emulsion	Multiple emulsion	d_{10}	d_{50}	d_{90}		
Arabic gum (4 % w/w)	84	42	10	10	0.67	3.43	7.53	25.34 ± 0.98	65.0 ± 0.5
	84	42	20	10	0.48	4.97	9.46	26.75 ± 1.84	68.8 ± 0.5
	108	54	10	10	0.48	5.48	10.48	24.75 ± 3.12	67.5 ± 0.5
	108	54	20	10	0.44	4.74	9.93	7.04 ± 0.01	63.8 ± 0.3
CMC (0.4 % w/w)	84	42	10	10	0.43	3.46	5.55	127.94 ± 3.98	31.3 ± 0.3
	84	42	20	10	1.91	2.72	5.57	48.45 ± 0.29	25.0 ± 0.0
	108	54	10	10	1.06	4.72	5.35	156.75 ± 1.59	25.0 ± 0.0
	108	54	20	10	0.29	5.08	6.17	166.65 ± 2.11	30.0 ± 0.5

3.2 Antifungal inhibition

Aspergillus niger inhibition with the addition of OEO in multiple emulsion was evaluated only with the more stable emulsions previously studied, the ones prepared with 108 μm of ultrasound wave amplitude and 10 min for the homogenization of the primary emulsion, for both stabilizers. A control was also prepared to compare the mold inhibition along time, for the model systems formulated with different pH (4.5 or 5.5) and a_w (0.95 or 0.97) but without the addition of the OEO multiple emulsions (TABLE 2). Results show that the mold grew after 1 day with no difference among the different model systems formulations. Even if *A. niger* growth started to appear in the second day of storage when OEO multiple emulsions was added, it was very clear that the multiple emulsions with AG as stabilizer was less effective than CMC for the mold inhibition. Relating the properties of the multiple emulsions with AG, the low effectiveness of this emulsion may be due to the presence of agglomerates which may delay the OEO release, allowing the mold growth. Multiple emulsions prepared

with CMC, which were more stable during storage, delayed *A. niger* to grow in 100 % up to 3 days for the model systems with higher pH (5.5) and up to 4 days for the lowest pH (4.5).

TABLE 2. Growth fraction of *Aspergillus niger* in PDA as model systems at different pH and a_w, in the presence of oregano essential oil microencapsulated in multiple emulsions.

Time (days)	Model systems		Stabilizer of multiple emulsion		Control
	pH	a_w	AG	CMC	
1	4.5	0.95	0.00	0.00	1.00
		0.97	0.00	0.00	1.00
	5.5	0.95	0.00	0.00	1.00
		0.97	0.00	0.00	1.00
2	4.5	0.95	1.00	0.67	1.00
		0.97	1.00	1.00	1.00
	5.5	0.95	1.00	0.83	1.00
		0.97	0.83	0.67	1.00
3	4.5	0.95	1.00	0.67	1.00
		0.97	1.00	1.00	1.00
	5.5	0.95	1.00	1.00	1.00
		0.97	1.00	1.00	1.00
4	4.5	0.95	1.00	1.00	1.00
		0.97	1.00	1.00	1.00
	5.5	0.95	1.00	1.00	1.00
		0.97	1.00	1.00	1.00

4. CONCLUSIONS

The homogenization conditions for the multiple emulsions preparation affects the size distribution of the dispersed phase and zeta potential, which are related to the stability of these during storage. The release of the active compounds, oregano essential oil in this case, may be explained by these characteristics and its application for mold inhibition presents interesting results and which may be studied further to achieve the desired mold delay inhibition in different environmental conditions.

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INNOVATION CHALLENGES FOCUSING ON EUROPE AND SMES

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ABSTRACT:

99.1% of European food and drink companies are small and medium sized enterprises (SME) and are generating 48% of the food and drink sectors' value added. Compared to other manufacturing industries the overall R&D expenditures (0.4% of food industries' output) and innovation intensity (e.g. Germany 1.6% of annual turnover) are low.

Overcoming bottlenecks to innovation is considered key task to safeguard long-term competitiveness of the sector on European and international markets. Various bottlenecks are identified like missing knowledge about needs and recognition and development of respective solutions for the food (processing) sector, provision of effective systems for transfer of knowledge and technology to SMEs, low absorption capacity for innovative technologies by food SMEs, lack of professional skills and competences of labour forces, no or only weak innovation culture established in companies, insufficient access or availability of R&D investment from the private and public sector and even weak R&D infrastructures in many regions.

There is no single solution to overcome specific bottlenecks for innovation. Results and findings of diverse trans-national and European projects dealing with this issue as well as own initiatives by DIL will be presented to demonstrate promising approaches to foster innovations in food processing industry, esp. SMEs.

Keywords: Innovation challenges, innovation bottlenecks, knowledge and technology transfer, food SMEs.

VARIETAL AND GEOGRAPHICAL ORIGIN DETERMINATION OF GREEK EXTRA VIRGIN OLIVE OILS BY DSC IN COMBINATION WITH GC

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ABSTRACT

The present study focused on the applicability of Differential Scanning Calorimetry (DSC) and Gas Chromatography (GC), singly or in combination, for the determination of the varietal and geographical origin of Greek extra virgin olive oil (EVOO) samples. Thermal parameters were obtained by application of cooling and heating protocols to EVOOs and GC was used for the determination of fatty acid contents. Statistical analysis aimed to identify relationships between content levels of fatty acids and thermal parameters. Furthermore, the discriminant ability of DSC, GC or the combination of both techniques, regarding Greek EVOOs' varietal and geographical origin, was investigated by means of Linear Discriminant Analysis (LDA). Results showed that the contents of various fatty acids influence specific thermal parameters of EVOOs, such as melting and crystallisation transitions ($p<0.05$). Specifically, saturated fatty acids' content (SFA) was shown to influence the range of crystallisation, while polyunsaturated fatty acids' content influenced the final stage of crystallisation, at lower temperatures. Results solely from DSC were shown to be promising for the determination of varietal and geographical origin of Greek EVOOs, correctly classifying ($p<0.001$) 100.0% and 86.7% of samples, respectively. Results solely from GC grouped EVOOs to their assigned to cultivar, by 86.7% ($p<0.001$), as well as in combination with DSC results, by 91.1% ($p<0.001$). GC alone did not prove to be effective in discriminating EVOOs in relation to geographical origin. The DSC technique was shown to have great potential of being used as a traceability monitoring tool for Greek EVOOs.

Keywords: DSC, GC, olive oil, geographical origin, cultivar.

1. INTRODUCTION

Traceability of EVOO is of growing interest among producers, since it leads to supply chain optimisation, increase of producers' competitiveness and prevention of mislabeling of geographical origin and olive varieties of products, so as to consequently assure correct information to the consumers (Regattieri et al, 2007). Furthermore, EC regulation on marketing standards for olive oil enables producers to market their extra virgin and virgin olive oils on the basis of geographic origin (EC Regulation, 2002). The complete field to fork traceability of olive oil involves the characterisation of the oils obtained from the main cultivars in each production zone, as the chemical composition of EVOO is well known to be influenced by genotype, different agronomic, environmental and technological factors (Kotti et al, 2009).

Greece is the third largest producer of virgin olive oils in the world, with about 353,000 tons in 2010, around 80% of which are extra virgin olive oils, thus making Greece one of the world's largest EVOO producing countries (FAOSTAT, 2012). In the case of Greek EVOO, the implementation of a reliable traceability system is very important, given the nutritional and economic value of this product for the Greek population. Among the parameters that should be recorded in such a system is the varietal and geographical origin of the product. Such data should be collected by means of analytical or other methods which must be simple, rapid and the results obtained should be accurate, sensitive and reproducible. Various analytical techniques have been used to confirm the varietal and geographical origin of edible oils, most of which are expensive, time consuming, require analytical expertise and have a high environmental impact. In this respect, Differential Scanning Calorimetry (DSC) could be a promising alternative. This technique, which allows the physical changes that occur upon heating/cooling of a sample to be determined, possesses the advantages of being accurate, repeatable, relatively quick and simple to carry out, with minimal sample preparation, involving no chemical treatments (Cerretani et al, 2011). It has been applied for the characterisation of oils (Tan and Che Man, 200) and a data bank with the calorimetric "fingerprints" of the main edible oils has been created (Dyszel and Baish, 1992). Gas chromatography (GC) is a traditionally used method for screening differences amongst olive oil samples (Chiavaro et al, 2011).

In the present study, the applicability of GC, DSC or the combination of both techniques for the determination of the varietal and geographical origin of Greek EVOO was investigated. Contents of fatty acids and thermal parameters were determined in order to identify

relationships between them and also, to determine, by means of linear discriminant analysis, which parameters provide efficient sample differentiation and grouping.

19. MATERIALS AND METHODS

2.1 Materials

EVOO samples included in this study were kindly donated by olive oil Cooperatives Unions from three different geographical regions of Greece (Crete, Peloponnesus and Northern Aegean islands). The olives were harvested during the crop period of 2009–2010 and belonged to three important Greek cultivars (Koroneiki, Adramitiani and Thasitiki). The samples acquired were of known cultivar, geographical origin, method and date of production and were shipped in amber glass bottles, without headspace. Specifically, samples of Koroneiki cultivar originated from Peloponnesus and Crete (Southwestern and Southern Greece, respectively) and of Adramitiani and Thasitiki from the islands of N. Aegean Sea (Northeastern Greece), Mytilene and Thassos, respectively. A total of 20 samples were collected and stored in the dark, at room temperature until the time of analysis.

2.2 Methods

2.2.1 DSC

Quantities (6–10 mg) of samples to be analysed per protocol described below were accurately weighed (0.01mg) into aluminum pans, covers were hermetically sealed into place and analysed with a DSC Q100 (TA Instruments, New Castle, DE, USA), equipped with a liquid nitrogen cooling system (LNCS). Dry nitrogen (99.999% purity) was used as purge gas at a flow rate of 50 ml/min. Indium (melting temperature 156.60°C, ΔH = 28.45 J/g) and *n*–dodecane (melting temperature 9.65°C, ΔH = 216.73 J/g) were used to calibrate the instrument and an empty pan was used as reference.

The two developed protocols of analysis, freezing and melting, involved cooling and heating of EVOO samples, respectively, at a scanning rate of 10°C/min. The freezing protocol, from which cooling profiles were obtained, involved equilibration at 50°C for 2 min, then cooling to -40°C and subsequent holding at -40°C for 10 min. The melting protocol, from which heating profiles were obtained, involved equilibration at -50°C for 10 min and then heating to 40°C. Three replicates were analysed per sample for each protocol. The resulting thermograms were analysed by the Universal Analysis software (Version 4.2E, TA Instruments, New Castle, DE, USA).

2.2.2 Gas chromatography

Fatty acid methyl esters (FAMEs) were prepared from samples following the AOAC Official Method of Analysis (1975). All chemicals were of analytical grade. FAMEs were analysed by capillary gas liquid chromatography (GLC). 2 μl of sample were injected into the gas chromatograph, a type GC-17A, Ver. 3 (Shimadzu Corporation, Kyoto, Japan), equipped with an FID detector and 50m fused silica capillary column, type CP-Sil 88 (Chrompack, Raritan, NJ, USA). The oven temperature was programmed initially at 150°C, raised to 170°C at 5°C/min, kept for 10 min and finally raised to 220°C at 5°C/min and kept for 25 min. Total time of analysis was 49 min. Injector and detector temperatures were set at 250°C. Helium was used as a carrier gas (0.1 kPa/min, constant flow). Identification of FAMEs was possible using appropriate analytical standards (Sigma, St. Louis, MO, USA). All determinations were performed in duplicate and the mean values were reported. Results are expressed as percentage (w/w) of total fatty acids detected.

2.3 Statistical analysis

Statistical analysis was conducted using SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA). One–way analysis of variance (ANOVA) and SNK's multiple range test, at a 95% confidence level ($p<0.05$), were employed in order to identify differences among groups of samples according to cultivar and geographical origin. At a second step, the method of linear discriminant analysis (LDA), using the stepwise approach, was applied in order to build predictive models of cultivar or origin group membership based on the values of appropriate sets of variables. Furthermore, cross–validation of the models, by the leave–one–out method, was used in order to ensure that models with the best fit generally include only a subset of independent variables that are deemed truly informative. The optimum model dimensionality was identified as that with the maximum classification success rate by cross-validation.

20.RESULTS AND DISCUSSION

Greek EVOO samples were assigned to three groups according to cultivar and into another three groups according to geographical region of origin. Similar DSC protocols were employed by other researchers (Chiavaro et al, 2008; Angiuli et al, 2006; Ferrari et al, 2007; Angiuli et al, 2009) studying the thermal properties of olive oil. Heating thermograms

exhibited a minor exothermic peak, followed by multiple endothermic events. The thermal parameters derived from heating profiles were maximum melting peak temperature (Tm), onset of maximum melting peak (Mon), temperature of end of melting transition (Mend) and melting range (Rm), all in °C. During cooling, all samples exhibited two well discernible exothermic peaks. Parameters from cooling profiles were peak and onset temperatures (°C) of the first exothermic event (Tf_1 and Fon_1, respectively), temperature of end of crystallisation transition (Fend), freezing range (Rf) and time that the second exothermic event peaks (tf_2, in minutes). One–way analysis of variance indicated that each of the thermal parameters studied partially differentiated samples according to cultivar, as well as all of them except Rm, provided discrimination according to geographical origin ($p<0.001$), but differences between fatty acid profiles were not appreciable. Results according to cultivar and geographical origin are shown in Table 1. Specifically, Mon, differed in all cultivars and Mend and Fend, discriminated all origins ($p<0.001$). Amongst groups of cultivars, Koroneiki was shown to have a lower amount of C18:2cis, while Thasitiki had lower contents of SFA ($p<0.05$). Amongst groups of geographical origin, Crete had the lowest amounts of C18:2cis and PUFA ($p<0.05$).

Statistical analysis also aimed to identify relationships between content levels of fatty acids and thermal parameters. Results revealed that saturated fatty acids' content (SFA) was shown to influence the range of crystallisation (Rf), where it was shown that Rf fairly negatively correlated with SFA (R2 = -0.631). Polyunsaturated fatty acids' content (PUFA) influenced the final stage of crystallisation, at lower temperatures. PUFA fairly correlated with tf2 (R2 = 0.652). Results are in agreement with previous findings that this freezing stage is attributed to the crystallisation of highly unsaturated TAG, in particular triolein (OOO) (Chiavaro et al, 2011).

In order to improve Greek EVOO group differentiation by cultivar and geographical origin, all parameters derived from DSC and GC were evaluated with the help of LDA, so as to reveal which single or combination of these would produce a significant EVOO differentiation and correct classification in their assigned groups.

Regarding results solely from DSC, the combination of four thermal parameters, namely Mend, Rm with Fon1 and Rf, resulted in correct classification of 100.0% of samples to their assigned cultivars (Wilks' $\lambda = 0.088$, $p<0.001$). Results are shown in Fig. 1, where a score plot of the two canonical discriminant functions of the LDA model, constructed for EVOOs' discrimination according to cultivar, is presented.

TABLE 1. Thermal parameters and fatty acid contents of Greek EVOOs, according to cultivar and geographical origin (means ± standard deviation).

	Cultivar			*Origin*		
	Koroneiki	***Thasitiki***	***Adramitiani***	***Crete***	***Peloponnesus***	***N. Aegean***
			DSC			
Tm	-4.96±0.71 ^{a}	-6.76±0.34 ^{b}	-6.27±0.37 ^{b}	-4.88±0.67 ^{a}	-4.96±0.67 ^{a}	-6.33±0.53 ^{b}
Mon	-15.05±0.58 ^{a}	-17.69±0.39 ^{b}	-16.61±0.80 ^{c}	-14.99±0.61 ^{a}	-15.09±0.47 ^{a}	-17.02±0.79 ^{b}
Mend	11.61±1.57 ^{a}	8.44±0.64 ^{b}	7.44±0.47 ^{b}	10.81±0.89 ^{b}	12.68±1.28 ^{a}	8.20±0.85 ^{c}
Rm	26.66±1.66 ^{a}	26.13±0.64 ^{a}	24.05±1.02 ^{b}	25.80±1.11 ^{b}	27.77±1.24 ^{a}	25.22±1.23 ^{b}
Tf_1	-19.64±1.94 ^{a}	-24.83±2.92 ^{b}	-19.82±0.60 ^{a}	-20.70±1.88 ^{b}	-19.05±1.71 ^{a}	-22.01±3.00 ^{b}
Fon_1	-16.42±1.47 ^{a}	-20.54±1.93 ^{b}	-16.19±0.78 ^{a}	-17.24±1.28 ^{b}	-16.09±1.45 ^{a}	-18.16±2.41 ^{b}
Fend	-31.76±1.25 ^{a}	-35.80±2.71 ^{b}	-32.77±0.68 ^{a}	-32.32±1.66 ^{b}	-31.23±1.05 ^{a}	-33.91±2.31 ^{c}
tf_2	10.50±0.11 ^{b}	10.93±0.14 ^{a}	11.00±0.39 ^{a}	10.55±0.08 ^{b}	10.54±0.21 ^{b}	10.97±0.25 ^{a}
Rf	15.35±1.01 ^{b}	15.26±1.00 ^{b}	16.58±1.06 ^{a}	15.09±0.96 ^{a}	15.14±1.21 ^{a}	15.74±1.13 ^{a}
			GC			
C16:0	15.39±1.84 ^{a}	11.90±0.42 ^{a}	15.00±0.14 ^{a}	15.40±1.66 ^{a}	18.21±5.11 ^{a}	14.22±2.33 ^{a}
C16:1	1.17±0.20 ^{a}	1.00±0.14 ^{a}	1.10±0.28 ^{a}	1.11±0.18 ^{a}	1.58±0.83 ^{a}	1.12±0.23 ^{a}
C17:0	0.01±0.03 ^{a}	0 ^{a}	0.10±0.14 ^{a}	0.01±0.01 ^{a}	0 ^{a}	0.08±0.07 ^{a}
C18:0	2.25±0.21 ^{a}	1.90±0.14 ^{a}	2.2±0.14 ^{a}	2.34±0.24 ^{a}	2.80±1.96 ^{a}	2.14±0.27 ^{a}
C18:1cis	67.03±1.75 ^{a}	67.10±0.71 ^{a}	63.5±0.28 ^{a}	66.97±2.31 ^{a}	60.91±8.21 ^{a}	63.40±4.63 ^{a}
C18:2cis	10.85±2.24 ^{b}	15.75±0.35 ^{a}	15.50±0.01 ^{a}	10.59±2.29 ^{b}	13.60±5.47 ^{ab}	16.28±1.48 ^{a}
C18:3	1.16±0.13 ^{a}	1.05±0.07 ^{a}	1.25±0.21 ^{a}	1.11±0.11 ^{a}	1.11±0.48 ^{a}	1.26±0.29 ^{a}
C20:0	0.53±0.18 ^{a}	0.25±0.15 ^{a}	0.50±0.00 ^{a}	0.50±0.23 ^{a}	0.56±0.25 ^{a}	0.54±0.36 ^{a}
C20:1	0.49±0.13 ^{a}	0.50±0.14 ^{a}	0.50±0.00 ^{a}	0.50±0.14 ^{a}	0.44±0.21 ^{a}	0.56±0.15 ^{a}
C22:0	0.13±0.10 ^{a}	0.05±0.03 ^{a}	0.10±0.14 ^{a}	0.86±0.11 ^{a}	0.38±0.58 ^{a}	0.1±0.1 ^{a}
C24:0	0.18±0.27 ^{a}	0.55±0.07 ^{a}	0.25±0.07 ^{a}	1.36±2.85 ^{a}	0.41±0.50 ^{a}	0.36±0.18 ^{a}
SFA	19.28±1.83 ^{a}	14.65±0.07 ^{b}	18.25±0.07 ^{a}	19.70±1.72 ^{a}	22.36±7.45 ^{a}	17.44±2.85 ^{a}
MUFA	68.69±1.75 ^{a}	68.60±0.42 ^{a}	65.10±0.01 ^{a}	68.59±2.34 ^{a}	62.93±7.72 ^{a}	65.08±4.33 ^{a}
PUFA	12.02±2.32 ^{a}	16.80±0.42 ^{a}	16.75±0.21 ^{a}	11.70±2.36 ^{b}	14.71±5.72 ^{ab}	17.54±1.73 ^{a}

*Different superscripts across rows of the three first and three three last columns denote statistically significant differences ($p<0.05$)

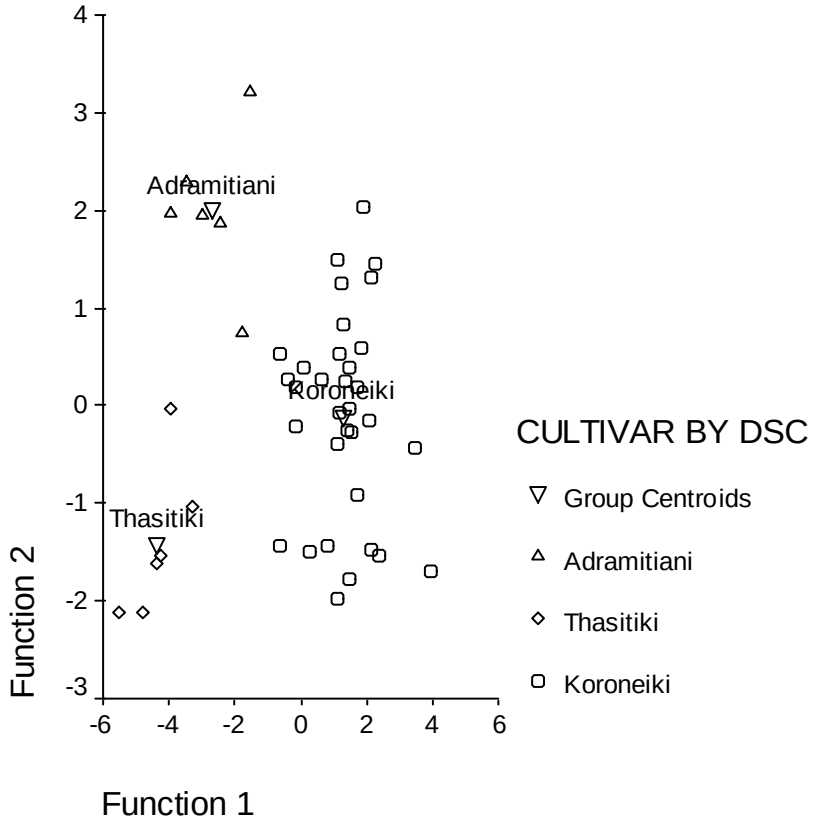

Figure 1. Score plot of two canonical discriminant functions of cultivar classification of Greek EVOOs, using variables Mend, Rm, Fon_1 and Rf.

Regarding results solely from GC, the combination of two variables, namely contents of C18:2cis and C20:0, resulted in correct classification of 86.7% of samples to their assigned cultivars (Wilks' λ = 0.200, p=0.001). Also, GC results in combination with DSC results, namely the variables SFA, Tm and tf_2, produced a prediction model which correctly classified 91.1% of samples (Wilks' λ = 0.041, p<0.001). The above suggest that GC analysis hindered sample differentiation and classification.

Discrimination of samples according to geographical origin of provenience was not possible by GC results, since no variables were qualified by the analysis (p=0.069). However, the combination of two thermal parameters derived from DSC, namely Mend and tf_2, resulted in correct classification of 86.7% of samples to their assigned origin groups (Wilks' λ = 0.116, p<0.001) (Fig. 2). Thus, the DSC technique was shown to have great potential of being used as a traceability monitoring tool for Greek EVOOs.

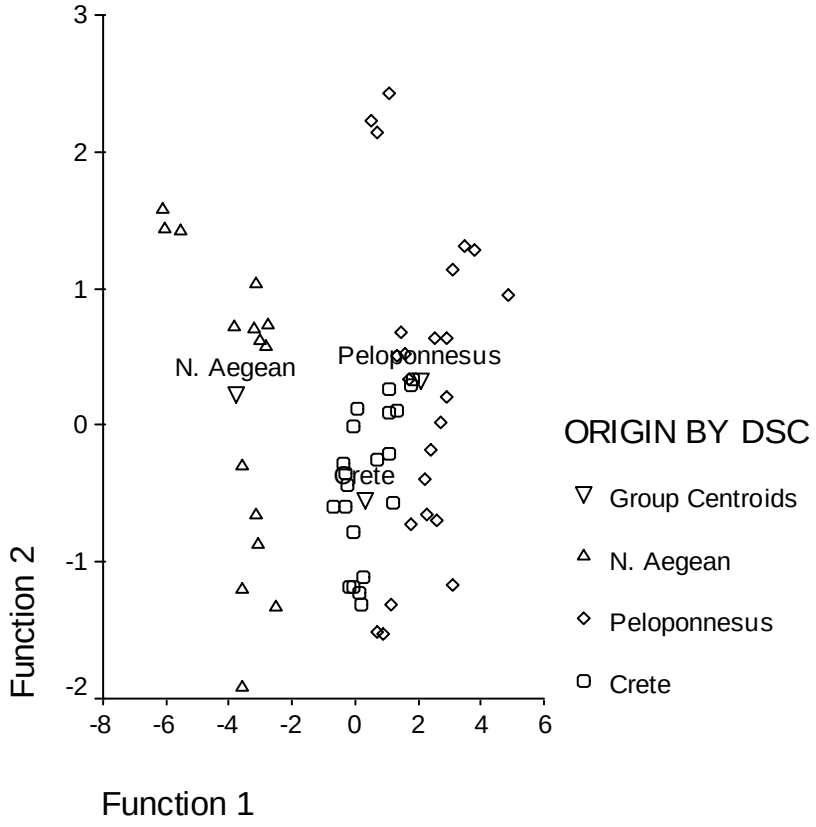

Figure 2. Score plot of two canonical discriminant functions of geographical origin classification of Greek EVOOs, using variables Mend and tf_2.

A recent paper on discrimination of Mediterranean EVOOs according to geographical origin and cultivar was published by Chiavaro et al. (2011). A multidisciplinary approach was also used, involving deconvoluted DSC results, during cooling down to -80°C, in combination with results from GC and isotope analysis. Correct classification of Italian EVOOs, by means of linear discriminant analysis of combined results, was reported (Chiavaro et al, 2011). In particular, LDA of combined results from DSC, GC and isotope analysis, produced correct classification of 94% of Italian EVOOs according to Italian region of origin (a total of 9 chosen variables) and of 100% of Italian EVOOs according to cultivar (a total of 10 variables). This multidisciplinary approach also proved to be efficient in discriminating EVOOs by to varietal and geographical origin.

The DSC techniques proposed in the present study revealed promising results, comparable to those produced using other multidisciplinary approaches. The present prediction models were produced by using techniques of increased simplicity and time efficiency. The findings of this

research could be further strengthened by creating larger databases of Greek EVOOs, incorporating monovarietal samples of additional cultivars and from different geographical regions of Greece.

21.CONCLUSIONS

GC alone did not prove to be effective in discriminating EVOOs in relation to geographical origin. Melting and freezing curves produced by DSC analysis of Greek monovarietal EVOO samples could discriminate samples according to cultivar and origin in a fast and simple way, suitable for oil industry and market traceability systems. Our observations, which are considered as results of an exploratory study with preliminary results, strengthen the premise that the sole use of DSC provides an efficient, fast, accurate and environmentally friendly method for characterising EVOO samples, with the help of multivariate analysis. The two proposed protocols of analysis were shown to be ideal for studying the thermal properties of EVOO samples originating from Greece.

22.ACKNOWLEDGEMENTS

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BIOCHEMICAL CHARACTERIZATION OF L-ASPARAGINASE FROM THREE *ASPERGILLUS* STRAINS

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ABSTRACT:

L-asparaginase (L-asparagine hydrolase, E.C.3.5.1.1) is an enzyme that is capable of selectively hydrolyzing asparagine resulting in the formation of aspartic acid and ammonia. L-asparaginase is claimed to significantly reduce the levels of acrylamide, a human carcinogen, in food containing reducing sugar and asparaginase and heated at temperatures above 100ºC. That enzyme converts L-asparagine into aspartic acid without altering the appearance and taste of the final product. The biochemical characterization is an important step in any bioprocess development. Hence, in the present study, three differentL-asparaginases from *Aspergillus oryzae LBA-01*, *Aspergillusoryzae 3940* and *Aspergillus niger LBA-02* were characterized. The enzyme production was carried out by submerged fermentation with modified Czapek-Dox medium at 30 ºC for 72-96 h. The L-asparaginases from*A.oryzae LBA-01*, *A. oryzae 3940* and *A. niger LBA-02* showed optimum activity at pH 7.0, 7.0-8.0 and 8.0-9.0, respectively. The pH stability tests revealed that the L-asparaginases from*A.oryzae LBA-01* and *A. oryzae 3940* were more stable around the pH 6.0-8.0, and the enzyme from *A. niger LBA-02 s*howed highest stability at pH 8.0-9.0. The L-asparaginases from *A.oryzae LBA-01, A. oryzae* TCC *3940, A. niger LBA-02* presented optimum activity at 40 ºC, 40-50ºC and 50ºC, respectively. The L-asparaginases from *A. oryzae 3940* and *A. niger LBA-02* retained 90% and 100% of initial activity after 1 hour at 50°C, respectively. The L-asparaginase from*A.oryzae LBA-01* showed lower stability retaining 60% of residual activity after 1 hour at 50°C. The results founded suggest that the enzymes studied have potential application in food industry.

Keyword: L-asparaginase,biochemical characterization, *Aspergillus oryzae, Aspergillus niger*

1. INTRODUCTION

L-asparaginase (L-asparagine hydrolase, E.C.3.5.1.1) is an extracellular enzyme that is capable of selectively hydrolyzing asparagine resulting in the formation of aspartic acid and ammonia (Capuano, Ferrigno, Acampa, Ait-Ameur, Fogliano, 2008). L-asparaginase has garnered substantial attention since it was presented by Broome (1961) and Mashburn and Wrinston (1963) that the bacterial enzyme can inhibit tumor growth. Since then, a large number of microorganisms has been investigatedaimed the production of L-asparaginase. The enzyme L-asparaginase has been isolated from various micro-organisms including Gram negative bacteria, Gram-positive, yeasts and fungi, as well as from plants (Baskar and Renganathan, 2011).

Asparaginase is claimed to significantly reduce the levels of acrylamide, a human carcinogen, in starchy food products containing reducing sugars and asparagine heated over 100ºC,by converting asparagine into aspartic acid without altering the appearance and taste of the final product. The addition of this enzyme in the food processing can reduce the formation of acrylamide up to 90% in starchy products heated over 100 ºC (Pedreschi, Mariotti, Granby and Risum, 2011, Pedreschi, Kaach and Granby, 2008).

Until the present day, L-asparaginase is subject of considerable medical interest. The enzyme is used as a chemotherapeutic agent for lymphocytic leukemia since it cleaves L-asparagine external to the cell (Ali, Rai, Soni, Kulshrestha and Lai, 1994; Shaffer, Arst, Estberg, Fernando, Ly, Sitter, 1988). Normal cells are able to produce all the asparagine they need whileneoplastic cells are unable to synthesize L-asparagine due to its low levels ofL-asparagine synthetase. L-asparaginase from*Escherichia coli* and *Erwiniacarotova*are used to treat leukemia and lymphoma dependent on L-asparagine (Keating, Holmes, Lerner and Image, 1993).However, the long term use of L-asparaginase from bacterial sources causes hypersensitivity that leads to anaphylaxis and allergic reactions in the patient. Eukaryotic microorganisms such as yeast and filamentous fungi have been described as producing significant amounts of extracellular L-asparaginase with fewer side effects (Sarquis, Oliveira, Santos and Da Costa, 2004, Ali, Rai, Soni, Kulshrestha and Lai, 1994; Shaffer, Arst, Estberg, Fernando, Ly, Sitter, 1988).

The biochemical characterizationis an important step for bioprocess andit is essential for improve thechemotherapeuticand food application of the L-asparaginase.

The demand for L-asparaginase will increase several fold in comingyears due to its potential application in food processing and its clinicalapplications. Hence, the present

studyassessed thebiochemical characterization of novel three different L-asparaginases from*Aspergillus oryzae LBA-01*, *Aspergillusoryzae 3940* and *Aspergillus niger LBA-02*aiming their possible food or chemotherapeuticapplications.

2. MATERIALS AND METHODS

2.1 Chemicals

Nessler Reagent was purchased from Merck (Darmstadt, Germany),L-asparagine was purchased fromVetec(Rio de Janeiro,Brazil).KH_2PO_4, KCl, $MgSO_4.7H_2O$, $CuNO_3.3H_2O$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$ and glucose wereobtained from Synth (Miami, U.S.A).

2.2Microorganisms and enzymaticcrudeextracts preparation

The filamentous fungi *Aspergillus oryzae* CCT 3940was obtained from theculturecollection ofthe FoundationAndréTosello- Campinas, Brazilandthe strainsof*Aspergillusoryzae*LBA-01 and *Aspergillus niger*LBA-02 were obtained from theculture collectionof the Laboratory ofFood Biochemistry, Department of Food Science, Faculty of Food Engineering,University ofCampinas. The strainsweremaintainedon inclined tubescontainingpotato dextrose agar (PDA)mediumwith 1% of asparagine, incubated at 30 ºC for 7 days, and stored at 4°C, withsubculturingevery 3months.

Conidial suspension was prepared from freshly raised seven day old culture on PDA medium by suspending in 5 mL of sterile Tween 80, 0,3% solution.The suspension was transferred aseptically to 250 mL Erlenmeyer containing 100 mL of modified CzapeckDoxmedium composed of: 2.0 g glucose, 10.0 g asparagine, 1.52 g KH_2PO_4, 0.52 g KCl, 0.52 g $MgSO_4.7H_2O$, 0.01 g $CuNO_3.3H_2O$, 0.01 g $ZnSO_4.7H_2O$ and 0.01 g of $FeSO_4.7H_2O$ per liter and the initial pH was adjusted to 6.2 (Gulati, Saxena and Gupita, 1997). The flasks were incubated at 30 °C and 150 rpm for 5 days. The enzymatic activity was measure every 24 h. The fungal cultures were filtered and the filtratewas calledenzymaticcrudeextract

2.3 Analytical methods

2.3.1 Determination of the activity of asparaginase

The L-asparaginase activity of crudeenzyme solution was assessed by Nesslarizationusingthe method described by Imada, Igarasi, Nakahama and Isono, 1973, with modifications. The reaction medium composed of 0.5 mL of 0.04 M L-asparagine, 0.5mL of0.1 M pH 8.0Tris-HClbuffer, 0.1mL of crude enzyme extract and 0.9mL of distilled water

was incubated at 37 °C for 30 minutes. The reaction was stopped with the addition of 0.5 mLof 1.5 M trichloroacetic acid. A blank was made with the addition of distilled water instead of the enzyme solution. The controlsamples were made with addition of the enzyme solution after addition of 1.5 M trichloraceticacid. A 125µL aliquot of reaction mixture was diluted with 1 mL of distilled water and 125µL of Nessler's reagent was added to it for quantification of ammonia. After 15 minutes, the absorbance wasmeasured at 450 nm in a spectrophotometer (DU®640, Beckman Coulter TM). An analytical curve with ammonium sulfate was used for the quantification of ammonia released (20-375 µmol ammonia/L). The enzyme activity was expressed in U/mL. One activity unit (U) of L-asparaginase is the amount of enzyme that catalyzes the formation of one micromol of ammoniaper mL per min.

2.3.2 Biochemical characterization of asparaginases

pH effect

The effect of pH on the L-asparaginase activity was assessed under standard assayconditions using a 50 mM sodium citrate buffer (pH 3.0-6.5), a 50 mMTris-HCl buffer(pH 7.0-9.0) and a 50 mM Borax-NaOH buffer (pH 10.0). The enzyme activity wasmeasured after 30 min at 37°C.

The effect of pH on asparaginase stability was assessed using the above mentionedbuffer systems over the pH range 3.0 to 10.0. The enzyme solutions were incubated at thevarious pH values for 60 min at 10 ºC without substrate. The remaining enzyme activitywas then measured at 37ºC using L-asparagine in a Tris-HCl buffer at pH 8.0.The residual activity was measured as described before.

Temperature effect

The effect of temperature on asparaginase activity was verified by assessing the activityat different temperatures (10 ºC and over the range from 30 °C to 70°Cat pH 8.0) using the reaction mixtures indicated previously.

Thetemperature stability was assessed by pre-incubating the L-asparaginase at various temperatures for 60 min, and immediately placing them on ice. The residual activity was determined as described before.

3. RESULTS AND DISCUSSION

3.1 Effect of time of fermentation on L-asparaginase activity

The maximumL-asparaginase production by *A. oryzae LBA-01*(20,6 U/mL) and *.A. oryzae 3940* (19,1 U/mL) was observed after 72 h fermentation at 30ºC The strain*A.nigerLBA-02* presented maximum L-asparaginase production (26,0 U/mL) after 96 h of fermentation (Figure 1).Siddalingeshwara and Lingappa (2011)observed maximum L-asparaginase production by*Aspergillus terreus*KLS2 after 72 h fermentation.Amena, Vishalakshi,Prabhakar ,Dayanand and Lingappa(2010) obtained maximum L-asparaginase production by *Streptomyces gulbargensis* after 120 h fermentation.

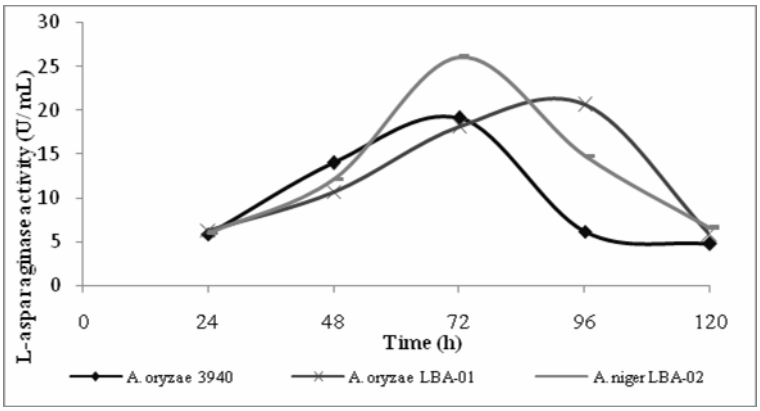

Figure 1. Production of asparaginase from *Aspergillus oryzae* TCC 3940, *Aspergillus oryzae* LBA-01 and *Aspergillus niger* LBA-02.

3.2 Effect of pH on asparaginase activity and stability

The L-asparaginases from*A.oryzae LBA-01*, *A. oryzae 3940* and *A. niger LBA-02* showed optimum activity at pH 7.0, 7.0-8.0 and 8.0-9.0, respectively (Figure 2a). In general, the range of optimum pH for L-asparaginase is from pH 6.5 to 9.0 (Maladkar, Singh and Nairk, 1993; Raha, Roy, Dey and Chakrabouty, 1990; Law and Wriston, 1971 Campbell, Mashburn, Boyse and Old, 1967) which is in accordance with the finds reported in this study. The L-asparaginases from *A. terreus* KLS2 (Siddalingeshwara and Lingappa, 2011), *Streptomyces gulbargensis* (Amena, Vishalakshi,Prabhakar ,Dayanand and Lingappa, 2010) and *Pectobacteriumcarotovorum*MTCC 1428 (Kumar, Dasu andPakshirajan, 2011) showed optimum activity in the range of pH 8.0 to 10.0. The L- asparaginases from *Helicobacter pylori* (Shibayama, Takeuchi, Wachino,Mori and Arakawa, 2011) and the L-asparaginase obtained from *Escherichia coli*, modified by linkage with palmitic acid chains

(Martins,Gonçalves e Cruz 1996) showed optimum activity at neutral pH. L- asparaginases showing activity at pH range from pH 5.5 to 8.0 are recommended to application in starch products (Vinci, Mestdagh and De Meulenaer, 2012; Food Standards, 2007).Mestdagh et al (2007) described that L-asparaginase activity at physiological pH is one of the pre-requisites for application of this enzyme as an antitumor agent.

The pH stability tests revealed that the L-asparaginases from*A.oryzae LBA-01* and *A. oryzae 3940* were more stable around the pH 6.0-8.0,which is desirable for food application aimed the acrylamide levels reduction. The enzyme from *A. niger LBA-02 s*howed highest stability at pH 8.0-9.0 (Figure 2b). It is observed that these enzymes were more stable at alkaline pH, retaining over 50% of residual activity at pH9.0 and 25% of residual activity at pH 4.0.

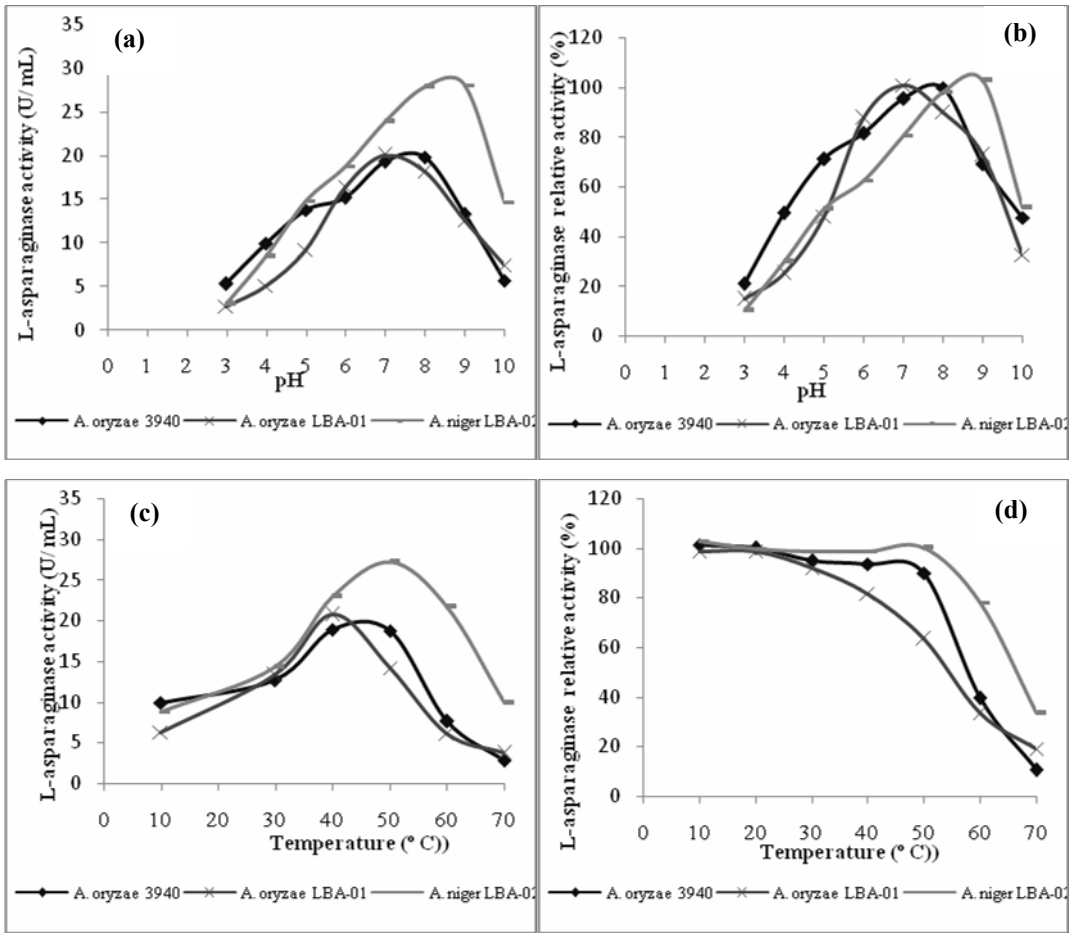

Figure 2. Effect of pH on the (a) activity and (b) stability and the effect oftemperature on (c) activity and (d) stability of crude asparaginase from*Aspergillus oryzae* TCC 3940, *Aspergillus oryzae* LBA-01 and *Aspergillus niger* LBA-02.

3.3 Effect of temperature on asparaginase activity and stability

The L-asparaginases from *A.oryzae LBA-01, A. oryzae* TCC *3940, A. niger LBA-02* presented optimum activity at 40ºC, 40-50ºC and 50ºC, respectively. The L-asparaginases from *Streptomyces gulbargensis* (Amena, Vishalakshi,Prabhakar ,Dayanand and Lingappa, 2010) and *Aspergillus terreus* (Siddalingeshwara and Lingappa, 2011) showed optimum activity at 40ºC and 37ºC, respectively.

The L-asparaginases from *A. oryzae 3940* and *A. niger LBA-02* retained 90% and 100% of initial activity after 1 hour at 50 °C, respectively. The L-asparaginase from*A.oryzae LBA-01* showed lower stability retaining 60% of residual activity after 1 hour at 50°C (Figure 2c).

Siddalingeshwara and Lingappa, 2011 found for L-asparaginase from *A.terreus* KLS2retained 50% and 80% when was incubated at 50 ºC for 30 and 60 minutes, respectively.

4. CONCLUSIONS

The L-asparaginases from*A.oryzae LBA-01* and *A. oryzae 3940* showed optimum activity at pH 7.0 and 40ºC and at pH 7.0-8.0 and 40-50ºC, respectively. The L-asparaginase from*A.niger*LBA 02 presented optimum activity at pH 9.0 and 50ºC.The L-asparaginases from*A.oryzae LBA-01* and *A. oryzae 3940* were more stable around the pH 6.0-8.0, and the enzyme from *A. niger LBA-02 s*howed highest stability at pH 8.0-9.0. The L-asparaginases from *A. oryzae 3940* and *A. niger LBA-02* retained 90% and 100% of initial activity after 1 hour at 50 °C, respectively. The L-asparagina from*A.oryzae LBA-01* showed lower stability retaining 60% of residual activity after 1 hour at 50°C. The results founded suggest that the enzymes studied have potential application in food industry for acrylamide levels reducing.

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VALORIZATION OF FLOUR-BASED INDUSTRIAL WASTE STREAMS FOR MICROBIAL OIL PRODUCTION

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ABSTRACT: Confectionary industries generate significant quantities of flour-rich waste streams, produced either during processing or as end-of-date products returned from the market. Such waste streams contain high amounts of starch, protein and micro-nutrients and can be utilised as renewable raw materials for the production of added-value products via fermentation. This study presents the hydrolysis of starch and protein, which are contained in the flour-rich waste streams, using crude amylolytic and proteolytic enzymes produced by a fungal strain of *Aspergillus awamori* through Solid State Fermentation (SSF). Hydrolysis was carried out using high concentrations of flour waste (25-35 % w/v), aiming to maximize the starch to glucose and protein to Free Amino Nitrogen (FAN) conversion yields. Hydrolysis experiments were carried out in fed-batch mode using different enzyme activities at optimum temperature and pH values. The obtained results showed that starch to glucose and protein to FAN conversion yields up to 96% and 45%, respectively, are feasible using the fed-batch hydrolysis process.

The produced hydrolysate was evaluated as fermentation feedstock for the production of microbial oil using an oleaginous strain of *Rhodosporidium toruloides*. Several bioreactor fermentations were carried out and different initial glucose and FAN concentrations were studied, aiming at maximizing microbial oil production. Lipid accumulation increased up to 50% (w/w), indicating that the integration of microbial oil production in existing confectionary industrial plants could lead to a viable and sustainable process.

Keywords: confectionery industry waste, enzymatic hydrolysis, microbial oil, bioreactor fermentation

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Biorefinery based on wine lees valorisation

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ABSTRACT: Wine lees (WL), produced during winemaking, are considered by-products and according to the European Council Regulation (EC) No 1493/1999 must be sent to alcohol distilleries, producing a solid waste, exhausted grape marc (EG) and a liquid waste, named vinasse (V). However, approximately 1365×10^3 tonnes/year of wine lees are included in the overall waste generation by wineries in EU-27, raising a serious environmental issue. The present study aimed to evaluate the potential of wine lees as renewable resource for biorefinery development. Wine lees produced from the wine making process were fractionated and evaluated for the production of various value-added products. After the extraction of ethanol, tartaric acid and antioxidants, research focused on the utilization of the remaining fraction, enriched in yeast cells, for the production of nutrient supplements for fermentation processes. Yeast cells were lysed using crude enzyme-rich extracts from solid state fermentation (SSF) of *Aspergillus oryzae*, producing a nutrient supplement that contained high concentrations of free amino nitrogen (FAN) and minerals (i.e. phosphorus).

The obtained hydrolysate was combined with crude glycerol in order to be evaluated as the sole nutrient supplement in shake flask fermentations using the bacterial strain *Cupriavidus necator* DSM 7237. The effect of initial FAN and IP concentration in microbial growth and polyhydroxyalkanoate (PHA) accumulation was investigated and PHA content higher than 50% (w/w) was obtained. Bioreactor fermentations also showed promising results regarding PHA production.

The present study suggests that crude glycerol combined with wine lees hydrolysate can substitute commercial nutrient supplements for PHA production and indicates that the integration of PHAs and other added-value compounds production in existing winery plants could lead to a viable and sustainable process.

Keywords: wine lees, tartaric acid, antioxidants, enzymatic hydrolysis, microbial bioconversion, PHA

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This work is part of the "Valorization of cheese dairy and winery wastes for the production of high added-value products" project (19SMEs2009), implemented within the National Strategic Reference Framework (NSRF) 2007-2013 and co-financed by National (Greek Ministry- General Secretariat of Research and Technology) and Community Funds (E.U.-European Social Fund). This work is also included in the Cost Action TD1203 entitled "*Food waste valorisation for sustainable chemicals, materials & fuels (EUBis)*"

PHYTOSTEROL AND γ-ORYZANOL STRUCTURED ORGANOGELS AND EMULSIONS: MICRO-STRUCTURAL FEATURES AND RHEOLOGICAL PROPERTIES

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ABSTRACT:

γ-Oryzanol and phytosterolshave been reported as oil structuring agents alternative to saturated fat. Previous research has shown that oryzanol-rich structurants of 60:40 weight ratio or 1:1 molar ratio (γ-oryzanol:phytosterols) demonstrate high structuring capacity creating transparent organogels. In the present study, two γ-oryzanol – phytosterols ratios, 60:40 and 30:70, were investigated in both sunflower oil organogels and emulsion gels by bulk rheology, texture analysis and polarised microscopy. The influence of total sterol content was also examined. Extensive phytosterol crystal formation was microscopically observed in phytosterol-rich systems. Texture analysis indicated thatan increase in total sterols content resulted in increased hardnessand gel strength of the organogels. Longer storage times and lower temperaturesincreased the organogels hardness, while no differences were observed in gel strength. With regard to organogel-based emulsions, rheological measurements showed that an increase in total sterol content resulted in a statistically significant increase in storage modulus (G'), yield stress(τ_y), and viscosity (η) of the emulsions. The 30:70 organogel-based emulsions had a substantially higher G' and $\tau_y$in relation to 60:40-emulsions. The presence of phytosterol crystals seems to play the dominant role in structuring and thereby the mechanical properties of the organogel-in-water emulsions.

Keywords: organogels, organogel-in-water emulsions, phytosterols, γ-oryzanol, rheology, microstructure

1. INTRODUCTION

Lipid phase structurantsas potential replacers of highly saturated triacylglycerol have attracted considerable attention in recent years. Low molecular weight structuring agents can variously serve this purpose (Pernetti*et al.*, 2007). Previous research has shown that γ-oryzanol and β-sitosterol can actually structure vegetable oils, forming edible oil gels or organogels (Bot &Agterof, 2006). γ-Oryzanol is extracted from rice bran oil and it is derived from a fraction containing ferulate (4-hydroxy-3-methoxycinnamic acid) esters of triterpene alcohols and plant sterols (Rogers *et al.*, 1993). β-Sitosterol is a phytosterolwidespread in plants (Moreau *et al.*, 2002). Phytosterols are non-nutritive compounds that occur naturally in vegetable products (such as fruits and nuts) and oils, with the same basic functions in plants as cholesterol in animals (Brufau*et al.*, 2008).

Oil structuring ability of γ-oryzanol and different phytosterols is attributed to the formation of hollow tubules of 7nm diameter and just under 1 nm wall thickness (Bot *et al.*, 2008). The formation of the tubules does not require an intermediate stage involving a tubular micelle, but rather "crystallization" directly from the solution (Bot *et al.*, 2009a). The tubule formation is a synergistic phenomenon between the phytosterol and γ-oryzanol, because individually neither is capable of entrapping the oil phase (Bot and Agterof, 2006). The oil phase is present both inside and outside of the tubule (Bot *et al.*, 2009a). The firmness and transparency of the organogel depend on the oryzanol-sitosterol ratio (Bot and Agterof, 2006; Bot *et al.*, 2008; Bot *et al.*, 2009b). The turbidity of the gel decreased with increasing γ-oryzanol concentration and the firmest gel is usually obtained at the ratio of ≈ 60-40 w/w oryzanol-sitosterolwhich corresponds to 1:1 molar ratio (Bot and Agterof, 2006; Bot *et al.*, 2008; Bot *et al.*, 2009b). Modifications to the ratio of phytosterol to γ-oryzanol affect not only the opacity of gels but also the aggregation into tubules and the activation energy of nucleation (Rogers *et al.*, 2010; Sawalha*et al.*, 2011).

γ-Oryzanol and phytosterols are capable of structuring o/w and w/o emulsions. Previous investigations have shown that the presence of water interferes with the capacity of the phytosterols to structure the emulsion (Bot *et al.*, 2009b; Duffy *et al.*, 2009; den Adel *et al.*, 2010; Bot *et al.*, 2011).It has been reported that a mixture of oryzanol and sitosterol(16% in total, ratio 60:40) forms crystals in water and in emulsions that can be related to the crystals of the pure compounds. In the case of emulsions, it is suggested that sterols have to diffuse from the oil phase into the aqueous phase, during and after the emulsification process, to form

the tubular structures or crystals (Duffy *et al.*, 2009). The formation of sterol tubular microstructure in the emulsion can be promoted by reducing the water activity and/or by using oil of low polarity (Sawalha*et al.*, 2012). However, in oil gels a completely different structure is formed which is not related to the structures that are formed by both individual compounds, and which can be identified as a self-assembled tubular structure involving both molecules of sitosterol and oryzanol(den Adel *et al.*, 2010).

The aim of this study was to investigate the microstructure and the rheological properties of sunflower oil organogels and organogel-based emulsions produced by two weight ratios of γ-oryzanol and phytosterols(30:70, 60:40). The 30:70 ratio was specifically chosen to be compared to the aforementioned ideal weight ratio 60:40, aiming at a phytosterol-enriched formulation due to dietary purposes for possible cholesterol-lowering properties.

2. MATERIALS AND METHODS

2.1 Materials and sample preparation

In the present study, sunflower oil (Minerva) obtained from a local super market, γ-oryzanol (Jan Dekker Nederland B.V., Wormerveer, the Netherlands) and phytosterols (commercial product Vegapure 867G, kindly offered by BASF Group, Ludwigshafen, Germany), were used for the preparation of organogels. In the organogel-based emulsion were additionaly used Tween® 20 (SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany), xanthan gum (SIGMA CHEMICAL CO., Saint Louis, Missouri, USA) and distilled water.

Two different γ-oryzanol:phytosterols weight ratios, 30:70 and 60:40, were used in the preparation of solutions of 5%, 10%, 15% and 20% total sterol content with sunflower oil. A given amount of γ-oryzanol and phytosterolswas added in oil at ambient temperature (25 ^{o}C). The solutions were heated and maintained at the temperature range of 90-95 ^{o}C for 30 min, under constant stirring. The samples were cooled and stored at 4 ^{o}C or 25^{o}C, depending on the experimental procedure.

Four sunflower oil and sterol solutions (10% and 20% total sterol content, 30:70 and 60:40 γ-oryzanol tophytosterols weight ratio) of temperature higher than 90 ^{o}C were prepared to produce organogel-in-water emulsions. Moreover, the aqueous phase of the emulsions were prepared by diluting the appropriate amount of Tween 20 (1.7% w/w in the emulsion) and xanthan (0.15% w/w in the emulsion) into distilled water and heating the solution up to 70 ^{o}C under stirring. Emulsions were prepared by homogenising the oil and the water phase using a high shear disperser (Ultra Turrax IKA T18 basic,IKA Works Inc., Wilmington, USA) for 1

min at 14.000 rpm at >70 ^{o}C. The produced emulsions were cooled at ambient temperature and stored at 4 ^{o}C until further examination.

2.2 Optical Microscopy

Polarised micrographs of 10% and 20% organogelswith 30:70 and 60:40 sterol weight ratio and organogel-structured emulsions were captured using anOlympus BX51(Olympus Optical Co Ltd, Tokyo, Japan) microscope equipped with a microscope digital camera (Olympus DP70, Japan). Freshly prepared organogelswere placed onto a slide with a cover glass and observed after 24 h at ambient temperature without any further preparation.

2.3 Penetration Test

Penetration tests were performed in organogel samples on a TA-XT2i Texture Analyser (Godalming, Surrey, UK) using a stainless steel cylindrical probe with a diameter of 6 mm. Hot solutions of 5%, 10%, 15% and 20% sterols (30:70 and 60:40 weight ratio) with sunflower oil were channeled into plastic cylindrical containers and stored at 4 ^{o}C or 25^{o}C. After a period of approximately 24 h,in which gelation has taken place, organogels were removed and cut to self-sustained cylinders of 10 mm height and 19 mm diameter. Penetration experiments were performed at a penetration speed of 0.5 mms^{-1} (probe's speed before and after penetration 1.0 mms^{-1} and 5.0 mms^{-1}, respectively) over a distance of 5 mm, corresponding to the 50% of the height of the samples. The parameters extracted were hardness (N) as the maximum force that occurred during penetration and total work for penetration, defined as the gel strength (mJ), represented by the surface under the force deformation curve (Van Camp &Huyghebaert, 1995; Verbeken*et al.*, 2006). At least five measurements were performed at ambient temperature and data were collected by using the software Texture Expert Version 1.22 of Stable Micro Systems (Godalming, Surrey, UK).

2.4 Rheological Measurements

The rheological properties of the organogel-based emulsions were studied by a rotational Physica MCR 300 rheometer (PhysicaMesstechnic GmbH, Stuttgart, Germany) using a parallel plate geometry (50mm diameter and 1 mm gap);thetemperature was regulated by a PaarPhysica circulating bath and a controlled peltier system (TEZ 150 P/MCR) with an accuracy of ± 0.1^{o}C. The data of the rheological measurements were analyzed with a supportingrheometer software US200 V2.21. Two recordings were made per sample and each measurement was carried out on two separate prepared samples (total four measurements per

sample). All tests were performed at 25 ^{o}C.Initially, strain sweep tests at 1 Hz were performed for all the samples in order to determine the linear viscoelastic region (LVR). A target strain of 1% and 0.5% for the 10% and 20% organogel-based emulsions respectively was used in the subsequent experiments, which was within the LVR for each sample examined. Small deformation oscillatory measurements for evaluation of the viscoelastic properties, G' (storage modulus), G" (loss modulus) and η* (complex viscosity) were performed over the frequency range 0.1 to 100 Hz at 25 ^{o}C.In addition, the flow behaviour of the samples was assessed by measuring steady shear viscosity η (Pa·s) over a range of shear rates 0.001–100 s^{-1}.

2.5Statistical Analysis

The data collected during measurements were analyzed using Minitab 16 (Minitab Inc.) for one-way ANOVA. Tukey's HSD test was used to identify significant differences ($p<0.05$) between samples.

3. RESULTS AND DISCUSSION

3.1 Organogels

Organogels of sunflower oil were prepared at concentrations of 5%, 10% and 20% w/w sterols at 60:40 and 30:70 weight ratios (Figure 1).

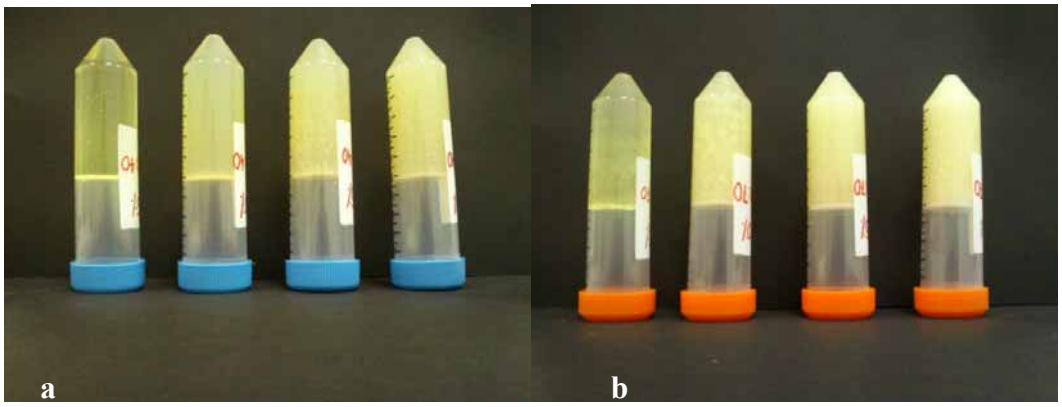


Figure 1. Sunflower oil organogels in various total concentrations 21 days after preparation at ambient temperature. Sterol concentration from left to right: 5%, 10%, 15%, 20% w/w. (a) 60:40 γ-oryzanol:phytosterolsand (b) 30:70 γ-oryzanol:phytosterols weight ratio.

Organogel with 5% sterol content in particular was not self-sustaining, and thusit could not be subjected to penetration test even after being stored at ambient temperature for 10 days. On the other hand, the organogels of 10% and 20% of sterols were hard enough to be subjected

topenetration test 24 h after preparation. The hardness and gel strength of the organogels increasestatistically significantly with increasing total sterol content (Figure 2).

As previous research has shown, the sterol mass ratio affects in a large extent the appearance and mechanical properties of organogels (Bot *et al.*, 2008). That is, the 60:40 ratio (Figure 1a) results in transparent gels while in the case of 30:70 ratio more turbid gels are formed (Figure 1b). This implies that the gel structuring blocks at 60:40 ratio are considerably smaller than the wavelength of visible light (Pernetti*et al.*, 2007). However, the turbidity at 30:70 gels can be explained by the crystallization of excess phytosterols to large-scale structures, much larger than the fiber structures of γ-oryzanol and phytosterol network (Bot *et al.*, 2008).

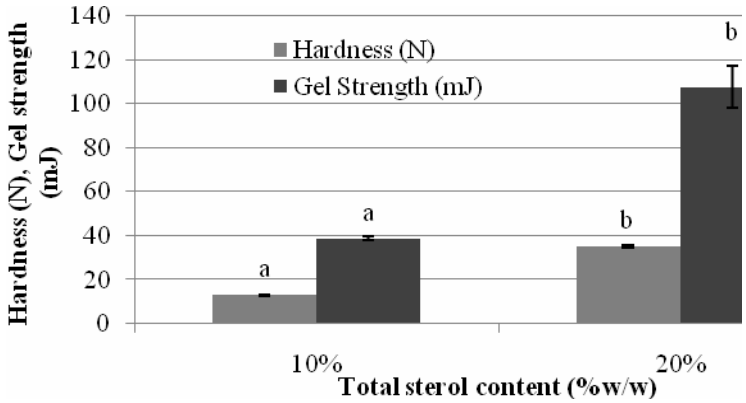


Figure 2. Effect of total sterol content (10% and 20% w/w) on sunflower oil organogels hardness and gel strength at 60:40 γ-oryzanol:phytosterols weight ratio, 24 h after preparation.

Similarly, the sterol ratio affected also the gelhardness and gel strength. According to macroscopic observation, the 30:70 gels were more brittle and softer than the 60:40 samples, with oily (greasy) texture resembling to that of wax. Indeed, the penetration test confirmed the increased (twofold) hardness and gel strength of 60:40 gels versus to 30:70 ones in both sterol contents examined (10% and 20%). Assuming that both sterols are involved in the fibrillar network at 60:40 ratiowhich corresponds to 1:1 molar ratio, then in 30:70 gels, the phytosterols are in excess and therefore they crystallize. Apparently,crystallizationof phytosterolscontributesto some extent to the mechanical propertiesof the gels,however the networkinduced by oryzanol and phytosterol seems to play the important role in network formation.

Moreover, hardness increased statistically significantly for both sterol concentrations and ratios with time. Although the absolute values of gel strengthincreased with time, no statistically significant differences were detected. Over time, gel structures are subjected to perpetual stabilization and enhancement. This phenomenon explains the fact that gels with

low structurant content, such as the 5% organogel sample mentioned above, reorganise and rearrange their structure and become more rigid with time, as seen in Figure 1, 21 days after preparation.

The effect of two storage temperatures, 4 ^{o}C and 20 ^{o}C for 48h, on hardness and gel strength was studied in 30:70 ratio organogels with 10% and 20% total sterol content.Samples showed no differences in the macroscopic examination. However, the penetration test indicated that by reducing the storage temperature, the hardness of organogels increased statistically significantly for both samples (Figure3). Undoubtedly, at low temperatures crystal growth occurs more rapidly and crystal-structured gels of 30:70 ratio become more resilient. Gel strength increased at 4 ^{o}C, and statistically significant differences were detected only for the 20% w/w sample. An increase in gel stability at low temperatures (5 ^{o}C) has been also observed in rapeseed organogels with 12-hydroxy-stearic acid (Rogers *et al.*, 2008).

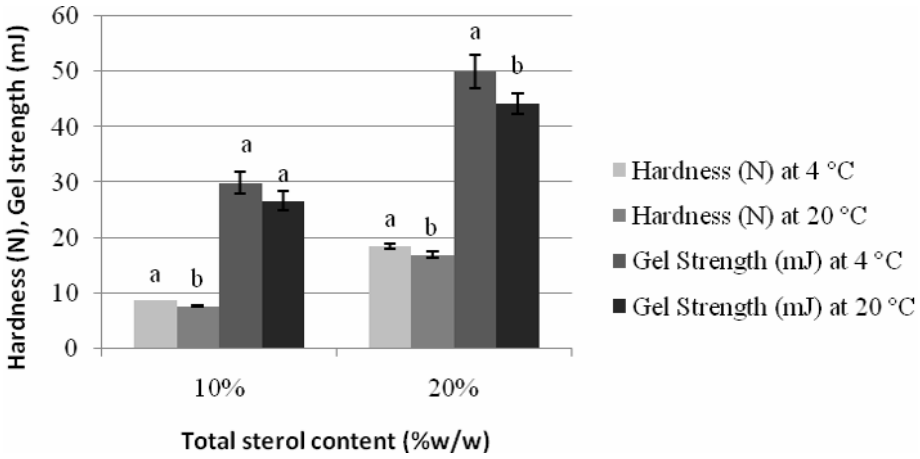
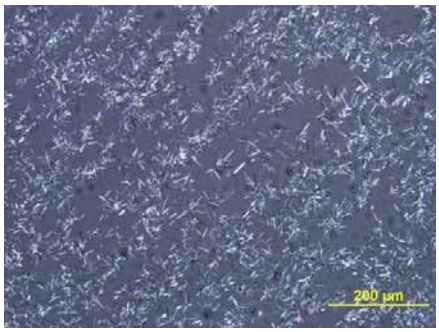
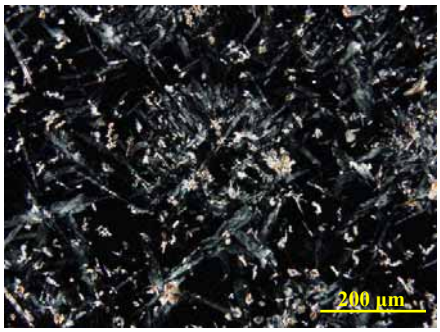


Figure 3. Effect of 48h storage temperature (4 ^{o}C and 20 ^{o}C) on gel hardness and gel strength of organogelswith 10% and 20% sterol content, at 30:70 γ-oryzanol:phytosterols weight ratio.

a 

b 

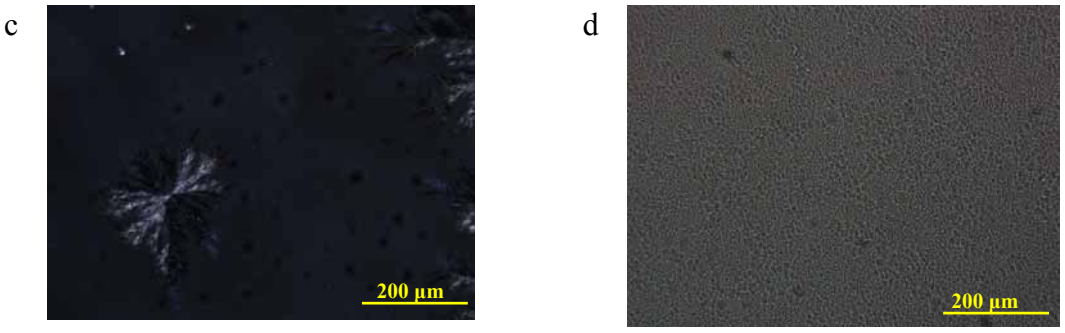


Figure 4. Polarized micrographs of organogels, 24 h after preparation, at ambient temperature: (a) 10% w/w sterol content 30:70 weight ratio, (b) 20% w/w - 30:70, (c) 10% w/w - 60:40 and (d) 20% w/w - 60:40.

Figure 4illustrates the microstructure of sunflower oil organogels with 10% and 20% structurants at different sterol weight ratios, 60:40 and 30:70, after 24 h at ambient temperature. In the 30:70 samples (Figure 4a & b), crystal formation of sterolsis favoured. The crystals resemble typical phytosterol crystals observed in phytosterol-saturated oil systems (Vaikousi*et al.*, 2007). At 20% sterol content samples (Figure 4b), the phytosterol quantity in excess is even greater. Consequently, sizable and diverse crystal formations occurin these samples, either in wide and elongated forms or as aggregates of smaller crystal structures. On the other hand, the 60:40 sterol weight ratio or 1:1 molar ratio has been characterised as ideal for creating sterol structured organogels (Pernetti*et al.*, 2007). Indeed, in this ratio, no crystals were observed (Figure 4 c & d), only some tufted formations. These formations are likely to be the result of the sterolsfibrillar network, described by other researchers (Bot *et al.*, 2008).

3.2 Emulsions

3.2.1 Optical Microscopy

Sterol-structured organogels emulsified with water in the presence of Tween 20 and xanthan led to organogel in water emulsions (Figure 5).

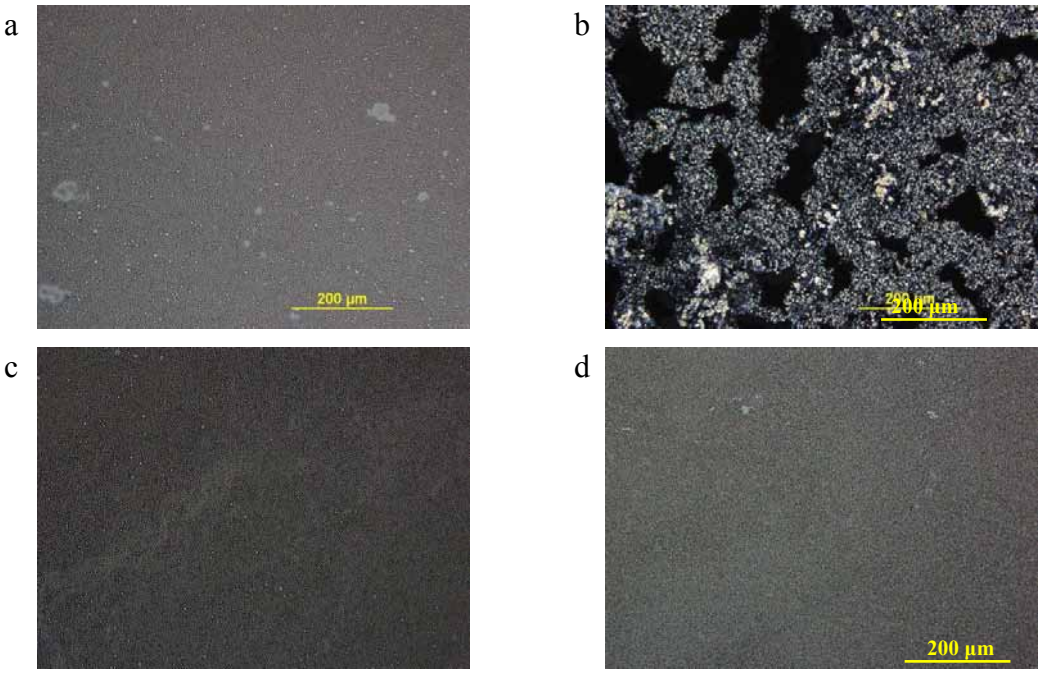


Figure 5.Polarized micrographs of emulsions, after storage at 4 ^{o}C for 24h. The emulsions contain 50% aqueous phase and 50% organogel: (a) 10% w/w sterol content 30:70 weight ratio, (b) 20% w/w - 30:70, (c) 10% w/w - 60:40 and (d) 20% w/w - 60:40.

Irrespective of sterol weight ratio, 10% w/w organogels(Figure 5 a & c) produced stable emulsions with small size of oil droplets dispersed in the aqueous phase, where no crystal formation was detected 24 h after storage at 4 ^{o}C. However, in 20% w/w organogel-based emulsions of 30:70 ratio (Figure 5 b), numerous crystalline structures were observed. On the other hand, at the 60:40 ratio, no crystal formation was noticed even at 20% w/w sterols. At 30:70 ratio (Figure 5a & b), only at high sterol content, i.e. 20% w/w (Figure 5 b), the crystal formation is extensive. This phenomenon could be attributed to two main factors. First factor is related to the 30:70 γ-oryzanol:phytosterols weight ratio in gel. As previously mentioned, the abundant phytosterols in the system are crucially involved in crystalline structures. The second factor refers to the presence of water. Previous research has shown that sterols (γ-oryzanol and β-sitosterol), in the presence of water, crystallise into forms similar to those that each sterol would individually form alone (Duffy *et al.*, 2009; den Adel *et al.*, 2010; Bot *et al.*, 2011). Basically, the water appears to inhibit or destabilize the tubular structures of sterols that occur in organogels. The absence of crystalline features in 60:40 weight ratio is probably due to the 1:1 molar ratio of sterols. In general, the crystallization of a structurant spreads rapidly in a continuous phase. However, in case of an oil-in-water emulsion, the oil phase is

dispersed into the aqueous phase and therefore crystallization or structure formation takes place more slowly since it initiates independently in each oil droplet (Bot *et al.*, 2009b). Of course, the absence of crystals at the time of the microscopic observation of the emulsions, i.e., 24 h after preparation, does not preclude their creation in the future. However, evolution of the emulsion microstructure over time was not further investigated in the present study.

3.2.1 Rheological Analysis

Strain sweep tests (results not shown) of 10% w/w organogel-based emulsions indicated a remarkable range of linear viscoelastic region (LVR) for both sterol ratios in which the 30:70 sample (G' ≈ 9.5 Pa) exhibited much greater LVR than the 60:40 sample (G' ≈ 6.0 Pa). On the other hand, tanδ (≈ 0.365) did not vary significantly between the two samples. Comparing the 20% w/w emulsion gels, the 30:70 emulsion exhibited much higher storage modulus values at the LVR (G' ≈ 735 Pa) than the 60:40 sample (G' ≈ 230 Pa). On the contrary, tanδ of 20% emulsions showed significant deviation. It is noteworthy that the 60:40 emulsion, despite the low values of storage modulus exhibited lower tanδ, i.e. it has a dominant elastic character.
With reference to the yield stress, defined as the stress required to initiate flow (Tabilo-Munizaga& Barbosa-Cánovas, 2005), during the strain sweep test, the 20%-30:70 emulsion gel exhibited the higher level of internal structure against all other samples (Table 1). In emulsions of the same sterol content, the30:70 samples were more stable than the corresponding 60:40 samples. Consequently, unlike to organogels, in emulsion gels the water obviously weakens the sterol tubular network (Bot *et al.*, 2011) and the 30:70 ratio indicates a stronger structure due to numerous phytosterol crystal formations.

TABLE 1. Yield stress (Pa) (± SE) of organogel-structured emulsions measured by strain sweep oscillatory tests.

Organogel composition in emulsion	Yield Stress (Pa)
10% - 30:70	0.79 ± 0.07
10% - 60:40	0.41 ± 0.01
20% - 30:70	2.40 ± 0.14
20% - 60:40	1.49 ± 0.18

Frequency sweep tests indicated that there is an effective gel state in 10% emulsion gels (Figure 6 a & b). In the present study, gel was defined as a material with G' exhibiting a pronounced plateau region extending to timescales of at least the order of seconds, and having a loss modulus G", which is considerably smaller than the G' in the plateau region (Moschakis *et al.*, 2005). Moreover, in 10% emulsions, both the elastic G' and the loss modulus G"

appeared to depend on the frequency, displaying a slight increase with increasing frequency. Emulsions of 20% sterol content in their organogel phase, showed also a slight increase of G' and G" with frequency (results not shown).

During large deformation steady-state viscometry, the measured viscosity of the emulsions was found to increase with sterol concentration and to show pronounced shear-thinning behaviour (results not shown). The low shear rate viscosity in 10%-30:70 emulsion was found to be 90 Pa·s while for the 10%-60:40 emulsion was 70 Pa·s illustrating the higher structural organization level in the first sample. The 20% emulsions did not exhibit shear-thinning behaviour, which is in agreement with their internal complex structure that was observed microscopically.

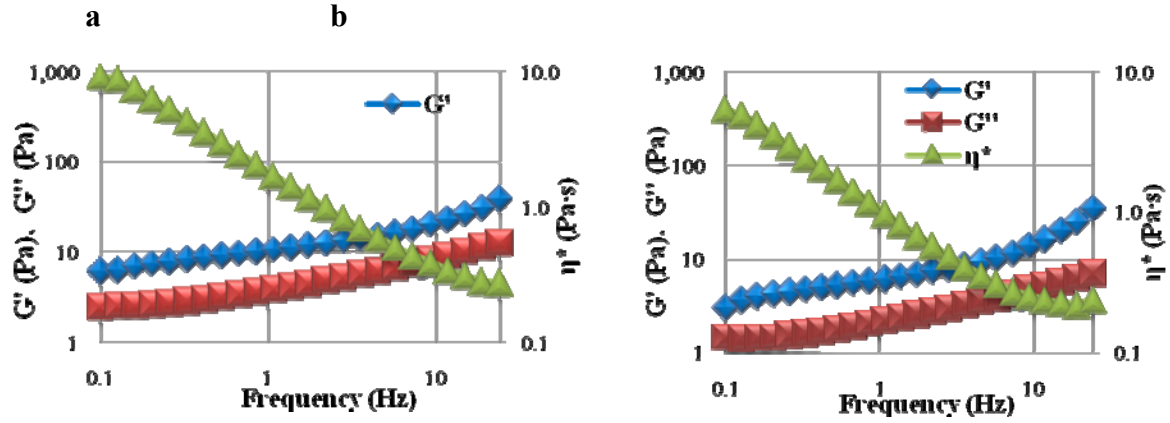


Figure 6. Variation of G', G" and η* as a function of frequency; (a) organogel (10% w/w, 30:70 sterol ratio) – based emulsion, (b) Organogel (10% w/w, 60:40 sterol ratio) – based emulsion.

4. CONCLUSIONS

γ-Oryzanol and phytosterols constitute an extremely interesting research field in terms of vegetable oil structuring. Sterol ratio and total sterol content define the properties of the oil matrix, both in oil gels and emulsions. In 30:70 sterol ratio, phytosterols in excess crystallize and therefore stabilize the system while the 60:40ratio favours fibril formation. Consequently,

the 30:70 ratioproduces stronger emulsion gels comparing to the 60:40 ratio due to the destabilising effect of water on fibrillar network. On the contrary, 60:40 ratio produces stronger gels indicating a remarkable fibril structuring effect on pure oil phase. An increase in total sterol content amplifies the structuring potential of both sterol ratios in organogels and emulsions. Vegetable oil gels could be utilized in foods containing relatively high amounts of solid fat. Organogel-based emulsions, on the other hand, are more appropriate for foods with emulsion composition such as margarine, spread and processed cheese, mayonnaise and dressings. With regard to sterol weight ratio, the 60:40 is undoubtedly more adequate in applications where firm organogels are required, while the 30:70 ratio is appropriate primarily for stabilising emulsions, and secondly for formulating functional foods.

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OSMOTIC TREATMENT OF BEEF MEAT WITH LIQUID SMOKE ADDITION: KINETICS, MICROBIAL AND OXIDATIVE STABILITY

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ABTRACT:

Osmotic dehydration, in the form of brining and marination, is traditionally used for meat processing and preservation. The objective of this study was to investigate the effect of liquid smoke on the kinetics of the osmotic process and to evaluate the subsequent microbiological and oxidative stability of the processed meat. Beef meat slices were immersed for 3h in 20% and 25% NaCl brines at 5^{o} and 15^{o} C, with or without 0.5% liquid smoke. Controls and osmotically treated samples were then stored at 4^{o} C and total viable counts (TVC) and oxidation levels (TBA test) were measured at 0, 1, 3, 6 and 9 d. The 25% NaCl brine resulted in increased moisture loss (ML) and solids gain (SG). Processing at 15^{o} C vs. 5^{o} C resulted in increased ML but it did not affect SG. The addition of liquid smoke resulted in increased ML, SG and salt pick-up at the lower processing temperature of 5^{o} C. Regarding the storage study, the 25% NaCl brine at 5^{o} C resulted in lower TVC. The osmotic process was effective in maintaining low TVC throughout the 9d storage period. The addition of liquid smoke in the brine did not affect the TVC, because of the drastic impact of the NaCl. However, due to the prooxidant effect of NaCl, osmotically processed samples in brines with no liquid smoke added, had increased oxidation levels (higher TBA values). Addition of 0.5% liquid smoke was effective in inhibiting oxidation and keeping the TBA values very low for the 9d of storage time. In conclusion, osmotic treatment of beef meat with liquid smoke can inhibit microbial growth and lipid oxidation. Adjustments in the osmotic process conditions need to be made, to account for the effect of liquid smoke on moisture loss and solids gain.

Keywords: osmotic process, beef, liquid smoke, TBA, TVC

1. INTRODUCTION

Osmotic processing aims at the removal of water in liquid form under mild temperatures (usually up to 50 ^{o}C). Consequently, the energy required for the dehydration can be decreased up to 2-3 times (Bolin et al., 1983; Lenart and Lewicki, 1988), especially when the osmotic solution is recycled. Moreover, dehydration without phase change leads to products of superior nutritional and organoleptic characteristics. Formulation of the product can also be applied, mainly in terms of candying (confectioneries) or salting (fish and meat). Emerging technologies such as ultrasound (Simal et al., 1998), γ-irradiation (Rastogi and Raghavarao, 2004), high intensity pulsed electric fields (Ade-Omowaye et al., 2002; 2003) and ultra-high hydrostatic pressure (Basak and Ramaswamy, 1998; Rastogi et al., 2000; Rastogi and Niranjan, 1998) offer some interesting pre-treatment alternatives for fast water activity reduction. They are all non-thermal treatments in order to meet consumers demand for minimum processed foods of high nutritional value and organoleptic quality.

Water activity of osmotically treated foods is significantly lowered, compared to the fresh product, due to the combined effect of water removal and solute incorporation, thus microbial growth is reduced (Collignan and Raoult-Wack, 1994; Rastogi et al., 2002). Additionally, during the osmotic treatment the microbial load can also be decreased due to the decontaminating effect of the high solute concentrations to the product/solution interface (Collignan et al., 2001). Shelf life of osmotically-treated food products proved to be sufficiently prolonged under refrigerated storage (Dermesonlouoglou et al., 2007a; 2007b). In a recent study, calcium alginate coating was found to slow down microbial growth during the osmotic treatment and refrigerated storage (Mitrakas et al., 2008). Elevated process temperatures may increase the rates of mass transfer phenomena, but attention should be paid to the increased risk of microbial growth, especially for meat products (Lawrie, 2005; Mujaffar and Sankat, 2005).

Smoking of meat and fish is one of the oldest preservation techniques that exist for more than 10,000 years (Pszczola, 1995). Smoke contains antioxidant and antimicrobial compounds (formaldehyde, carboxylic acids, phenols) which are deposited on the product's surface (Ellis, 2001; Sunen et al., 2003). Smoking techniques include traditional vaporous smoke alongside with the newly developed liquid smoke. Nowadays, the main purpose of smoking is to enrich the taste, odour and appearance of the food product; while on the other hand, the preservation aspects are diminishing in importance due to alternative preservation procedures (Simko, 2009).

Liquid smoke application is regarded as a more economic, easier and faster process compared to the traditional smoking procedure. Additionally, the characteristics of the smoked product are more uniform and with high reproducibility in terms of physical and chemical properties (Knowles et al., 1975; Meier, 2009), while the antimicrobial activity is preserved (Sunen et al., 2003). Liquid smoke application also addresses the concern for the toxicity of the polycyclic aromatic hydrocarbons (PAHs) present in vaporous smoke (Simko et al., 1991; Pszczola, 1995). The use of liquid smoke is allowed in most countries inside and outside the European Union, with the limitation that the 3,4-benzo[α]pyrene concentration should not exceed 10 μg/kg (Meier, 2005).

The objective of this study was to investigate the effect of the addition of liquid smoke in the osmotic solution on the kinetics of the osmotic process and to evaluate the subsequent microbiological and oxidative stability of the meat processed in the presence of liquid smoke.

2. MATERIALS AND METHODS

2.1 Osmotic processing

Meat slices, 1cm thick, from beef round muscle were cut into 5 x 5 x 1 cm pieces. The meat pieces were individually weighed and immersed in the appropriate osmotic solutions. The osmotic processing conditions (solution composition and temperature) are presented in Table 1. The ratio of osmotic solution to samples was more than 10:1, thus assuring constant concentration of the osmotic solution throughout the 3h process. The osmotic process duration was 3h. Every 1h, samples were drawn out of the osmotic solution for analysis.

TABLE 1. Experimental design – osmotic solution composition and temperature.

Treatment	Temperature (^{o}C)	Nacl (%)	Liquid smoke (%)
1	5	20	0
2	5	20	0.5
3	5	25	0
4	5	25	0.5
5	15	20	0
6	15	20	0.5
7	15	25	0
8	15	25	0.5

2.2 Physicochemical analyses

Moisture content was determined by air drying at 105 ^{o}C until constant weight (18-22h). Moisture and solids content of the samples were expressed as g/g i.d.m. (initial dry matter).

The NaCl content of the trout fillets was determined by the Mohr method. Briefly, a 10g sample was extracted with hot distilled water and an aliquot was titrated with $AgNO_3$, after addition of K_2CrO_4 as indicator (Skoog and West, 1976).

2.3 Storage stability evaluation

For the evaluation of the storage stability, untreated controls and osmotically processed samples (5 x 5 x 1 cm) were stored under aerobic conditions at 4 ^{o}C for 9d. The microbial load and lipid oxidation levels were determined at 0, 1, 3, 6 and 9 d of storage, since microbiological spoilage and rancidity are the two most important factors that limit fresh meat's shelf-life.

Total microbial viable counts (TVC) of the samples were determined with a variation of the standard plate count agar (PCA) procedure (Mitrakas, *et al*., 2008). Namely, samples of 5 x 5 x 1 cm dimensions with a total surface of 70cm^2, were placed in sterile bags with 100ml of ringer solution (Lab-M, Lancashire, UK) and they were stomached (Lab-Blender 400, Seward Medical UAC, London, UK) for 60 s. For the enumeration, 1ml volumes of appropriate serial dilutions of homogenized samples were incorporated in petri-dishes of Plate Count Agar (PCA, Lab-M, Lancashire, UK) and incubated for 3d at 30°C (Model FOC 225E, Velp Scientifica, Usmate, Italy). Results were reported in log(cfu/cm^2). In order to account for sample (meat batch to batch) variability, results were expressed as $\log(N/N_o)$, where N is the microbial load at time t, and N_o is the initial microbial load of the meat sample.

The 2-thiobarbituric acid (TBA) test, according to Salih *et al.* (1987), was used to determine the extent of lipid oxidation in the meat pieces. Briefly, 10 g of sample were extracted with 35 ml perchloric acid 3.86%, 1 ml BHA solution (to prevent lipid oxidation during analysis) and 5 ml of H_2O. Five (5) ml of the filtered extract were reacted with 5 ml of 0.02 M TBA solution in a water bath (95 ^{o}C) for 30 min. Absorbance readings were made by a Shimadzu UV-1700 spectrophotometer (Shimadzu Europe GmbH, Germany), at 532 nm. Results were expressed in ppm of malonaldehyde. Duplicate determinations were conducted. In order to account for sample (meat batch to batch) variability, results were expressed as ΔTBA (= TBA_t - TBA_o), where TBA_t is the TBA value of the meat sample at time t and TBA_o is the TBA value at time 0.

2.4 Statistical Analysis

The effects of osmotic process temperature, NaCl concentration and the presence of liquid smoke on moisture loss, solids gain, NaCl gain, TVC and TBA levels were analysed by ANOVA using the General Linear Model. Means were compared using Tukey's multiple range test. For the statistical analysis, the MINITAB v.16 (Minitab, Inc., State College, PA, USA) statistical software was used.

3. RESULTS AND DISCUSSION

3.1 Osmotic process kinetics

The NaCl concentration of the osmotic solution, the processing temperature and the addition of liquid smoke had significant impact ($p<0.01$) on the moisture content of the samples. Specifically, processing of beef meat in 25% NaCl brine resulted in increased moisture loss (Fig. 1) and solids gain compared to the 20% NaCl brine. This is in agreement with other researchers (Nguyen et al., 2010). Osmotic processing at a higher (15 ^{o}C) temperature also resulted in increased ($p<0.001$) moisture loss (Fig. 2), but it did not affect solids gain, compared to processing at 5 ^{o}C. Addition of 0.5% liquid smoke in the 15 ^{o}C brine did not have a significant impact on moisture (Fig. 2) or NaCl content of the samples. In the 5 ^{o}C brine, however, the addition of liquid smoke resulted in a statistically significant ($p<0.03$) increase in moisture loss (Fig. 2) and NaCl content (Fig. 3).

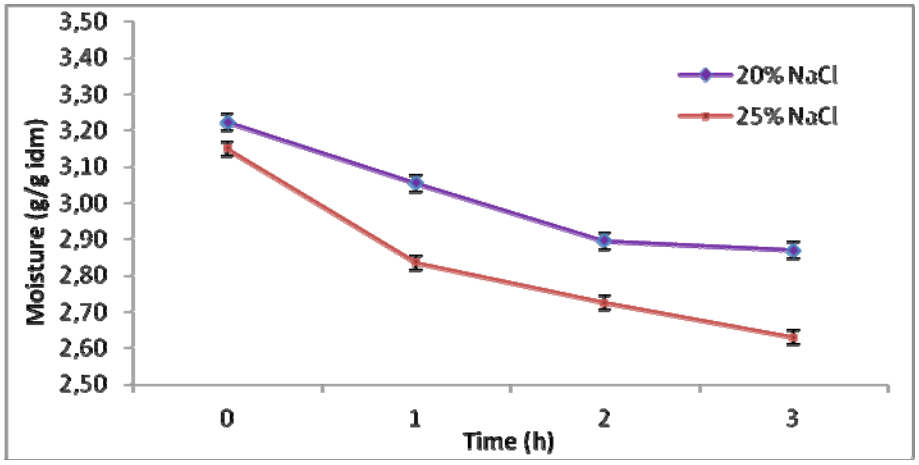

Figure 1. Effect of % NaCl in the osmotic solution on the moisture content of beef meat.

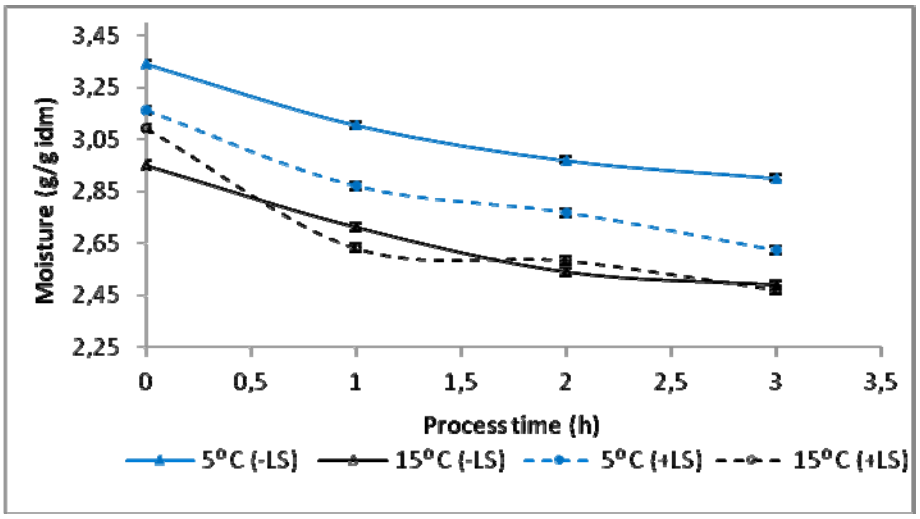

Figure 2. Effect of processing temperature and liquid smoke (LS) on the moisture content of beef meat processed in 25% NaCl osmotic solution.

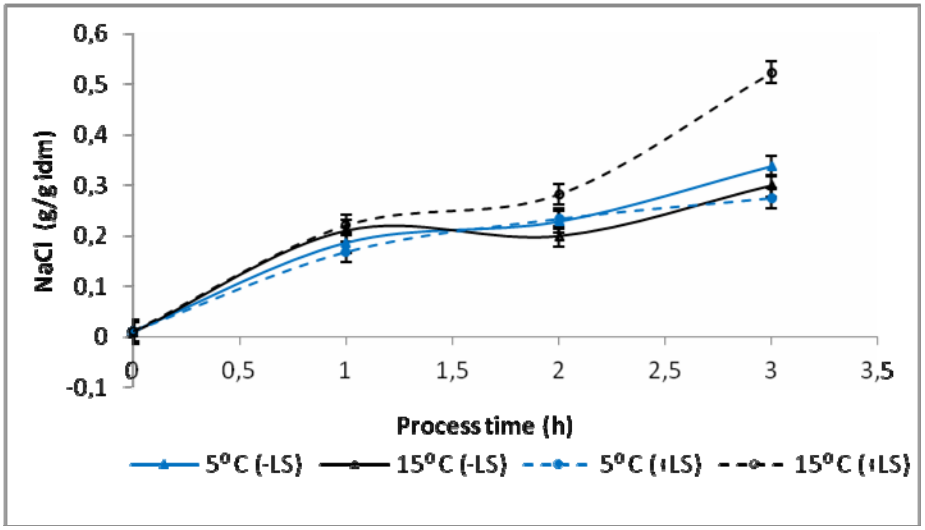

Figure 3. Effect of processing temperature and liquid smoke (LS) on the NaCl content of beef meat processed in 25% NaCl osmotic solution.

3.2 Storage stability evaluation

In osmotically treated samples, when liquid smoke was not present in the osmotic solution, lipid oxidation progressed quickly and TBA values were higher than the untreated control, due to the pro-oxidant effect of the NaCl content of the treated samples. Addition of liquid smoke in the osmotic solution resulted in lower ($p<0.001$) TBA values and practically complete inhibition of lipid oxidation for the duration of the study. This was true for both 20 and 25% NaCl brines, proving the very strong antioxidant effect of liquid smoke even in the presence of high concentrations of NaCl, a strong pro-oxidant. The lipid oxidation profile of

untreated controls and osmotically treated samples at 15 °C are presented in Figure 4. Similar results were obtained for samples treated at 5 °C (data not shown).

The microbiological profile (TVC) of the untreated samples and osmotically treated samples at 15 °C are presented in Figure 5. It is clear that osmotic treatment, even at 15 °C, results in lower TVC levels throughout the 9d storage period. This is attributed to the presence of NaCl and the consequent lowering of the water activity of the treated samples, which acts as an added hurdle for microbial growth (Collignan and Raoult-Wack, 1994; Rastogi et al., 2002). Osmotic treatment in the 25% NaCl brine resulted in lower TVC levels than the 20% NaCl brine, when liquid smoke was not used. When liquid smoke was added in the osmotic solution, TVC levels remained practically steady for the 9d storage period. The antimicrobial effect of liquid smoke is not obvious in the samples treated in the 25% NaCl brine due to the strong inhibitory action of the NaCl on meat microflora. For the 20% NaCl samples though, the addition of liquid smoke resulted in a significant lowering of the TVC levels, confirming the antimicrobial effect of liquid smoke.

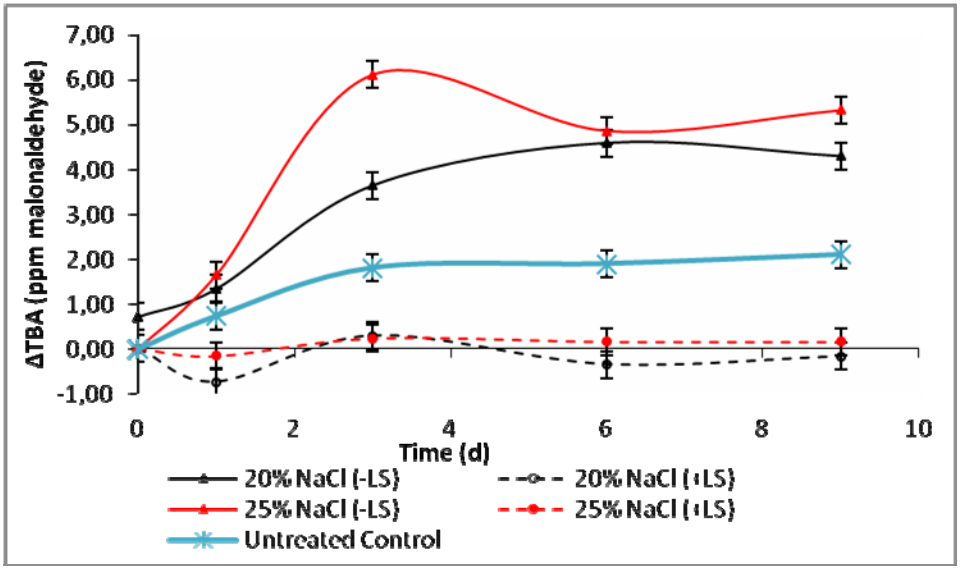

Figure 4. Effect of NaCl concentration in the osmotic solution and liquid smoke (LS) on lipid oxidation of beef meat processed at 15 °C and stored at 4 °C for 9d.

4. Conclusion and outlook

In conclusion, the use of liquid smoke during the osmotic treatment of beef meat offers advantages, prolonging the shelf-life of the treated meat by effectively inhibiting lipid oxidation and by adding an extra hurdle for microbial growth. Care should be exercised in

designing such a process, since the addition of liquid smoke may affect the mass transfer rates for water removal and NaCl pick up.

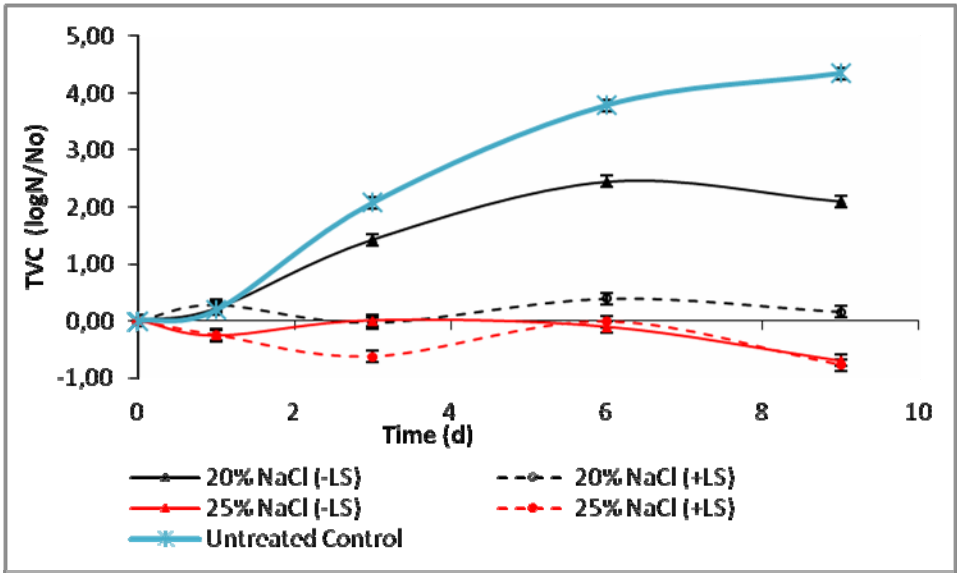

Figure 5. Effect of NaCl concentration in the osmotic solution and liquid smoke (LS) on total viable counts (TVC) of beef meat processed at 15 ^{o}C and stored at 4 ^{o}C for 9d.

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THE EFFECT OF PH AT COOKING ON THETEXTURAL PROPERTIES OF BLOCK-TYPE PROCESSED CHEESES WITH COKELEK DURING STORAGE PERIOD.

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ABSTRACT:

Block-type processed cheeses were prepared from curd (70 %), Cokelek (30 %), emulsifying salts (2.5 %), distilled water, salt (2 %), clourement by using a processed cheese cookerunder varying pH values (5.3pH, 5.4pH, 5.5pH and 5.6pH). On the other hand, Cokelek which is a dairy product like cheese has got high denaturated whey proteins. Inthisstudy, two replicates of the block-type processed cheeses were manufactured. The main objective of this research was to determine the effect of different cooking pH values on the textural properties of the processed cheeses during storage period. A composition alandtextural characterisation of the block-type processed cheeses wasre ported and measured during 90-day storage period. Thetexturalanalyses (hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewnessandresilience) of theeachprocessedcheeseweresubjected on 7th, 15th, 30th, 60thand 90thdays of storageperiodwhereasthecompositonalanalyses (drymatter, fat, total protein, salt and total ash) oftheeachprocessedcheeseweresubjected on 1st and 90thdays of storageperiod. Drymatter, total protein, salt and total ash of theprocessedcheesesampleswereincreased at theend of storageperiod. The hardness and fracturability values of the cheese samples increased during storage period.Varying the cooking pH conditions had a significant modification in hardness, springness, gumminess and chewness values of the processed cheese samples statistically (p□0.05). Dry matter acted as a important role on the textural properties except adhesiveness parameter. Thet extural properties of the processed cheeses could be markedly modified by varying the cooking pH values during processing but these textural modifications could not be explained solely by the changes in pH values. It was also proposed that compositions of the cheeses and the interactions between storage period and pH values effected the textural properties of the cheese samples. The seinteractions in cheese system were also effected by varying the cooking pH of theprocessedcheeses.Consequently, varyingthecooking pH values had a significantinfluence on thetexturalproperties of thecheesesamples. Thevaryingthecooking pH in the processed cheeses containing Cokelek could effect interactions to form different structures between case in sand whey proteins, which ultimately effectt hetextural properties of the cheese samples during storage period.

Keywords:Cokelek, chemicalcomposition, pH, processedcheese, storageperiod, texturalproperties.

USE OF SIMULATION MODELS TO STUDY THE DYNAMIC OF RECALL OF NON-CONFORM PERISHABLE PRODUCE THROUGH THE SUPPLY CHAIN

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ABSTRACT:

Traceability allows to sketching the manufacturing process through a system documentary, enabling to identify the operational structures the products and the lots involved, and to define the flows of production, packaging and distribution. Although researches have been conducted dealing with investigating the traceability within the firm, little work has been carried out on the investigation of the traceability system over the whole supply chain.

The supply-chain of fresh produce is constituted of many links: producer/grower, warehouse, packing centre, distribution centre, retailers and finally the consumer. Each of these is a system itself that interacts with the other components of the supply-chain. The non-conformity could occur in each of these links.

Because of processing plant requirement, storage requirements, and because of savings in the traceability process, often small size lots are merged together to form a large size lot at some points in the supply-chain. Larger lot size could imply higher risk for the consumers in case of recall of the produce and much higher recall time and cost for the supply-chain.

When a non-conformity occurs, the time to recall the produce depends on many factors: lot size, lead time for information spreading from link to link, product transit time among links, product storage procedures and times, and the point in the supply chain where the problem occurred. To study with a system approach different scenarios for the recall procedure, a discrete event dynamic simulation model using Extendsim® has been developed. Here, the model framework is described and a selected example is presented.

Keywords: Traceability, Logistics, Dynamic simulation.

Introduction

The request by the consumer for fresh products with elevated qualitative standard implies a supply chain managed in such way that keeps the produce safe and of high quality up to the consumer. Often consumers have insignificant control on important quality criterions such as food safety, nutritional value, and origin of the product (Heyder et al., 2009). For this reason the European Community provided in the past years a number of regulations to protect consumers against food hazards. The Regulation (EC) 178/2002 define traceability as "*the possibility to build up and follow the pattern for food produce, feed produce and animal used for food production or for the production of a ingredient or animal feed, through the production, processing and distribution phases*". The main drivers to implement traceability systems are: legislation, risk management strategies, the requirements of certification systems, improvements in internal and external business processes, differentiation strategies and stakeholder demands (Theuvsen and Hollmann-Hespos, 2007).

For what concern the risk management strategies, public product recalls are a major threat to food manufacturers. Product recalls mainly result in fewer sales due to out-of-stocks and higher costs due to backhaul and disposal of defective products, additional laboratory analyses, ad hoc process improvements, compensation payments, and crisis communication with supply chain partners and consumers (Heyder et al., 2009). Often the recall of a produce interest the whole chain of distribution (McGarry, 2007).

To this extent, the use of traceability could be a cost saving tool, not just for the firm but for the whole supply chain, that is usually constituted of many links: producer/grower, warehouse, packing centre, distribution centre, retailers, and finally the consumer. Each of these is a system itself that interacts with the other components of the supply chain. There is a flow of orders and information that goes from the consumer to the producer and a flow of product that it goes to the opposite direction, from producer to consumer (Prussia and Mosqueda, 2006).

In the distribution of the fruit and fresh vegetable, along the supply chain, logistics play an important role. Logistics is the discipline that studies and optimises the management of the flow of products considering production, maintenance, transformation, transport and distribution of the products and information related to them (Busato and Berruto, 2006). The effective information exchange is the key to improving value chain performance and competitiveness in today's complex and rapidly changing environments (Busato et al., 2007; Hofstede, 2003). Traceability could also help to extract information from the system and make it available for improving the supply chain (Berruto and Busato, 2006).

The traceability system involves the supply chain logistics, and it is a cost, both in terms of investments and in term of operations. The implementation of a tracing system within the firm, has been extensively studied since this is mandatory by law (Heyder et al., 2009). For what concern the whole supply chain the research are still limited. However, the study of the implementation of traceability on supply chain scale could lead up to many advantages: sharing some information and knowledge along the chain, improving the performance in the tracking and tracing procedures, and in the recall operations.

The traceability operations in a supply chain could be considered as a system. The system is an interrelated set of objects, and has the properties of being interactive, complex, and dynamic. For this reason, the traceability of a produce is certainly a discipline where the simulation can make important contributions to the organization of the processes and information, as well as the ways in which targeted interventions may be implemented to obtain efficiency throughout the whole supply chain. Examples concerning simulation used to study food supply chain are related to fresh produce (Busato and Berruto, 2006; Busato et al., 2007; Prussia et al., 2001; Prussia and Mosqueda, 2006) and dairy production (Monroy et al., 2008).

With the aim to provide a tool to assess the traceability system, the associated risks and costs, the authors worked to implement a discrete event simulation model able to predict the efficacy of different traceability scenarios for the supply chain of fruits and vegetables.

Methods

Simulation software

For the model implementation, the Extend system simulation software (Imagine That Corporation) was used. Extend is a general-purpose package suitable for modelling discrete, continuous, and mixed systems, and utilize blocks connected together to build the models. The entities move in the model from one block to the next through the connection lines. Groups of blocks can be combined into hierarchical blocks (H-blocks) with unlimited layers of nesting (Krahl, 2001). In 2006 the authors developed the FruitGame, a set of customs blocks in a library, dedicated for the simulation of supply chain for the distribution of fruits and vegetables (Busato and Berruto, 2006). The model simulates and tracks the supply chain performance by simulating the production and distribution of each single box of fruits and vegetable.

The discrete event simulation models embed all the features needed to simulate a system, i.e. complexity, interactivity, and dynamicity. In these models the object are moving in the system and have their own attributes. This helps to differentiate them and their behaviour inside the model. The entities moving in the model could be stored in a queue, waiting for some activities, or could be used/subjected to some operations. So the main components of the model will be the queue blocks and the activity blocks.

The paper presents an extension of the FruitGame library in order to implement the tracking and tracing system for the whole supply chain, and to study the effect of recall procedures under technical, logistic and economic point of view.

Parameters used to describe tracking and tracing systems in the model

When addressing the problem of recalling of a non-conform produce, the performance of the reverse logistics of the supply chain is important. Some factors have influence in this process, and the model should be able to represent them. For the purpose of modelling the recall process, in Table 1 are described the relevant factors and assumptions for the recall process (no other aspects of supply chain performance related to quality, shelf-life or costs are described). How much each parameter matter the tracking and tracing system vary also from link to link in the supply chain. So, the parameters listed in Table 1 have to be specified for each single link.

TABLE 1. Parameters determining effectiveness of traceability and recall procedures, for each single link in the supply chain.

Parameter	Description	Information needed
Time when the problem occurs	The tracking system should notice a problem and record it	Time of the event, the batch (group of items) that present the problem, and the location
Time when the problem is evident	The tracing system should start the recall process by tracing back through the supply chain all the items of the same batch that present the problem	Time of the event, the batch of items, the location
Transit time	The longer the transit time, the easier to stop the produce before it reaches the consumers, if the problem was evident	Delay for transit, location of origin, location of destination

	before the retailer. Not important if the produce was already available on the shelf	
Intermediate storage of produce	The intermediate storage of produce could be an advantage because we can stop the produce there before sell. This is the case of a distribution center between suppliers and retailers	Location, amount of non-conform produce
Information retrieval	Longer time to retrieve the information make the recall problem bigger and the risk of selling non conform produce higher	Delay for information retrieval, location
Information lead time (backward)	Longer time to transmit the information back in the chain make the recall problem bigger and the risk of selling non conform produce higher	Delay for information transmission, location of origin, location of destination
Information lead time (forward)	Once the problem location is known and the lot that present the problem is known, it is important to spread the information down to the links in the supply chain. Longer time for passing the information make the recall problem bigger and the risk of selling non conform produce higher	Delay for information passing, location of origin, location of destination
Batch size – lot size	Big lot size means big recall activities and expenses in case of problems. On the other hand the cost of recall depends on the probability to make mistakes in the processing. Small lot size increment the tracking costs and reduces the recall costs. Another issue is the batch reversibility, in the case the single items preserve its uniqueness after the batch or not	Location, lot size, and batch reversibility
Time to segregate non conform produce	This activity affect the costs of the recall	Location, delay for the activity
Time to transport non conform produce	This activity affect the costs of the recall	Delay for the activity, location of origin, location of destination

Model for the simulation of tracking and tracing systems

An example of the layout of the model is presented in Figure 1. Each link of the supply chain is represented by a group of blocks. The lines that connect the blocks represent the flow of product or the flow of information.

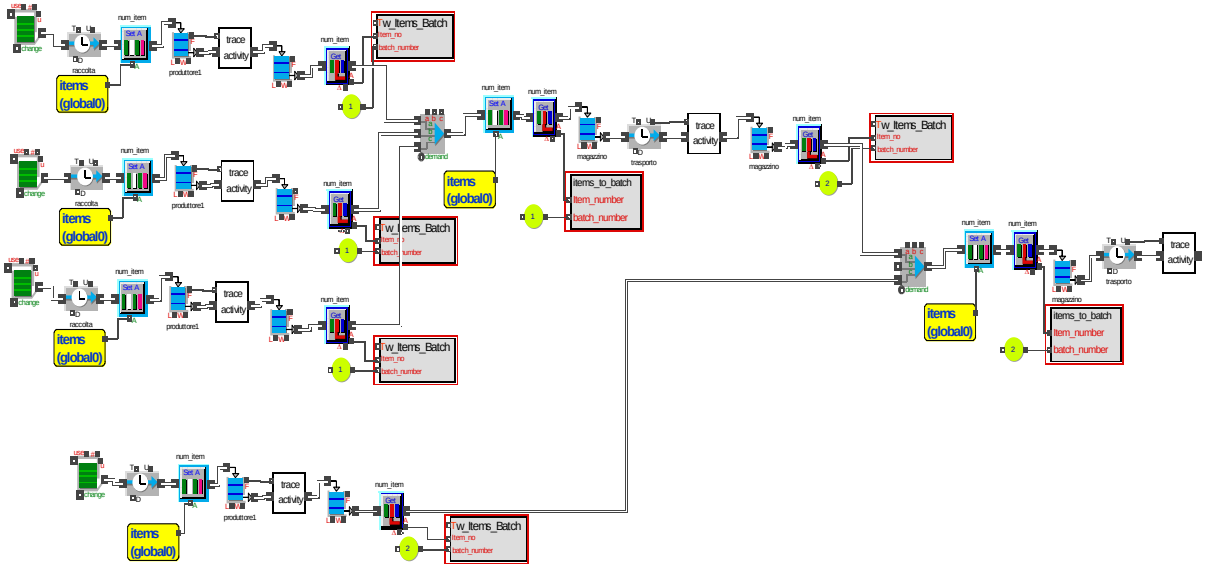

FIGURE 1. Portion of the main layer of the prototype model.

The information could be stored on each single item on in a batch. Batch represent in the simulation a group of items, that behave as a single item in the model until unbatch occurs. The produce is batched together in some points, for example when it is loaded on the truck or is stocked on a pallet, so the unit to be moved is no longer the single object, but the batch itself. When the produce is in a batch, the information related to a single object cannot be read or written, and this is a problem for this type of simulation, where all the events and activities related to a single item have to be tracked.

Usually, in the discrete events models it is more important the system performance as a whole or in single location of the supply chain, rather than the flow of information that travels with each single item in the system. This framework structure is different since allows for complete tracking and tracing activities along the simulated system, for each item in the simulation.

Custom blocks for the simulation of tracking and tracing system

In order to simulate the traceability system, custom blocks that are part of the library of the FruitGame have been built. The custom blocks built so far addressed the problem to associate information and activities to the correct items when they are batched along the supply chain. They are described as follows:

Global arrays manager. All the information is stored in a global array. The main one called item array, allows storing all the data in a matrix. The columns of the array are the following:

Item_origin. This is the progressive number of the item in the array which can represent a single item or a batch.

Item_destination. It describes the destination number of the item. If this number is the same of origin, this means the current array row refers to an activity or storage, without batching or un-batching of items.

Location. It refers to the location (activity, queue, storage) where the item just passed through.

Simulated_time. It records the simulated time the item passed thru the tracking block.

Monitor. This block monitors each part of the model. It records the item number; the location and the simulated time an item pass through it.

Batch_read. The batching activity is more important since it has to be recorded which items are in which batch.

Batch_write. A progressive number is assigned to the batch and this block updates the global array, associating to each item (item_origin) the number of the current batch (item_destination).

Unbatch. The unbatch is done in one step. The block search in the global array for the creation on an item (batch) and read all the items belonging to this one

Trace. The trace block acquires the item number to trace back all the activities and locations recorded in the global array that may have the no-conform produce. The output of this block is a first step toward the recall process. An example of how it works is presented in table 2.

Track. This block, with an item/batch number, is tracking forward all the activities and locations recorded in the global array that may have the non conform produce.

Notify_recall. Taking into account the transit time of information from link to link and the information retrieval time this block alert each single location interested by the non conform produce to start the recall process. The locations are provided in the output of the track block.

Recall. Based on the received notification, this block segregate items/batches that have non conform produce inside, stopping the delivery of this to the following links in the chain.

Results of the first trial

A simple supply chain was simulated to test the ability of the model to track single items and batches passing through the chain. The chain presented in the Figure 1 consists of three producers that send their produce in a single load to the wholesaler. Another product is shipped directly to the wholesaler, as depicted in the figure. The produce is batched and then is shipped to a distribution centre. At the distribution centre, one produce goes to one retailer directly, while the other produce was transported to another distribution centre and then shipped to three different retailers. The traceability output is presented in Table 2.

Table 2. Results from the simulation of the supply chain presented in Figure 1. All the rows where the time and locations are not present refer to batch/unbatch activities.

item origin	item destination	location(*)	simulated time (h)
1	1	2	0,53
1	5	-	-
2	2	83	2,28
2	5	-	-
3	3	124	4,22
3	6	-	-
4	4	44	4,25
4	5	-	-
5	5	71	12,45
5	6	-	-
6	6	174	25,01
6	3	-	-
6	5	-	-
5	5	232	27,75
5	1	-	-
5	2	-	-
5	4	-	-
1	1	253	27,75
2	2	357	27,75
4	4	373	27,75
3	3	208	36,34

(*) this is a unique block number assigned by the model

Beside these results it is possible to see the resource utilization and waiting times like in all the traditional models (e.g. storage space utilization, transport utilization, etc.). The performance of the simulated supply-chain can be also evaluated with the following indexes

(Busato and Berruto, 2006): residual shelf-life, time in system and lead time, distribution costs.

In addition, the new model framework, built to fulfil the traceability requirements, allow more specific evaluations in the chain, such, for instance, the cost of the transport in a specific phase, the quantity of product stored in a link, and so on.

Conclusions

In the discrete events models it is, generally, more important the system performance as a whole or in single location of the supply chain, rather than the flow of information that travels with each single item in the system.

This framework structure is different since allows for complete tracking and tracing activities along the simulated system. This is the starting point to study with a system approach the traceability and the recall of a produce, considering also working time, cost and risks associated with the production, distribution and processing of perishable produce.

With the built structure it is possible also to investigate many aspects related to the supply chain, like food quality, shelf life of the produce, chain performance and distribution costs, traceability performance and costs, and environmental aspects, such are the CO_2 emissions for the chain as a whole of for single produce distributed.

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PREPARATION AND EVALUATION OF SPREADABLE PROCESSED FETA CHEESES

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ABSTRACT

The development of novel processed cheese products, with the use of Feta cheese, was studied. Seven different samples of spreadable processed cheeses based on Feta cheese were prepared, using a Stephan mixer-cooker. Mixtures of raw materials comprised of Feta cheese (50-100%), butter (0-20%), sodium caseinate (0-10%) and water (0-30%). In addition, trisodium citrate was added (3%) to mixtures, as an emulsifying salt. Mechanical properties of samples were determined with lubricated squeezing flow and stress relaxation in combination, as well as texture profile analysis (TPA). Colour of samples was also measured and, finally, sensory tests were carried out. Regression analysis showed that the mechanical properties of processed cheeses are highly dependent ($P<0.05$) on chemical composition. Protein, as well as fat content, enhances most mechanical properties. It is concluded that the increase of protein content renders the protein network denser and stronger. Also, since tests were performed at 5-8°C, where fat is mostly in solid state, fat particles were also found to strengthen the protein network. In contrast, moisture softens the product and improves spreadability. Also, increase of fat content lead to an increase of the product's yellow hue intensity. Results of sensory tests revealed that higher panelist scores were ascribed to samples with 70% Feta cheese – 25% water – 5% sodium caseinate, as well as 90% Feta cheese – 10% water.

Keywords: Processed cheese, Feta cheese, mechanical properties.

1. INTRODUCTION

Processed cheese is the product that is normally produced with grinding, mixing and melting of one or more natural cheeses, in the presence of emulsifying salts, as well as with optional

addition of other ingredients (dairy ingredients, vegetables, meat products, stabilisers, food colourings, preservatives), until a smooth and homogenous mixture is obtained (Guinee *et al*., 2004). Due to their preparation technology, processed cheeses present a plethora of advantages. The most important are longer shelf life (because of thermal processing), a great variety of textures (cheeses sliceable, spreadable, in powder form), the huge range of presentation forms (blocks, slices, portions, cups, squeeze tubes, cans), the endless ability for new product development (with numerous ingredients) and good dietary properties (which are due to the ingredients' state) (Thomareis, 1986). For the above mentioned reasons, processed cheeses are foods widely consumed, in a global scale (Kosikowski & Mistry, 1997).

Almost all natural cheese types can be used for the preparation of processed cheeses (Berger *et al.*, 1998). Most commonly used natural cheeses include Cheddar, Emmental, Gruyère, Gouda, Edam, Colby, Mozzarella, Monterey Jack, Tilsit, Trappist, Fontina and Provolone (Meyer, 1973; Nogueira de Oliveira *et al*., 2011). However, during literature review, natural Feta cheese did not appear to have been used as a raw material for processed cheese preparation.

Aim of the present study is the investigation of the possibility of development of such a spreadable processed cheeses based on Feta cheese. In this respect, seven different samples were prepared and evaluated instrumentally, regarding texture and colour, as well as by sensory analysis.

2. MATERIALS AND METHODS

2.1 Materials

Three-month-old Feta cheese (moisture 60.51%; fat in dry matter 53.18%; proteins 19.31%, pH 4.56) and butter supplied from a local dairy industry, sodium caseinate (Lactalis Ingredients, France) and distilled water were used as raw materials for the preparation of the spreadable processed cheese samples. Analytical grade trisodium citrate (BDH, England), added at 3%, was used as emulsifying salt. All samples were manufactured in a jacketed mixer-cooker UMC 5 Universal Machine (Stephan, Germany). After removal of air by vacuum, mixture was heated to 80°C, under continuous mixing at 1000 rpm. Then, mixing was continued for 5 min, at 2000 rpm, at constant temperature (80°C). Seven samples of 1800 g were prepared in total. Table 1 shows the amount of each ingredient in the raw materials' mixtures.

TABLE 1. Quantities (%) of ingredients in the raw materials' mixtures used during the production of experimental processed cheeses.

Sample	*Feta cheese (%)*	*Butter (%)*	*Sodium caseinate (%)*	*Water (%)*
1	100	-	-	-
2	90	10	-	-
3	80	20	-	-
4	90	-	-	10
5	70	-	5	25
6	60	-	10	30
7	50	10	10	30

At each mixture, a supplementary amount of 3% of trisodium citrate was added.

For instrumental texture evaluation, the cheese melt was transferred into cylindrical stainless steel moulds, previously lubricated with paraffin oil and on a smooth glass surface. Once the hot cheese samples were poured into the moulds, they were covered with Parafilm®, equilibrated at room temperature and stored at 4°C for 24 h before measurement. For chemical analyses, colour measurement and sensory evaluation, samples were placed into food grade plastic containers (225 g capacity), equilibrated at room temperature and stored at 4°C for one or two days prior to analysis.

2.2 Methods

2.2.1 Chemical composition

Feta cheese and processed cheese samples were analysed for moisture, fat and protein content. Moisture content was determined by drying at 102 ± 2 °C to a constant weight (IDF, 1982). Fat content was assayed by the van Gulik method (ISO, 1975). Total nitrogen (TN) (IDF, 1964) and non-protein nitrogen (NPN), that is the fraction of TN soluble in 12% trichloroacetic acid (Ardö & Polychroniadou, 1999), were assayed by the Kjeldahl method. Protein content was calculated as 6.38 × (TN – NPN). The pH of processed cheese was measured using a glass electrode Microprocessor pH-meter SER-NR (WTW, Germany).

2.2.2 Determination of mechanical properties

For the determination of mechanical properties lubricated squeezing flow, stress relaxation and texture profile analysis (TPA) were employed (Steffe, 1996), with the aid of Instron

Universal Testing Machine model 1140 (Instron Ltd, UK). The two first methods were used in combination.

During lubricated squeezing flow, cylindrical specimens of 10mm height and 10cm diameter were subjected to compression between two metal discs of 10cm diameter, with a speed of 5mm/min, to 80% deformation. Straight after, the stress relaxation test followed, during which the specimens were kept at constant deformation (80%) for 3 min. Two forces were obtained during the test: force at end of lubricated squeezing flow (initial relaxation force, F_0) and force at end of stress relaxation (residual relaxation force, F_3). The ratio F_3/F_0 is a measure of elasticity and can range from 0 for ideal viscous materials (fluids) to 1 for ideal elastic (solids).

During texture profile analysis (TPA), cylindrical specimens of 22mm height and 22mm diameter were subjected to compression between two metal discs of 10cm diameter, with a speed of 20mm/min, to 80% deformation. The test comprised of two consequent compression-decompression cycles, as mastication simulation. The determined mechanical properties were: hardness 1 (H_1), hardness 2 (H_2), compression work 1 (A_1), compression work 2 (A_2), cohesiveness (A_2/A_1), springiness (S_1), stringiness (S_2), adhesiveness (A_3), chewiness (K) and gumminess (G).

Temperature of samples during all tests ranged from 5-8^{o}C and tests were performed in eight replicates.

2.2.3 Colour measurement

Colour measurement of processed cheese samples was determined with the help of Micro Color colourimeter (Dr. Lange, Germany). The instrument provides the three parameters (C.I.E.) L* (lightness), a* (green to red) and b* (blue to yellow).

2.2.4 Sensory evaluation

Immediately after refrigeration, the seven samples of processed cheese were organoleptically evaluated by a panel of fourteen testers, members of the Department of Food Technology. Each panelist simultaneously compared four samples. Thus, each sample was evaluated eight times on each sensory attribute. Rating tests were employed on the intensity and acceptance of characteristics, using unstructured scales (0-10). Regarding intensity, sensory attributes such as colour (yellow hue), saltiness, consistency, resistance in spreading and stickiness were evaluated, while regarding acceptance, flavour, texture and overall acceptance were rated.

2.3 Statistical analysis

One-way ANOVA was applied to the experimental data using the general linear model, while the Tukey's multiple comparison test determined whether statistically significant differences occurred among means. All differences considered as significant are at least $p<0.05$. Multiple regression equations were obtained using three procedures: best subset regression, forward selection and backward elimination. The best regression equation was derived each time by taking into account the results from all the above methods. Simple regression and correlation analyses were also used. Statistical analysis of the experimental data was performed using Minitab 16 statistical software.

3. RESULTS AND DISCUSSION

3.1 Chemical composition

Mean chemical composition of the prepared processed cheese samples is given in Table 2. Moisture ranges from 45.97 to 61.97%, fat content from 12.67 to 35.00% and protein content from 12.75 to 18.85%. Since the product should not be either liquid or a hard solid, but an in-between state, thus a spreadable solid, the variation of the three basic ingredients is subject to certain limitations. In this respect, between the two extreme samples, the moisture content increase was by 34.8% and protein content by 47.8%. In contrast, fat content was spread over a wider range and almost tripled. All processed cheese samples exhibited constant pH value (5.9–6.0).

All physical properties (texture and colour) studied were correlated with chemical composition, in order to elucidate the effect of the three main ingredients. In this respect, multiple regression analysis was employed, which revealed that the simultaneous correlation of all three ingredients with each physical property always leads to a VIF factor greater than 7.

Thus, in all regression equations only two ingredients were introduced: fat and protein content, since they, combined, give the highest coefficient of determination (R^2). In the present study, only regression equations with R^2 values higher than 60% were considered important and thus, are shown in the following sections.

TABLE 2. Chemical composition of processed cheese samples.

Sample	*Moisture (%)*	*Fat (%)*	*Proteins (%)*
1	$51.47^{d} \pm 0.30$	$23.50^{c} \pm 0.50$	$15.91^{c} \pm 0.13$
2	$51.10^{d} \pm 0.59$	$28.17^{b} \pm 0.76$	$13.86^{e} \pm 0.05$
3	$45.97^{e} \pm 0.23$	$35.00^{a} \pm 0.43$	$12.75^{f} \pm 0.30$
4	$57.70^{c} \pm 0.30$	$20.67^{d} \pm 1.23$	$14.04^{e} \pm 0.06$
5	$61.97^{a} \pm 0.35$	$16.17^{e} \pm 0.29$	$15.15^{d} \pm 0.26$
6	$60.32^{ab} \pm 1.43$	$12.67^{f} \pm 0.58$	$18.85^{a} \pm 0.03$
7	$59.10^{bc} \pm 1.65$	$19.58^{d} \pm 0.80$	$17.60^{b} \pm 0.07$

Values are means of three replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

3.2 Lubricated squeeze flow - stress relaxation

In Table 3, the three mechanical properties, determined by the combination of lubricated squeezing flow and stress relaxation, are shown. Values of residual relaxation force (F_3) are much lower than the corresponding values of the initial relaxation force (F_0), thus samples have shown increased relaxation during the 3 min of the test. Ratio of F_3/F_0 is lower than 0.5, which means that samples are viscoelastic materials with low elasticity. This is expected, since these products are spreadable.

TABLE 3. Mechanical properties of processed cheese samples by lubricated squeeze flow and stress relaxation.

Sample	F_0 *(N)*	F_3 *(N)*	F_3/F_0
1	$410.40^{b} \pm 82.52$	$177.73^{bc} \pm 45.17$	$0.441^{a} \pm 0.020$
2	$359.84^{b} \pm 31.11$	$146.52^{c} \pm 10.97$	$0.398^{bc} \pm 0.026$
3	$747.63^{a} \pm 79.12$	$302.88^{a} \pm 21.12$	$0.385^{c} \pm 0.009$
4	$229.07^{c} \pm 4.03$	$99.39^{d} \pm 3.24$	$0.434^{ab} \pm 0.010$
5	$239.88^{c} \pm 12.50$	$98.93^{d} \pm 10.27$	$0.411^{abc} \pm 0.023$
6	$694.63^{a} \pm 50.75$	$199.79^{b} \pm 16.07$	$0.307^{d} \pm 0.010$
7	$739.83^{a} \pm 35.69$	$212.51^{b} \pm 16.98$	$0.286^{d} \pm 0.012$

Values are means of eight replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

The resulting regression equations reveal that as initial relaxation force (F_0), as well as residual relaxation force (F_3), which both express hardness of samples, are increased with protein and fat content:

$$F_0\,(N) = -2656 + 147.0 \cdot \text{proteins}\,(\%) + 39.0 \cdot \text{fat}\,(\%) \qquad (R^2=73.0\%)$$

$$F_3\,(N) = -697.0 + 37.0 \cdot \text{proteins}\,(\%) + 13.6 \cdot \text{fat}\,(\%) \qquad (R^2=75.2\%)$$

Proteins increase the product's hardness, because the structure and, as such, the texture of cheese, mainly depend on the casein network which is made denser and stronger with increase of content in proteins. The positive effect of proteins on the rheological properties of processed cheeses was also reported in other studies (Joshi *et al*., 2004; Dimitreli & Thomareis, 2007; Dimitreli & Thomareis, 2008). At a lesser extent, fat also increases the product's hardness; this is attributed to the fact that tests were performed at low temperature (5-8^{o}C), where fat is found mainly in solid state, thus strengthening the casein network. Regarding the effect of fat on the rheological and mechanical properties of spreadable processed cheeses, similar results have been reported by other researchers (Brighenti *et al*., 2008; Gliguem *et al*., 2009; Bayarri *et al*., 2012).

3.3 Texture profile analysis (TPA)

In Table 4, the mechanical properties that resulted from TPA, such as hardness 1 (H_1), hardness 2 (H_2), compression work 1 (A_1), compression work 2 (A_2) and cohesiveness (A_2/A_1) of processed cheese samples are given. Mean values of H_1 range from 23.95 to 75.70 N, H_2 from 18.54 to 61.04 N, A_1 from 0.135 to 0.315 J, A_2 from 0.015 to 0.046 J and A_2/A_1 from 0.068 to 0.147. The highest values of H_1, H_2, A_2 and A_2/A_1 are presented by sample 7 with the lowest content of Feta cheese.

Statistical analysis of multiple regression produced two important prediction equations:

$$H_1 = -199.0 + 11.7 \cdot \text{proteins}\,(\%) + 2.99 \cdot \text{fat}\,(\%) \qquad (R^2 = 74.1\%)$$

$$A_1 = -0.911 + 0.048 \cdot \text{proteins}\,(\%) + 0.019 \cdot \text{fat}\,(\%) \qquad (R^2 = 72.1\%)$$

Both mechanical properties (H_1, A_1) refer to the specimen's resistance during the first compression and are increased with increasing protein and fat content. Both equations agree with the corresponding of lubricated squeezing flow – stress relaxation.

The remainder mechanical properties, namely springiness (S_1), stringiness (S_2), adhesiveness (A_3), chewiness (K) and gumminess (G) are presented in Table 5. Mean values of S_1 range from 1.14×10^{-3} to 2.79×10^{-3} m, S_2 from 0 to 1.78×10^{-3} m, A_3 from 0 to 0.024 J, K from 3.35 to 29.43 J and of G from 2.35 to 12.67 N. Samples 6 and 7, which were produced with the highest amount of added sodium caseinate (10%), are those presenting the highest values of springiness, chewiness, and gumminess and presented no stringiness and adhesiveness.
Statistical analysis of multiple regression produced three notable prediction equations:

$$S_1 = -3.38 + 0.289 \cdot \text{proteins (\%)} + 0.029 \cdot \text{fat (\%)} \qquad (R^2 = 61.2\%)$$
$$K = -92.7 + 5.70 \cdot \text{proteins (\%)} + 0.744 \cdot \text{fat (\%)} \qquad (R^2 = 67.1\%)$$
$$G = -32.5 + 2.01 \cdot \text{proteins (\%)} + 0.325 \cdot \text{fat (\%)} \qquad (R^2 = 60.2\%)$$

The above show that springiness (S_1), chewiness (K) and gumminess (G) of samples are increased with increase of protein and fat content. These results are in agreement with all previous and explain the structural role of protein and fat in processed cheese.
During multiple regression, regarding all mechanical properties, when moisture and protein contents were used as independent variables, moisture always has a negative sign, i.e.:

$$F_0 = 858 + 55.5 \cdot \text{proteins (\%)} - 23.0 \cdot \text{moisture (\%)} \qquad (R^2 = 60.3\%)$$
$$H_1 = 80.7 + 4.83 \cdot \text{proteins (\%)} - 2.00 \cdot \text{moisture (\%)} \qquad (R^2 = 73.1\%)$$

TABLE 4. Mechanical properties (hardness 1, hardness 2, compression work 1, compression work 2 and cohesiveness) of processed cheese samples by TPA.

Sample	*Hardness 1 (H_1, N)*	*Hardness 2 (H_2, N)*	*Compression work 1 (A_1, J)*	*Compression work 2 (A_2, J)*	*Cohesiveness (A_2/A_1)*
1	$41.33^{c} \pm 5.43$	$30.57^{c} \pm 5.50$	$0.222^{c} \pm 0.023$	$0.015^{b} \pm 0.003$	$0.068^{d} \pm 0.015$
2	$43.60^{c} \pm 7.34$	$31.92^{b} \pm 5.40$	$0.221^{c} \pm 0.029$	$0.019^{b} \pm 0.002$	$0.088^{cd} \pm 0.009$
3	$63.57^{b} \pm 8.93$	$50.26^{a} \pm 7.76$	$0.428^{a} \pm 0.053$	$0.038^{a} \pm 0.010$	$0.088^{c} \pm 0.019$
4	$23.95^{d} \pm 2.73$	$18.54^{d} \pm 1.80$	$0.135^{d} \pm 0.013$	$0.021^{b} \pm 0.004$	$0.099^{bc} \pm 0.012$
5	$33.96^{cd} \pm 8.19$	$34.68^{bc} \pm 11.05$	$0.156^{d} \pm 0.027$	$0.018^{b} \pm 0.003$	$0.119^{b} \pm 0.016$
6	$59.98^{b} \pm 6.03$	$41.93^{ab} \pm 3.87$	$0.265^{bc} \pm 0.021$	$0.038^{a} \pm 0.003$	$0.146^{a} \pm 0.012$
7	$75.70^{a} \pm 11.61$	$61.04^{a} \pm 19.03$	$0.315^{b} \pm 0.042$	$0.046^{a} \pm 0.006$	$0.147^{a} \pm 0.015$

Values are means of eight replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

Decrease of rheological properties with increase in moisture of spreadable processed cheeses has been observed in other studies (Sutheerawattananonda & Bastian, 1998; Pereira *et al*., 2001; Lee *et al*., 2004; Dimitreli & Thomareis, 2008). This is attributed to the softening of the casein network by the insertion of water molecules amongst protein-protein interactions.

TABLE 5. Mechanical properties (springiness, stringiness, adhesiveness, chewiness, gumminess) of processed cheese samples by TPA.

Sample	*Springiness* (S_1, 10^{-3} m)	*Stringiness* (S_2, 10^{-3} m)	*Adhesiveness* (A_3, J)	*Chewiness* (K, J)	*Gumminess* (G, N)
1	$1.14^{c} \pm 0.22$	$1.25^{bc} \pm 0.27$	$0.011^{a} \pm 0.004$	$3.35^{c} \pm 1.15$	$2.92^{d} \pm 0.70$
2	$1.53^{b} \pm 0.09$	$1.65^{ab} \pm 0.16$	$0.015^{a} \pm 0.005$	$5.88^{bc} \pm 1.17$	$3.84^{cd} \pm 0.78$
3	$1.58^{b} \pm 0.40$	$1.65^{ab} \pm 0.61$	$0.020^{a} \pm 0.009$	$9.75^{b} \pm 2.06$	$5.90^{c} \pm 1.70$
4	$1.47^{bc} \pm 0.26$	$1.78^{a} \pm 0.29$	$0.012^{a} \pm 0.004$	$4.92^{bc} \pm 1.24$	$2.35^{d} \pm 0.37$
5	$1.29^{bc} \pm 0.28$	$0.96^{c} \pm 0.27$	$0.014^{a} \pm 0.006$	$5.50^{bc} \pm 1.69$	$4.32^{cd} \pm 1.14$
6	$2.79^{a} \pm 0.08$	0	0	$24.46^{a} \pm 2.83$	$8.77^{b} \pm 0.97$
7	$2.37^{a} \pm 0.32$	0	0	$29.43^{a} \pm 3.69$	$12.67^{a} \pm 2.72$

Values are means of eight replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

3.4 Colour

The colour parameters (L^*, a^*, b^*) of processed cheese samples are presented in Table 6. All samples exhibit high and similar brightness, since means of L* parameter range from 86.76 to 90.23, meaning that all samples have an intense white colour. Furthermore, they present similar light green hue, since a* parameter has a negative sign with a small absolute value ranging from –3.18 to –2.34. On the contrary, yellow hue is increased with increasing fat content of samples, since b* parameter is increased from 5.94 to 10.18. In fact, simple regression analysis produces the following equation:

$$b^* = 3.33 + 0.212 \cdot \text{fat}\ (\%) \qquad (R^2 = 90.7\%)$$

TABLE 6. Colour parameters (L^*, a^*, b^*) of processed cheese samples.

Sample	L^*	a^*	b^*
1	$90.22^{a} \pm 1.10$	$-2.88^{b} \pm 0.27$	$8.96^{b} \pm 0.31$
2	$89.74^{a} \pm 1.22$	$-2.97^{b} \pm 0.26$	$9.80^{a} \pm 0.28$
3	$87.96^{ab} \pm 0.77$	$-2.92^{b} \pm 0.17$	$10.18^{a} \pm 0.46$
4	$89.01^{ab} \pm 0.90$	$-3.18^{b} \pm 0.14$	$8.02^{c} \pm 0.22$
5	$90.23^{a} \pm 0.98$	$-2.85^{b} \pm 0.15$	$6.53^{d} \pm 0.18$
6	$86.76^{b} \pm 3.28$	$-3.09^{b} \pm 0.26$	$5.94^{e} \pm 0.24$
7	$88.43^{ab} \pm 2.81$	$-2.34^{a} \pm 0.27$	$6.83^{d} \pm 0.41$

Values are means of eight replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

3.5 Sensory evaluation

Mean scores of sensory characteristics of processed cheese samples are shown in Table 7. Yellow hue's intensity scores given by panelists are strongly correlated with the parameter b* (r=0.87). Saltiness is highly correlated with the percentage of Feta cheese in raw materials' mixtures (r=0.89), since salt of processed cheeses originates exclusively from Feta cheese. Consistency is strongly correlated with the following mechanical properties: cohesiveness A_2/A_1 (r=0.84), hardness H_1 (r=0.84), chewiness K (r=0.92) and gumminess (r=0.95).

Moreover, resistance in spreading is highly correlated with the same mechanical properties: cohesiveness A_2/A_1 (r=0.79), hardness H_1 (r=0.90), chewiness K (r=0.94) and gumminess G (r=0.97). In this respect, consistency strongly correlates with the resistance in spreading (r=0.96). However, because moisture softens the cheese, it reduces its resistance in spreading and improves its spreadability.

Table 8 shows the acceptance (liking) scores regarding samples' flavour. Samples 4 and 5 had the highest scores, while 6 and 7 had the lowest. Regarding texture, panelists preferred samples 2, 4 and 5, while having given the lowest scores to samples 6 and 7. On overall acceptance, samples towards which the panelists showed incerased preference were samples 4 and 5, with high differentiation from all other five samples. In general, samples with a high content in Feta cheese (70-90%) were preferred, but not the sample containing exclusively Feta cheese. In contrast, the lowest scores were given to samples with low content in Feta cheese (50-60%) and high in sodium caseinate (10%).

TABLE 7. Panelists' scores on the intensity of sensory characteristics of processed cheese samples (scale 0 – 10).

Sample	*Colour (yellow hue)*	*Saltiness*	*Consistency*	*Resistance in spreading*	*Stickiness*
1	$1.91^{ab} \pm 0.65$	$7.74^{a} \pm 0.70$	$3.21^{ab} \pm 0.98$	$3.28^{c} \pm 0.93$	$6.19^{a} \pm 0.69$
2	$3.24^{ab} \pm 1.06$	$6.43^{a} \pm 1.39$	$3.80^{ab} \pm 1.18$	$3.03^{c} \pm 0.64$	$6.73^{a} \pm 1.01$
3	$4.53^{a} \pm 1.28$	$7.10^{a} \pm 0.77$	$4.50^{ab} \pm 0.87$	$5.56^{abc} \pm 1.40$	$6.40^{a} \pm 0.83$
4	$2.31^{ab} \pm 0.40$	$5.80^{a} \pm 0.90$	$2.41^{b} \pm 0.69$	$2.44^{c} \pm 0.47$	$7.08^{a} \pm 1.62$
5	$2.00^{ab} \pm 0.63$	$4.86^{ab} \pm 0.35$	$4.74^{ab} \pm 0.67$	$4.31^{bc} \pm 0.88$	$6.31^{a} \pm 1.02$
6	$0.948^{b} \pm 0.26$	$1.15^{c} \pm 0.42$	$6.25^{ab} \pm 1.68$	$7.04^{ab} \pm 1.09$	$1.44^{b} \pm 0.47$
7	$1.19^{ab} \pm 0.26$	$2.28^{bc} \pm 0.52$	$7.12^{a} \pm 1.34$	$8.19^{a} \pm 0.91$	$1.21^{b} \pm 0.30$

Values are means of eight replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

TABLE 8. Panelists' scores on the acceptance of sensory characteristics of processed cheese samples (scale 0 – 10).

Sample	*Flavour*	*Texture*	*Overall acceptance*
1	$4.92^{b} \pm 0.45$	$5.67^{b} \pm 0.43$	$4.88^{b} \pm 0.41$
2	$4.98^{b} \pm 0.43$	$7.16^{a} \pm 0.60$	$4.63^{b} \pm 0.38$
3	$5.05^{b} \pm 0.41$	$5.38^{b} \pm 0.45$	$3.83^{c} \pm 0.36$
4	$5.98^{a} \pm 0.49$	$7.10^{a} \pm 0.63$	$6.80^{a} \pm 0.59$
5	$6.20^{a} \pm 0.57$	$6.82^{a} \pm 0.44$	$6.96^{a} \pm 0.58$
6	$2.49^{c} \pm 0.28$	$2.95^{c} \pm 0.27$	$2.78^{d} \pm 0.34$
7	$2.68^{c} \pm 0.22$	$1.87^{c} \pm 0.19$	$2.42^{d} \pm 0.21$

Values are means of eight replications ± standard deviations.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

4. CONCLUSIONS

Conclusions of the present study can be summarized in the following:

- Preparation of processed cheese samples based on Feta cheese is plausible and two of the samples studied were shown to have increased scores on overall organoleptic acceptance by the panelists.

- Protein, as well as fat, strengthens most mechanical properties. Conclusively, the increase in protein content leads to a denser and stronger protein network. Furthermore, since samples were tested at 5-8^{o}C, where fat is mainly found in solid state, fat particles strengthen the protein network. In contrast, it appears that moisture softens the product and improves its spreadability.
- Sensory properties of texture are highly correlated amongst them, as well as with mechanical properties.
- Yellow hue's intensity, as evaluated by panelists, is highly correlated with fat content and colourimetric measurements.

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THERMOCHEMICAL STABILITY OF STARCH DEGRADING ENZYMES OF TEFF (ERAGROSTIS TEF) MALT DURING ISOTHERMAL MASHING

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ABSTRACT:

Enzymatic hydrolysis of malt-starch has many applications in food industries, particularly in the production of soft and alcoholic drinks. The rate of enzyme-catalysed reaction increases with temperature. However, at higher temperatures the rate is retarded due to the destabilization and irreversible deactivation of the enzyme caused by its thermo-sensitive protein nature. Inhibitors are also useful in brewing and beverage industries for regulating proportions of fermentable sugars. This research was aimed to study the thermal stability of starch degrading enzymes of teff malt, and investigate the stability of the enzymes towards chemical inhibitors. Isothermal mashing at temperatures ranging between 30 and 75 °C with sampling in 15 min interval for a total of 90 min were conducted to study thermal stability of the enzymes. Chemicals such as oxalic acid (0.01 mol/L), mercuric chloride (100×10^{-6} mol/L and 10×10^{-3} mol/L), and sodium acetate (0.2 mol/L and 0.5 mol/L) were tested as inhibitors of alpha-amylase, beta-amylase, and limit-dextrinase, respectively. Silver nitrate (10×10^{-3} mol/L) and copper (II) sulfate (10×10^{-3} mol/L) were also tested as potential inhibitors of alpha- and beta-amylases. The reaction mixtures were allowed to react at 37 °C for 90 min with continuous stirring. The thermal stability study revealed that the deactivation rate constants (k_d) of alpha- and beta-amylases for temperatures ranging from 40 to 75 °C were in the ranges of 0.0003 to 0.0409 min^{-1}, and 0.002 to 0.032 min^{-1}, respectively. The rate of deactivation of limit dextrinase was not significant at temperatures lower than 60 °C but showed high deactivation at higher temperatures with rate constants ranging from 0.02 to 0.1 min^{-1}. The thermal deactivation energies (E_d) of alpha-amylase, beta-amylase, and limit dextrinase were found to be 148, 84.4, and 144 kJ/mol, respectively. Based on the results, the Arrhenius rate constant equations for thermal deactivation of these enzymes were determined to be $k_d = 7.98\times10^{20}\exp(-1.48\times10^5/RT)$, $k_d = 1.89\times10^{11}\exp(-8.44\times10^5/RT)$, and $k_d = 7\times10^{20}\exp(-1.44\times10^5/RT)$, respectively. The study on their stability towards

chemical inhibitors showed that the enzymes appeared to be inactivated by most of the inhibitors used in this experiment. Oxalic acid inhibited α-amylase and limit-dextrinase activities by about 98 and 63%, respectively, but largely conserved β-amylase activities. Mercuric chloride failed to cause complete inhibition of β-amylase in teff malt. Sodium acetate didn't show any effect on limit-dextrinase activity, but slightly inhibited alpha- and beta-amylases at both studied concentrations. Silver nitrate and copper (II) sulfate showed almost complete inhibition on alpha-amylase, and more than 87% on both beta-amylase and limit dextrinase activities. The results of this study showed that mercuric chloride is unsuitable for determination of α-amylase activity by selective inactivation of β-amylase. The present findings have significant applications in commercial processes where determination of the upper temperature limits for amylolytic enzyme activities, and controlling proportions of fermentable sugars are required.

Keywords: amylolytic enzymes, chemical inhibitors, malt, teff, thermal stability

NEURAL NETWORK MODELS WITH IMAGE PROCESSING TECHNIQUES FOR DISCRIMINATION OF CONVENTIONAL AND ORGANIC FOODS

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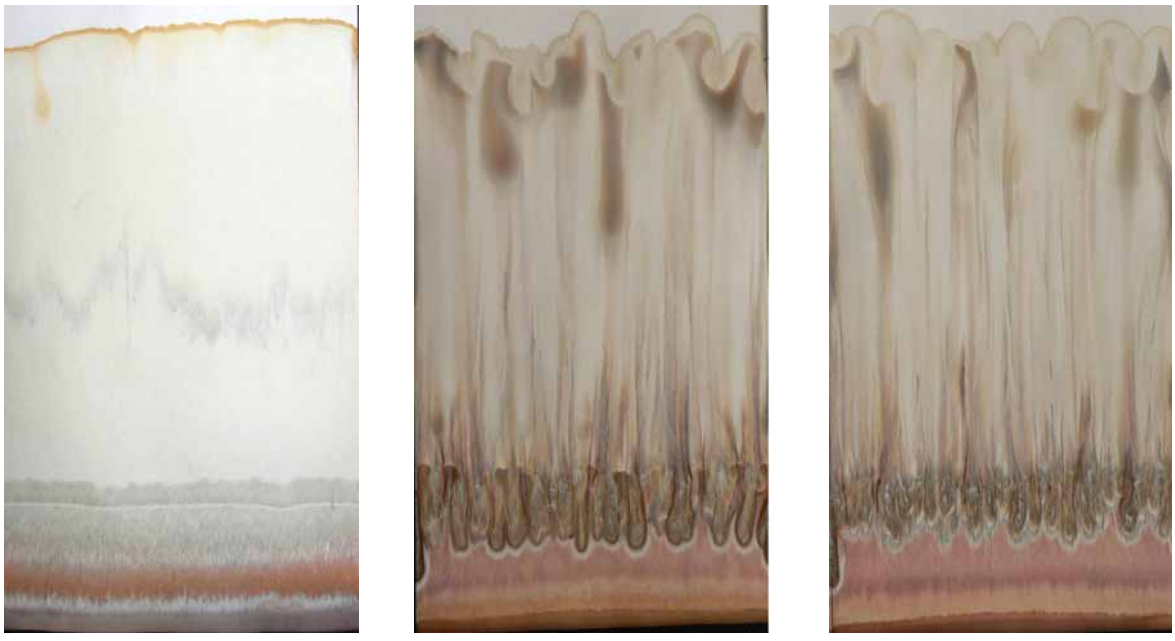
ABSTRACT

There is an increasing interest on the quality of foods among customers. Especially, organic farming receives a considerable attention in recent years. The quality of foods from conventional and organic production is compared in numerous studies. In this study, different neural network models were employed in the holistic methods (picture developing methods) such as bio-crystallization (copper chloride crystallization) and capillary dynamolysis. The first novel methodology called the Image Neural Network (INN) has been developed for texture image analysis to differentiate the crystallograms from pairs of fresh red pepper fruits from conventional and organic farms. However, the visual description and definition of bio-crystallogram images by human-beings has major disadvantages. The INN has been developed to overcome these shortcomings. The second neural network model called Gram-Charlier Neural Network (GCNN) has been studied to classify the fresh tomatoes from conventional and organic farms by using a capillary rising picture method. The INN and GCNN reaches a 100% recognition performance under testing. This high level of recognition suggests that the neural network methods coupled with image processing techniques are very effective tools in the discrimination of capillary dynamolysis and bio-crystallization images.

Keywords: Organic tomato; Conventional tomato; Capillary dynamolysis; Fresh red pepper fruits; Bio-crystallization; Neural network; Bayes optimal decision rule, Back-propagation algorithm.

1. INTRODUCTION

Holistic methods (picture developing methods) including bio-crystallization (copper chloride crystallization) and capillary dynamolysis have been used to examine foods from organic cultivation (Zalecka et al., 2006). These methods are developed on the basis of images of the 'inner structure' of an organism. The inner structure of a living organism is believed to be related to the growth (i.e. farming system) and development of the organism. Different imaging methods are developed to reveal this 'inner structure' (Doesburg P, 2007). The capillary dynamolysis is one of these methods. The method involves capillary dynamolysis first with an aqueous extract of plant sap followed, with intermediate drying, by a metal salt, usually silver nitrate, which serves as an indicator to develop the structured pattern. Any kind of additive will change the chromatogram. The process is influenced by the qualitative and quantitative variations in the macromolecules of the biological extracts, thus allowing food quality assessment (Meelursarn, 2006). The pictures are evaluated qualitatively describing the different form elements and their interactions (Skjerbaek K et al., 2004). Figure 1 illustrates the chromatograms produced on the basis of biological substances, such as plant extracts, exhibit distinctive structures covering the chromatography paper (Kuscu A, 2008).



(a) (b) (c)

Figure 1. (a) Chromatogram obtained from basis of aqueous silver nitrate, iron sulphate (blank) (b) conventional growing fresh tomato chromatogram (c) organic growing fresh tomato chromatogram (Kuscu, 2008).

The pictures obtained by capillary dynamolysis method are evaluated visually by trained people, using a 3-4 zone concept and characteristic criteria's for describing the differences (Figure 2). Although capillary dynamolysis method is promising to distinguish between organic and conventional food, they need further development and validation for routine application in food quality assessment. Even if the methodological problems have been overcome there remains the cognitive task of how the images and patterns are to be read (pattern recognition).

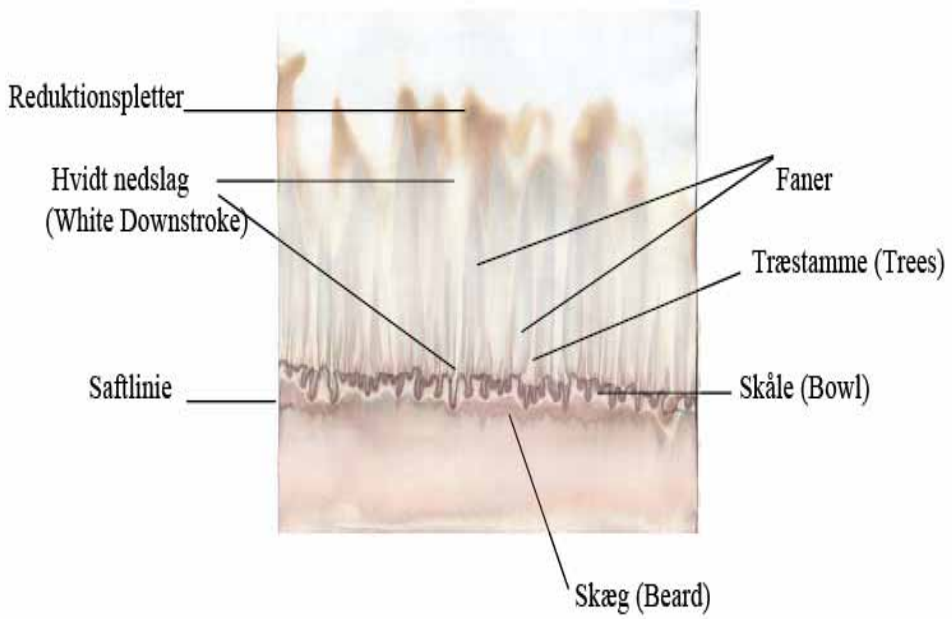


Figure 2. Definition of zone in capillary dynamolysis method (Andersen et al., 2004)

In literature several trained authors have shown to be capable of judging and interpreting capillary pictures by connecting them correctly to different farming systems (Skjerbaek K et al., 2004). Although visual examination by trained people can be used as a tool for evaluation of the images, it requires high expertise and practice. Additionally, there is a lack of validated methodology for the quality assessment. On the other hand, computerized image analysis with neural networks can be an alternative approach increasing the objectiveness of the picture forming methods and allow the analysis of large numbers of chromatogram images (Unluturk et al., 2011).

The other holistic method called bio-crystallization was originally introduced by Pfeiffer (1931), which is also called "sensitive crystallization" and "copper chloride crystallization." Engqvist (1970) later initiated the bio-crystallization method. This method was developed from the viewpoint that living organisms have directing and organizing

"structuring forces." These structuring forces direct the form and function of the organism. The method is based on the crystallographic phenomenon that when adding specific ionic substances, or in general all organic substances, to an aqueous solution of dehydrate $CuCl_2$, bio-crystallograms with reproducible dendritic structures are formed during crystallization. Crystallograms that are produced on the basis of pure $CuCl_2$ exhibit a merely peripheral distribution of crystals on the circular glass underlay, with a diameter of 90 mm. In contrast, bio-crystallograms produced on the basis of biological substances, such as plant extracts (fresh sweet red pepper samples in this study), exhibit crystal structures covering the entire glass underlay (Figure 3).

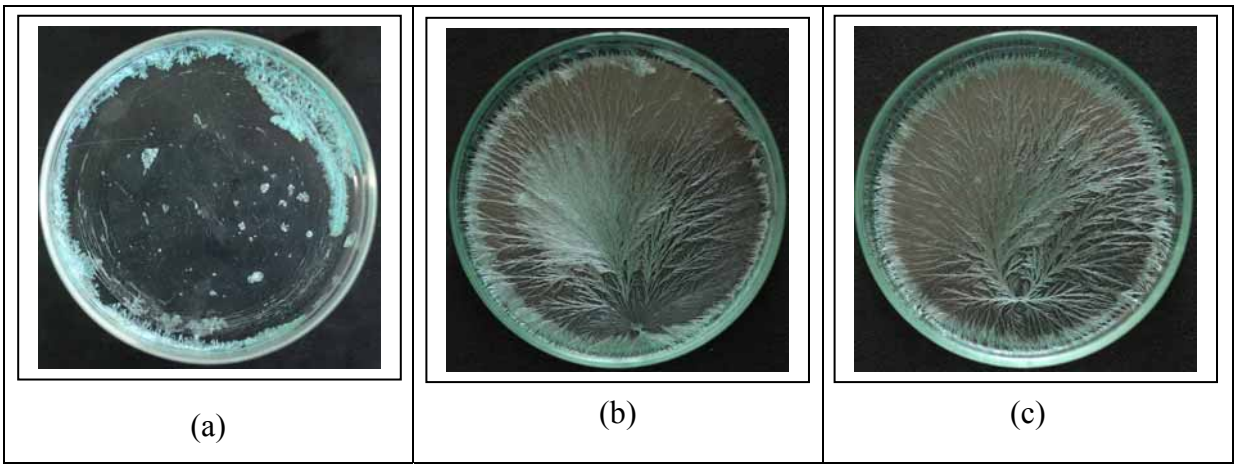


Figure 3. (a) Crystallogram obtained from basis of aqueous dehydrate $CuCl_2$ (blank) (b) conventionally growing fresh sweet red pepper bio-crystallogram (c) organically grown fresh sweet red pepper bio-crystallogram (Kuscu, 2008)

The bio-crystallograms that are produced from agricultural products, such as vegetables, grains, fruits, and milk samples are based on three components: (a) an aqueous solution or extract of the sample in question; (b) an aqueous solution of dehydrate copper chloride; and (c) purified water**.** Any kind of additive will change a copper chloride crystallization. The process is influenced by the qualitative and quantitative variations in the macromolecules of the biological extracts, thus allowing food quality assessment. The phenomena of bio-crystallograms are based on ramification patterns, which may be divided into three major stages, extending from the center in all directions to the periphery of the image (Figure 4).

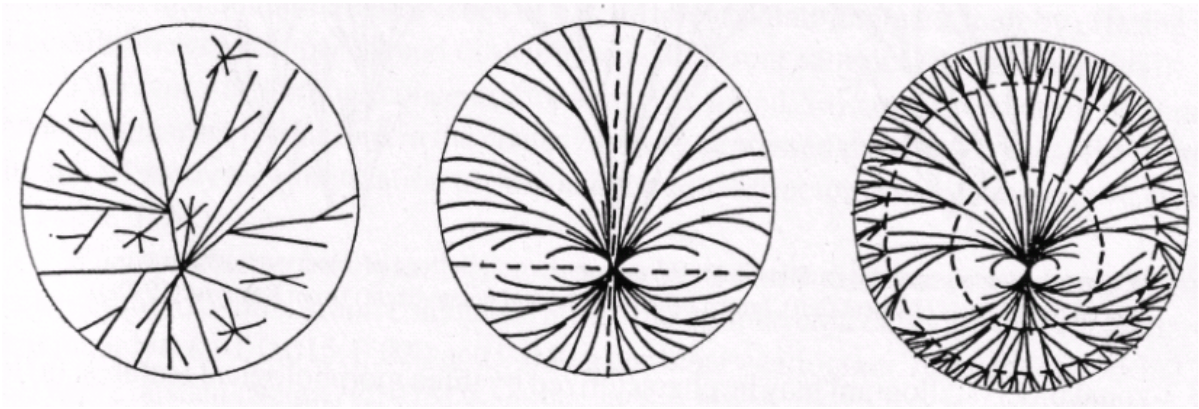


Figure 4. Bio-crystallization method based on a dendritic pattern phenomena that are formed during crystallization from an aqueous solution containing plant extracts and $CuCl_2$ (Engqvist, 1970).

In the first or 1-zone bio-crystallogram, when increasing concentrations of agricultural extracts are applied relative to a given fixed concentration of $CuCl_2$, transparent needles with enormous star-like formations extend in all directions to the periphery. The second, 2-zone structure goes through the crystallization center when it is divided by a vertical and horizontal axis. The needles are pointed, predominantly transparent, and of relatively equal length in the middle zone. These morphological features may be described by means of plant morphological terms, such as stems, branches, and needles. The last stage of a bio-crystallogram is an optimal degree divided into a 3-zone structure with a center zone, middle zone, and marginal zone. In the third stage, a bio-crystallogram exhibits various macro and microscopic morphological features that reflect the quality of the sample in question (Meelursarn, 2006). The bio-crystallization method comprises two main parts (Figure 5). The first is pattern formation, which starts in a laboratory and continues until the crystallogram picture is completely generated in the chamber. The second part is pattern recognition. This is where the evaluation tools will be responsible for perceiving and differentiating between images (Engqvist, 1970).

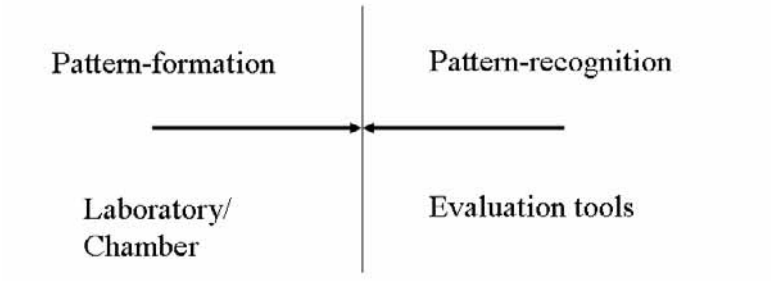


Figure 5. The bio-crystallization method (Doesburg, 2007)

There are two tools currently used to evaluate an image, visual evaluation and computerized image analysis. In visual evaluation the images in question are evaluated based on the judgment of a trained human using discrete reference scales arranged in connection with picture phenomena. Computerized image analysis interprets the image by using the fundamental knowledge of texture analysis. Such techniques have been explored and applied with the bio-crystallization method (Unluturk et al., 2013). Computerized image analysis techniques may meet the demand for such methods. Ideally an image analysis procedure should reflect all of the characteristics of a bio-crystallogram as a three-dimensional, colored ramification structure, coordinated with zones relative to the center. Computerized image analysis tools increase the objectivity of the method and allow the analysis of large numbers of crystallization images (Doesburg, 2007). This paper presents unique neural network models, called an image neural network and a Gram-Charlier neural network, as effective analysis tools.

2. MATERIALS AND METHODS

2.1. Tomato fruit sample preparations

Conventional and organic tomato fruits were obtained from Ege University Agricultural Faculty Research, Applied and Production Farm in Menemen (Izmir, Turkey) during the period of 2005 and 2006. Tomato fruits were quickly transferred to the laboratory. The fresh fruits were cleaned, chopped into small pieces and passed through a kitchen type blender (Braun MR 404). The homogenate was first passed through a cloth to remove debris particles and then filtered. The fruit juice was diluted to 30-100% with bi-distilled water. Silver nitrate and iron sulphate solutions were also prepared at a 0.25-0.75% concentrations with bi-distilled water. The combination ratios for the sample extract, silver nitrate and iron sulphate influence specific patterns of the chromatograms. Therefore, different ratios of sample, silver nitrate and iron sulphate concentrations were studied. The concentration levels were between 30 to 100%, 0.25 to 0.75% and 0.25 to 0.75% for sample, silver nitrate and iron sulphate, respectively. Sample extracts are prepared at different concentrations ranging from 30% to 100% with bi-distilled water. 0.6 ml of these aqueous extract is allowed to rise in chromatographic paper (Whatmann no. 2 CHR) up to 2cm from the base line and dried for 3 hours in a room at 20 °C and 60 % RH. Next the chromatographic paper is placed in a solution of 0.7 ml of silver nitrate solution prepared at different concentrations (0.25-0.75 %). Silver nitrate is allowed to migrate vertically up to the level approximately 1 cm over the juice-line in a glass

bowl for 30 min. After drying, 2 ml of iron sulphate solution (0.25-0.75 %) is added and allowed to rise nearly to the top of the paper (12 cm) in 0.5 to 2 hours (Figure 3). Then the paper is dried for 3 hours. The last two stages take place in a dark room. A total of 23 paper chromatograms were produced from organic and conventional fresh tomato fruits.

2.2 Sweet red pepper extracts and copper chloride concentrations

Conventional and organic sweet red pepper fruit were also obtained from Ege University Agricultural Faculty Research, Applied and Production Farm in Menemen (2005-2006; Izmir, Turkey). Fresh sweet red pepper fruit were quickly transferred to the laboratory. The fresh fruit were cleaned, chopped into small pieces and passed through a kitchen type blender (Braun MR 404). The homogenate was first passed through a cloth to remove debris particles and then filtered. The fruit juice was diluted to 1% with bi-distilled water. Copper chloride dehydrate solution was also prepared at a 16% concentration with bi-distilled water. The optimal mixing ratio for the sample extract and copper chloride influences the crystallization pattern. Therefore, the optimum sample and $CuCl_2$ dehydrate concentrations were determined to be 1% and 16%, respectively. For each bio-crystallogram, a 1 ml sample (1% concentration) was mixed with 3 ml $CuCl_2$ dehydrate (16% concentration) per plate at chamber conditions of 25 °C and 55% relative humidity (Doesburg, 2007). The copper chloride was crystallized after 16-18 hours. A total of 23 bio-crystallograms were produced from organic and conventional fresh sweet red pepper fruit in 2005 and 2006. Only single centered bio-crystallograms were evaluated, the others (multi-centered) were discarded.

2.3. Image acquisition and capture

Images were captured using a digital camera model DMC-FZ5 (5 MPs) (Panasonic, NJ, USA). The camera was positioned vertically over the sample at a certain distance. The angle between the camera lens, the lighting source and ambient illumination were fixed and kept the same for all the sample pictures. After then the images were transferred and stored in a PC as a JPEG format of ‘high resolution’ and ‘superfine quality’.

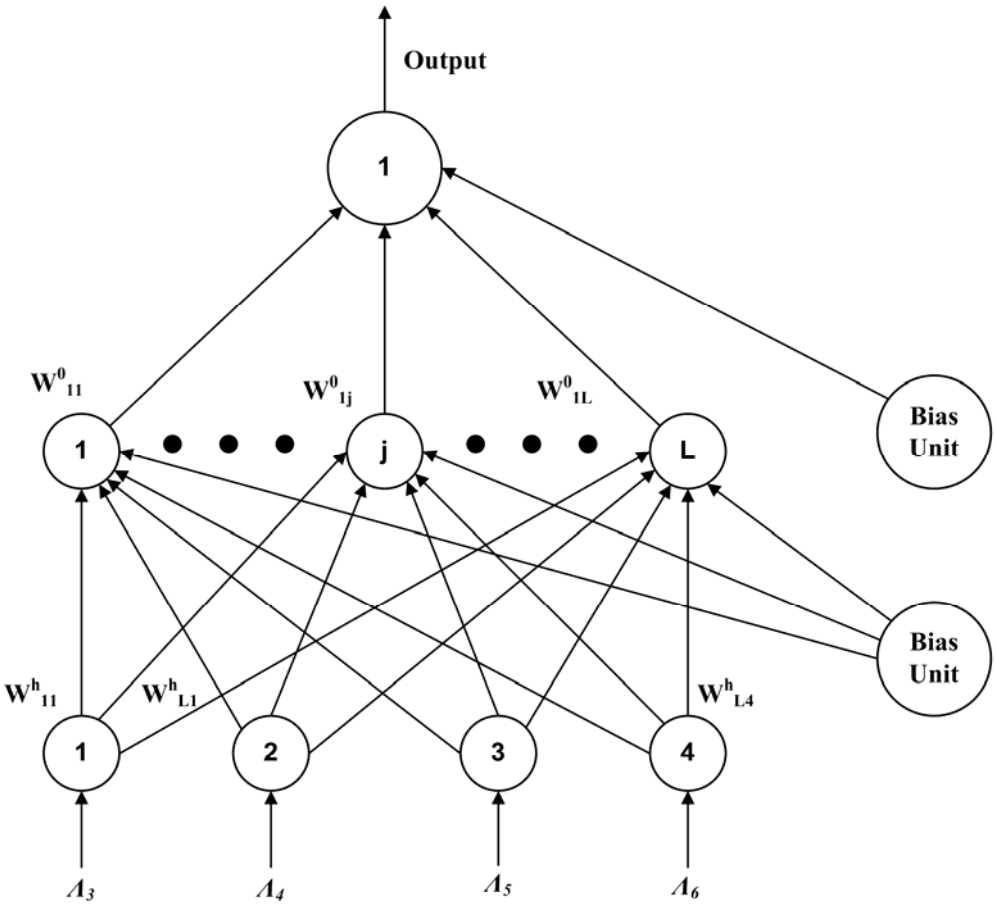
3. THE NEURAL NETWORK ARCHITECTURE

The neural network model has three processing stages. The first stage preprocesses images and the second stage converts these images into row vectors and estimates Gram-Charlier coefficients (Masters, 1993) corresponding to these row vectors. In the third

processing block, these parameters are applied to a back propagation neural network to classify the images (Figure 6). This neural network is a fully connected feed forward neural network. When the Gram-Charlier coefficients are presented as an input to the neural network, each output of the hidden neurons is a weighted sum of the input nodes and the bias node passes through a hyperbolic tangent function. Then, the output of the neural network is the weighted sum of all the hidden neurons and the bias node for the output layer. This actual output of the neural network is then compared to the desired output for every set of input values. Furthermore, the neural network error is defined as the difference in these outputs. Then, the weights of the neural network are updated using the gradient of this output error (Masters, 1993). The results obtained when testing the neural network show that images of organic and conventional targets can be detected with 100% accuracy. Out of the 23 images, 19 were used for training and 4 were used for testing. The number of hidden neurons for the back propagation neural network was chosen to be 10, based on experience. During testing, when the output of the neural network ≥ 0, then the input Gram-Charlier coefficients was belonged to class Organic. If the output of the neural network was less than 0, then the input Gram-Charlier coefficients was belonged to class Conventional.

4. CONCLUSION

In this study we developed neural network models that are designed to classify the conventional and organic targets. However, the information is not readily quantifiable and lacks uniquely recognizable features. Therefore, a neural network becomes appealing for classifying these images, because it is trainable. The optimal values for the neural network weights were estimated using the back propagation algorithm. Experimental measurements of the images were utilized to train and test the neural networks. This network showed a remarkable 100% classification performance. Parallel classification performance was also achieved when training the neural network. These results are encouraging and suggest that neural networks are potentially useful for detecting conventional/organic food samples and in quality control. Furthermore, the neural network renders practical advantages such as real-time processing, adaptability, and training capability. It is important to point out that similar neural network designs can be used in medical ultrasonic imaging for tissue characterization and diagnosis.



Gram-Charlier coefficients

Figure 6. Back propagation neural network. If Output ≥ 0, then input type of sample image belongs to organic class or it belongs to conventional class.

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AN ALTERNATIVE FRAMEWORK TO CONDUCT INFERENTIAL STATISTICS FOR LOW MICROBIAL COUNTS IN FOODS: THE POISSON-GAMMA REGRESSION

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ABSTRACT

The objective of this article was to compare four Poisson-gamma regression models to assess the effect of chilling on the concentration of coliforms from beef carcasses. A total of 600 carcasses were sampled before and after chilling at eight large Irish abattoirs, and the total coliforms were determined. With a coded variable (pre-chill/post-chill) as treatment, and extracting the variability of batches nested in abattoirs, random-effects models confirmed that chilling had a decreasing effect on the overall recovery of coliforms. Furthermore, the expected coliforms concentrations on pre-chill and post-chill carcasses were estimated on a CFU/cm^2 scale, as well as their between-batch variability. This study introduced an alternative conceptual framework that can find interesting applications in stochastic risk assessment and in the design of more efficient sampling plans.

Keywords: Negative binomial, chilling, coliforms, carcass.

1. INTRODUCTION

In the evaluation of microbiological quality of foodstuffs, bacterial concentration is conventionally expressed in terms of logarithm of the colony forming units (CFU). Logarithmic transformation is believed to approximate data normality, which is fundamental for the application of inferential statistical data analysis such as the analysis of variance (ANOVA). This may lead to the widely-held practice that whenever bacterial colonies are not observed (zero counts), a low log value corresponding to the limit of enumeration of the microbiological test can be used. This statistical practice for 'censored' observations is known

as imputation, and, depending on the proportion of zero counts or censored points, the mean values are normally overestimated. On the other hand, Gonzales-Barron and Butler (2011a,b) have demonstrated that the lognormal (and Poisson-lognormal) distributions are only appropriate for the representation of high microbial counts, while the Poisson-gamma distribution performs much better for low microbial counts. Thus, for bacterial counts of lower occurrence (such as some hygiene indicators and pathogens), an alternative conceptual framework based on the Poisson-gamma distribution should be explored.

The Poisson-gamma models correspond to a family of flexible count data regressions that can model more variability than the nominal variance under the simple Poisson model (which is equal to the expected value). Heterogeneity or clustering causes a condition called over-dispersion, meaning that the variance of the observed count data normally exceeds the mean. A heterogeneous Poisson model loosens the Poisson restriction by allowing the expected number of counts (λ) to be a function of some random variable. If this random variable follows a gamma distribution, the resulting heterogeneous Poisson will be a Poisson-gamma, also known as a negative binomial distribution. The objective of this work was to introduce an alternative count data regression framework to conduct inferential statistics on microbial counts that did not approximate to a normal distribution after logarithmic transformation. Specifically, the effect of chilling on the coliforms counts recovered from Irish beef carcasses, was assessed by fitting mixed-effects Poisson-gamma regression models.

2. METHODOLOGY

2.1 Sampling of pre-chill and post-chill beef carcasses and microbiological analysis

Eight beef export Irish abattoirs were visited, and a total of 600 beef carcasses were sampled for the quantification of the total coliforms present at the pre-chill and post-chill stage (Gonzales-Barron et al., 2010). All abattoirs were visited at least twice, and during each visit, 30 animals were randomly sampled at the end of the slaughter line.

2.2 Poisson-gamma regression model

In its simplest form, the Poisson distribution models the number of events from a memory-less exponential process where the event rate λ is constant. Assuming that (i) there are no losses in the transfer of bacterial cells from the swabs to the homogenate; (ii) the bacterial cells extracted from the swabs are randomly distributed in the 200-ml neat homogenate; and (iii) each of the plated cells will become a colony after incubation, let Y_i be the random

variable for the number of bacterial colonies *i*, counted on a Petri dish. If Y_i follows a Poisson distribution, the probability mass function is,

$$\Pr(Y_i) = \frac{Exp(-\mu_i) \times \mu_i}{Y_i!} \qquad \ldots(1)$$

$$\mu_i = \frac{A}{V} dt \times \lambda_i \qquad \ldots(2)$$

where A is the swabbed area of the carcass (cm^2), which was measured for each of the sampled carcasses, V is the homogenate volume (200 ml), d is the dilution level at which the respective plate count Y_i was made (i.e., 10^0, 10^{-1}, 10^{-2}, etc.), t represents the aliquot volume poured onto the Petri dish (1 ml), and λ_i is the unknown mean bacterial concentration in $cell/cm^2$.

In a Poisson regression model, the mean parameter λ_i would be a function of a vector of covariates X_n, where β is a parameter vector. In our case, the only covariate is a coded variable X (0 as pre-chill, 1 as post-chill) to quantify the effect of the chilling treatment β_1.

$$\lambda_i = \exp(\beta_0 + \beta_1 X) \qquad \ldots(3)$$

However, carcasses within a batch do not share the same true unknown microbial concentration λ_i (CFU/cm^2) due to the heterogeneity in contamination. Thus, the basic Poisson regression model is generalised by including a dispersion parameter to accommodate the heterogeneity in the count data. Then, a generalised Poisson distribution lets the expected microbial concentration λ_i be a function also of some unobserved random variable e_i (Hinde and Demetrio, 1998),

$$\lambda_i = \exp(\beta_0 + \beta_1 X + e_i) = \exp(\beta_0 + \beta_1 X)\exp(e_i) \qquad \ldots(4)$$

When $exp(e_i)$ follows a gamma distribution $\Gamma(1/k, k)$ with expected value 1 and dispersion parameter k, the above modification to the Poisson distribution yields a more flexible distribution, the Poisson-gamma. The discrete probability mass function for the microbial plate count Y can then be estimated as,

$$\Pr(Y_i) = \frac{\Gamma(Y_i + k^{-1})}{\Gamma(Y_i + 1)\Gamma(k^{-1})}\left[\frac{k^{-1}}{k^{-1} + \lambda_i}\right]^{k^{-1}}\left[\frac{\lambda_i}{k^{-1} + \lambda_i}\right]^{Y_i} \quad \ldots(5)$$

where $\lambda_i = \exp(\beta_0 + \beta_1 X)$ and the gamma function $\Gamma(a) = \int_0^\infty e^{-s} s^{a-1} ds$. The Poisson-gamma and Poisson models are considered to be nested because as *k* approaches to zero, the Poisson-gamma converges to Poisson.

2.3 Fitted variants of Poisson-regression models

2.3.1 Model 1

This model presumes that the measure of dispersion *k* is common for all the sampled batches of beef carcasses. Nevertheless, this model takes into account that there is some heterogeneity in the observed plate counts *Y* that originate from the production batches *b* nested in the different abattoirs *a*. To extract this source of heterogeneity, the fitted function of the expected microbial concentration λ becomes,

$$\lambda = \exp(\beta_0 + \lfloor \beta_1 + u_{b(a)} \rfloor X) \quad \ldots(6)$$

where the chilling effect β_1 is allowed to take up different independent values batch to batch. The nested random effects $u_{b(a)}$ are assumed to have a normal distribution with mean zero and standard deviation σ_u.

2.3.2 Model 2

This model does not only extract the batch-to-batch variability in the chilling effect β1 but also in the intercept β0. Thus, the expected microbial concentration becomes,

$$\lambda = \exp(\lfloor \beta_0 + v_{b(a)} \rfloor + \lfloor \beta_1 + u_{b(a)} \rfloor X) \quad \textbf{...(7)}$$

where the random effects $v_{b(a)}$ model the shifts in the intercept β_0 for the logarithm of the contamination level in each batch. The $v_{b(a)}$ are assumed to have a normal distribution with

mean zero and standard deviation σ_v. The correlation ρ between both random effects was assessed.

2.3.3 Model 3

This is a flexible model in which both the mean λ and the dispersion parameter k are affected by chilling. The model then becomes,

$$\lambda = \exp\left(\beta_0 + \left[\beta_1 + u_{b(a)}\right]X\right)$$
$$k = \exp\left(\alpha_0 + \alpha_1 X\right) \qquad ...(8)$$

where the intercept of the logarithmic expression defining k is λ_0, and the slope describing the chilling effect on k is α_1.

2.3.4 Model 4

In this model, the between-batch heterogeneity in the chilling effect affects both the mean λ and the dispersion parameter k. The model then becomes,

$$\lambda = \exp\left(\beta_0 + \left[\beta_1 + u_{b(a)}\right]X\right)$$
$$k = \exp\left(\alpha_0 + \left[\alpha_1 + v_{b(a)}\right]X\right) \qquad ...(9)$$

The nested random-effects $v_{b(a}$ are assumed to have a normal distribution with mean zero and standard deviation σ_v. The correlation ρ between both random effects was assessed.

The four models were fitted to the coliforms counts data set using the SAS procedure PROC NLMIXED for non-linear mixed models (SAS version 9.1, SAS Institute Inc, NC, USA).

3. RESULTS AND DISCUSSION

As forecast by Gill et al. (1998), who pointed out that for there to be an approximation to normal distribution, the bacteria of interest should be counted in at least 85% of the samples, the microbial data set of coliforms in beef, consisting of 67% of positive counts, did not fit to a normal distribution. For plotting the histogram of frequencies in log CFU/cm^2, the zero counts were replaced by the microbiological limit of enumeration of −2.0 log CFU/cm^2. As

shown in Figure 1, neither for the pre-chill nor the post-chill group, the microbial concentration could be approximated to a normal distribution. Here, it is worthy to mention that such practice of replacing zero counts by the limit of enumeration has been very common for statistical treatment either merely descriptive or inferential.

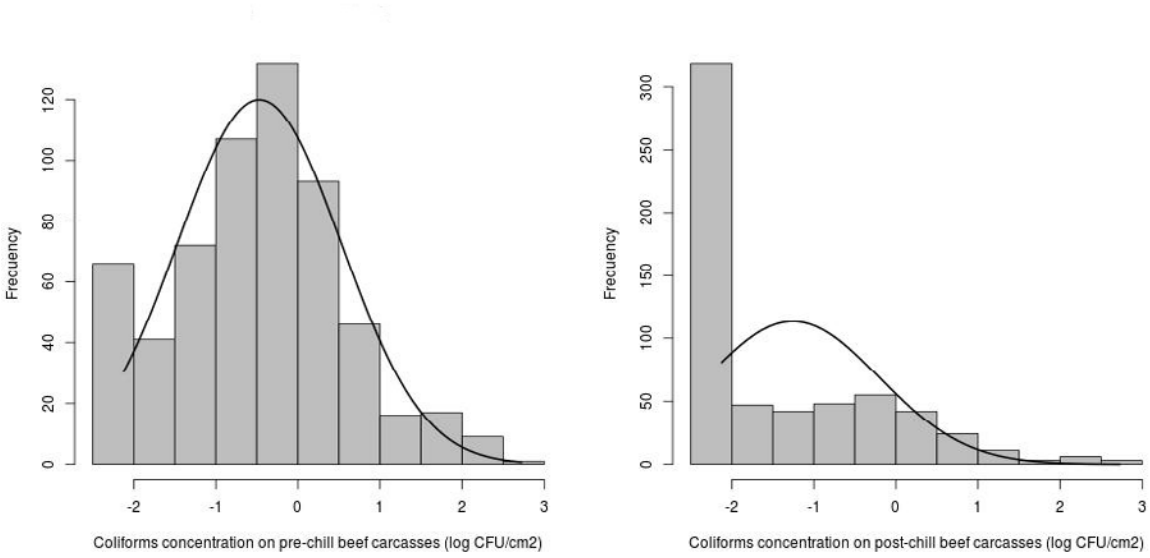

FIGURE 1. Histograms of frequency of the concentration of total coliforms on beef carcasses before (left) and after chilling (right) sampled from eight Irish abattoirs. Zero counts were replaced by the microbiological limit of enumeration of -2.0 log CFU/cm^2. Lack of fit to a normal distribution ($p<0.001$) is graphically shown

Due to the high level of zero counts (equivalent to less than −2.0 log CFU/cm^2), the application of the Box–Cox method (Peltier et al., 1998) failed to approximate the data to a normal distribution. It may be argued that inferential statistics in these cases can be conducted using non-parametric statistics (e.g. Mann–Whitney, Kruskal–Wallis tests) or even outcome categorisation. However, although they may be acceptable in certain applications, these techniques normally result in loss of information, which is detrimental in prediction and modelling applications. Thus, it is clear that, while logarithmic transformation to induce data normality can be suitable for bacterial counts of high occurrence such as mesophile or total viable counts (Gonzales-Barron and Butler, 2011a), this approach may be inappropriate for bacterial counts of lower occurrence such as coliforms or pathogens. Gonzales-Barron and Butler (2011a,b) and Gonzales-Barron et al. (2012; 2013) have demonstrated that the Poisson-gamma distribution is much more suitable than the lognormal distribution to represent low microbial counts and even highly clustered microbial data consisting of a large proportion of zero counts. Thus, to carry out inferential and predictive statistics without incurring loss of information, an alternative approach is to conduct count data Poisson-gamma regression

models. For the fitting of these count data models, the coliforms counts cannot be expressed in terms of a continuous variable (log CFU/cm^2). A discrete response variable must be used, which is defined as the number of colonies counted on a Petri dish originated from the 1-ml aliquot (Y in CFU).

The simplest Poisson-gamma regression model (Model 1), with a significant dispersion parameter k (Table 1), suggests that the over-dispersion of the plate count data is indeed accounted for by the Poisson-gamma distribution. It also evidences the significant decreasing effect that chilling has on the coliforms viability by an overall factor of 0.133 ($\exp(\beta_1)=\exp(-2.017)$). Nevertheless, there is some uncertainty around this value due to the significant heterogeneity among the batches nested in the abattoirs, represented by a standard deviation σ_u of 1.99 (Table 1). The fluctuation found batch to batch in the reducing effect of chilling is not unexpected as there are many factors contributing to the variability in the detected coliforms: hygiene of the abattoirs, size of the abattoirs, type and capacity of the chilling systems, logistics, carcass surface dryness, proximity of contaminated carcasses, etc.

The objective of the second Poisson-gamma model was not to estimate the effect of chilling but to test whether there is any correlation between the intercept β_0 and the slope β_1 of the logarithmic function of the mean concentration λ (Equation 7). For this, random effects for the batches (nested within abattoirs) were placed in both parameters. The significant coefficient of correlation $\rho=-0.7$ suggested that as the initial (pre-chill) contamination increases ($\exp(\beta_0)$), the reducing effect of chilling ($\exp(\beta_1)$) on the viability of coliforms tends to be weaker. The association can be clearly visualised in Figure 2, where each of the markers represents the exponential values of the paired random effects ($\exp(v_{b(a)})$, $\exp(u_{b(a)})$) of a batch. Notice that in Model 2, both β_0 and β_1 were not significantly different from zero, and hence they were dropped.

When a log-linear predictor for the dispersion parameter k was introduced (Model 3), the goodness-of-fit measure BIC was considerably improved (in relation to Model 1) since chilling significantly affected k. Thus, the dispersion parameter for the pre-chill carcasses group was lower (4.6) and significantly different from the one for the post-chill group (9.7; Table 1). As explained in Gonzales-Barron and Butler (2011a), the higher the proportion of zeros in a data set, the more skewed the Poisson-gamma distribution becomes, and hence, the higher the dispersion parameter.

TABLE 1. Mean parameter estimates and standard errors (in brackets) of the four random-effects Poisson-gamma regression models fitted to the plate count data of total coliforms on Irish beef carcasses

	Model 1	Model 2	Model 3	Model 4
No. parameters	**3**	**4**	**5**	**6**
Parameters				
β_0	1.820***	0	1.816***	1.816***
	(0.103)		(0.089)	(0.017)
β_1	-2.017***	0	-1.950***	-1.834***
	(0.470)		(0.465)	(0.061)
k	6.300***	5.121***	-	-
	(0.243)	(0.204)		
α_0	-	-	1.533***	1.533***
			(0.048)	(0.027)
α_1	-	-	0.744***	0.810***
			(0.081)	(0.037)
Random effects				
σ^2_u	3.961*	3.626*	3.786*	2.922***
	(1.328)	(1.195)	(1.318)	(0.058)
σ^2_v	-	7.904*	-	0.599***
		(2.602)		(0.009)
ρ	-	-0.698*	-	0
		(0.120)		
Goodness-of-fit				
BIC	11245	11034	11159	11076
Other estimates				
λ before	6.171	-	6.148	6.148
(CFU/cm^2)	(0.636)		(0.544)	(0.106)
λ after	0.822	-	0.875	0.982
(CFU/cm^2)	(0.376)		(0.400)	(0.061)
k before	-	-	4.633	4.633
			(0.220)	(0.123)
k after	-	-	9.748	10.415
			(0.639)	(0.241)

We could then generalise that, should the effect of chilling be different batch to batch – as already suggested by the previous models, the proportion of zero counts in the pre-chill carcasses relative to the post-chill carcasses will be different batch to batch, and this will lead to different pre-chill to post-chill dispersion parameter rates batch to batch. With this in mind, in Model 4, random effects $v_{b(a)}$ were added to the slope (effect of chilling α_1) of the dispersion parameter predictive equation (Equation 9). In this Model 4, all parameters were significant and the goodness-of-fit measure BIC was considerably improved (in comparison

to Model 3). No correlation was found between the random effects for the chilling effect on the mean microbial concentration ($u_{b(a)}$) and the random effects for the chilling effect on the dispersion parameter ($v_{b(a)}$). Overall, the parameter estimates of Model 4 did not differ considerably from those of Model 3, although the additional random effects of Model 4, accounting for the batch-to-batch heterogeneity in the effect of chilling on the dispersion parameter, decreased the standard errors of the parameter estimates (Table 1). It can be said that the chilling operation reduces the viability of total coliforms on beef carcasses by a mean factor of 0.16 (exp(-1.834)). On average, before chilling the beef carcasses sampled from the Irish abattoirs presented a concentration of coliforms of 6.15 CFU/cm^2, and after chilling, this concentration dropped significantly to 0.98 CFU/cm^2.

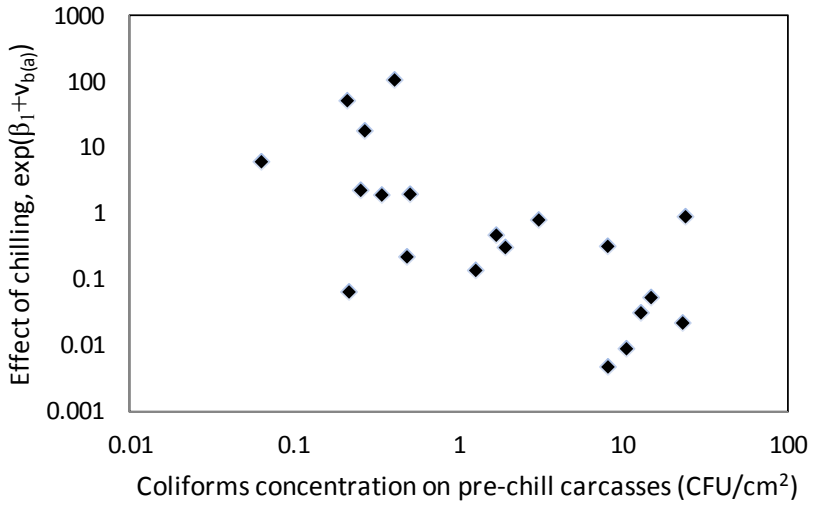

FIGURE 2. Correlation between the initial concentration of coliforms on a batch of pre-chill beef carcasses and the effect of chilling (ρ=-0.7)

The Poisson-gamma regression framework constitutes a neat approach to tackle statistical analysis of low counts microbial data because it does not make use either of the ordinary log-transformed data or the imputation of the lowest limit of enumeration for the zero counts, and instead it uses directly the observed number of colony forming units and takes into analysis all the zero counts.

4. CONCLUSIONS

This work introduced an alternative framework based on the Poisson-gamma regression to conduct inferential statistics on plate count data from microorganisms of low recovery. As an

illustration of this type of count data models, we estimated the effect that chilling has on the mean counts of the total coliforms on beef carcasses as well as its between-batch variability. Such a neat approach has a potential utility in risk assessment modelling of pathogens and in the design of sampling plans.

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INFLUENCE OF FERMENTATION CONDITIONS ONFERMENTED CARROT JUICE QUALITY USING *LACTOBACILLUS PARACASEI*AS A STARTER CULTURE

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ABSTRACT:

Carrot juice is one of the most important vegetable juicesdue to its high nutritional value.The fermentation of vegetable juices by lactic acid bacteria as starter cultures (lacto-fermentation) improves the juice nutritional value and sensory properties. On the other hand, *Lactobacillus paracasei* is one of the most used lactic acid bacteria for the fermentation of milk-based products. However, using it for the preparation of other than dairy products is still uncommon. The aim of this study was to investigate the influence of fermentation conditions onthe growth of*Lb. paracasei* in carrot juice and evaluate the fermented juice quality attributes. The response surface methodology was used for this purpose. Three fermentation parameters were tested at three levels,i. e.fermentation temperature of 22, 30 and 38 °C; initial pH valueof 4, 5 and 6; and inoculation dosage of 0.2, 0.6 and 1 OD_{600}. The face-centred cube design was used with six replicated centre points. The criteria for the optimisation were to obtain high bacterial cell concentration, high lactic acid formation and final pH value between 3.5 and 4. Results showed that the optimum fermentation conditions were obtained at fermentation temperature 36°C, pH value 6 and inoculation dosage0.7 OD_{600}. This fermentation regime yielded the highest bacterialcell concentration, lactic acid6.1 g/L, sugar content 23.4 g/L and final pHvalue 4.The models showed high R^2value and *p*-value was significant. The studyshowed that the fermentation of carrot juice by *Lb. paracasei* can offer a suitable alternative to lactic acid fermented dairy products. Further efforts will be made to evaluate the effect of fermentation on the sensory properties of carrot juice.

Keywords:fermentation, response surface methodology, carrot, *Lactobacillus paracasei*

OAT STARCH EDIBLE FILM: WATER VAPOR PERMEABILITY, PHYSICAL AND MECHANICAL PROPERTIES AS AFFECTED BY PLASTICIZER

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ABSTRACT:

Starch-based films have promising application on food packaging, because of their environmental appeal, low cost, flexibility and transparency. Nevertheless, their mechanical and moisture barrier properties should be improved. Therefore, the aim of this work was to enhance these properties by reinforcing the oat films with glycerol. Besides, the influences of glycerol on mechanical properties, water vapor permeability (WVP), moisture uptake and optical properties were investigated. Films were prepared by the so-called casting technique, from film-forming suspensions of oat starch, glycerol (0, 20, 30, 40 and 50 g/100 g starch) and water. The findings of this study showed that the addition of glycerol in oat starch solutions has a significant effect on film properties ($P<0.05$). Determination of the mechanical properties of the films at 25 ^{o}C and relative humidity 74% revealed that increasing the relative humidity and the presence of glycerol improved the mechanical properties of the films. The oat starch films showed the lowest WVP values as the glycerol percentage increased from 0 to 30% (W/W). Water adsorption at 25 ºC, degree of lightness (L), total color difference (ΔE) and whiteness (WI) were improved significantly ($P<0.05$) with the increase in glycerol concentration.

Keywords: Oat starch; Edible film; Mechanical properties; Water vapor permeability (WVP)

1. INTRODUCTION

Any type of material used for enrobing (i.e., coating or wrapping) various food to extend shelf life of the product that may be eaten together with food or without further removal is considered an edible film or coating (Debeaufort, Peroval, Despre, Courthaudon and Voilley, 1998). Edible films and coatings enhance the quality of food products and protect them from moisture migration, microbial growth on the surface, light-induced chemical changes, oxidation of nutrients (Kester and Fennema, 1986). Films and coatings are produced from edible biopolymers and food-grade additives. Proteins, polysaccharides (carbohydrates and gums) or lipids can be used as film-forming biopolymers.

Starch is one of the most plentiful natural polysaccharides (Narayan, 1994). As chief constituents or additives, native or modified starches have been playing important roles in the food industry. In addition, starches and their derivatives have been used to modify physical properties of food products, contributing principally to texture, viscosity, gel formation, adhesion, binding, moisture maintenance, product homogeneity, and film formation (Thomas and Atwell, 1997). Starch films are often transparent (Myllairinen, Bulcon, Lahtinen and Forssell, 2002) or translucent (Rindlav, Hulleman and Gatenholm, 1997), odorless, tasteless, and colorless (Wolff, Davis, Cluskey, Gundrum and Rist, 1951). They have been utilized in the packaging and coating of food products, because of their edibility and low permeability to oxygen (Roth and Mehltretter, 1970).

Recently, the popularity of oat as a part of the human diet has enhanced because of information illustrating the advantageous nutritional characteristics of oat β-glucans (Anderson, Deakins and Bridges, 1990). Oats is recognized as a functional cereal and its advantage on health have been well documented. Oat starch configures an attractive alternative for starch edible films because it includes about 1 to 3% of lipids in its native composition, a high value when evaluated with other ordinary starches, moreover the lipid portion in oat starch is complex with amylose, which would restrain phase separation (Galdeano, Mali, Grossmann, Yamashita and Garcia, 2009). However, less information is obtainable on the physicochemical properties of edible films based on oat starch.

The most important shortcoming of oat starch edible films similar to other biodegradable starch-based films is their hydrophilic nature. Consequently, the incorporation of a plasticizer is obligatory to conquer the fragility of these materials. A number of additives can be added in the formulation of a coating solution, which will alter the characteristics of the resulting coating layer or freestanding film to improve performance.

The objective of the current work is to investigate the effects of different concentrations of glycerol on moisture sorption, water vapor permeability (WVP) and mechanical properties of oat starch films.

2. MATERIAL AND METHODS

2.1 Materials

Oats were grown in the Lavark experimental field of Isfahan University of Technology. Glycerol and other chemicals including analytical grade were purchased from Merck, Darmstadt, Germany.

Extraction of starch from oat grain was done after screening via hand. The method illustrated by Hoover and Vasanthan (1992) was employed.

The moisture content, Ash, crude protein and lipid were determined according to AACC procedures (AACC, 2003). Amylose content of the starch was determined in triplicate using the method of Williams, Kuzina and Hlynka (1970).

2.2 Preparation of films

The films were prepared by the method of Farahnaky, Saberi and Majzoobi (2012).

2.3 Water vapor permeability (WVP)

The method of ASTM E96-95 (1995) was used to analyze WVP of films.

2.4 Moisture content

The procedure described by Galdeano, Mali, Grossmann, Yamashita and Garcia (2009) was utilized to determine the moisture content of films at 25±2 °C under desired relative humidity conditions (11, 22, 38, 64, 74 and 84% RH). All tests were conducted in triplicate.

2.5 Mechanical properties

The mechanical properties were measured using a TA.TX2i Stable Micro Systems texture analyzer, UK, operated according to ASTM D882-91 (1996) method. Filmstrips (40×15 mm) were cut from conditioned samples (at 74% RH, 25 °C) and tested. The thickness of each specimen was measured in five points along its length and the average was reported. Crosshead speed was set to 10 mm/s. The mechanical properties of eight specimens from each sample were measured.

2.6 Color

The color parameters of the films (L: lightness, a: redness-greenness and b: blueness-yellowness) were determined according to the method described by Farahnaky, Saberi and Majzoobi (2012).

2.7 Statistical analysis

The experiments were performed in a much-randomized design. Analysis of variance (ANOVA) was carried out and the results were separated using the Multiple Ranges Duncan's test ($P<0.05$) by using statistical software of Statistical Package for Social Science (SPSS) 16 (SPSS, Inc., New Jersey, USA).

3. RESULTS AND DISCUSSION

3.1 Chemical composition

The yield of starch extracted of oat based on initial material was 29.3%. The chemical composition of oat starch was; moisture (10.25 ± 0.26%), ash (0.40 ± 0.09%), protein (0.46 ± 0.01%) and lipids (1.85 ± 0.32%). The lipid content in oat starch was higher when compared with other botanical sources, which agrees with data reported by others (Mali, Grossmann, Garcia, Martino and Zaritzky, 2006). Most of the lipids are present within native starch granules in the cavity of amylose helix, which prevent phase separation and correspond to a benefit for film making (Wang and White, 1994).

3.2 Water vapor permeability (WVP)

As shown in Table1, addition of 20% or 30% glycerol provided a noticeable decline in the permeability of the film. This would improve the appropriateness of the plasticized oat starch film for use as a food packaging film, particularly in a highly humid environment. However, the effect was not obvious above 40% glycerol, because of the high hydrophilicity of glycerol molecules, which helps the adsorption of water molecules; could also contribute to the increase in the WVP of the film (Gontard, Guilbert and Cuq, 1993).

TABLE 1.Water vapor permeability (WVP) of OS films as function of glycerol concentration at 25 °C and 74% RH*

OS film	Glycerol (%w/w)				
	0	20	30	40	50
Thickness $\times 10^{-3}$ (m)	0.17±0.03	0.19±0.04	0.22±0.06	0.21±0.07	0.21±0.04
WVTR $\times 10^{-5}$ ($gh^{-1}m^{-2}$)	8.54±0.12^{e}	6.15±0.47^{d}	3.47±0.41^{c}	9.09±0.59^{b}	11.47±1.23^{a}
WVP $\times 10^{-12}$ ($gh^{-1}m^{-1}Pa^{-1}$)	8.28±0.11^{c}	6.66±0.04^{d}	4.35±0.38^{e}	10.89±0.18^{b}	13.74±0.26^{a}

* Values are the average of triplicates ± standard deviation. Means at same line with different capital letters are significantly different (P□0.05).

3.3 Moisture content

Moisture content (%M) of OS films in different relative saturated humidity was explained in Figure 1. It could be found that moisture content of OS films enhanced by the increase of glycerol content, when the films were placed under a particular relative saturation humidity condition. Under lower relative humidity of 11%, the %M changes, from 1.10% to 3.30%, was not noticeable with increasing glycerol content. However, under high relative humidity (84%) when glycerol content was over 20%, the alteration of %M was more understandable (from 28.4% to 48.9%). Under constant glycerol content, %M of OS films grew with increase of the relative saturated humidity. Moreover, the addition of glycerol caused the rising of moisture content of OS films, because glycerol had outstanding water-holding capability and made more favor to the moisture absorbability of films.

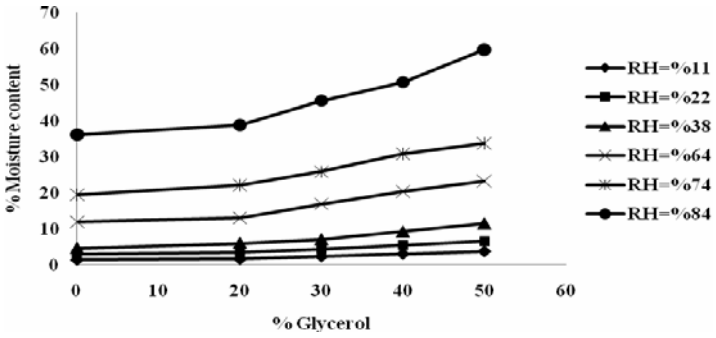

Figure 1. %Moisture content of OS films as a function of glycerol content and different relative humidities.

3.4 Mechanical properties

The mechanical properties of the OS films with the different glycerol contents were shown in Table 2. As shown in Table 2, a decline in tensile strength and breaking factor was

noticed when glycerol content enhanced in all formulations. Hydrogen bonds between the starch chains brought about the high tensile strength and breaking factor value in unplasticized oat starch film. It should be mentioned that there is an obvious synergistic interaction between glycerol and water molecules at high relative humidity. This relationship indicates that films containing glycerol in the presence of high level of water acted mechanically as films with larger amount of plasticizers (with higher % elongation and lower tensile strength).

The unplasticized OS films showed the distinctive pattern of delicate materials, since they displayed low values of strain at maximum breaking force, that is low elongation values (Mali, Sakanaka, Yamashita and Grossmann, 2005). Table 2 signifies that the percent elongation at break of plasticized OS films depends on glycerol concentration. The elongation behavior of films with further 20% w/w of glycerol could be related to those of ductile polymers because elongation at break increased considerably ($P<0.05$) compared with unplasticized films. Glycerol contributes to polymeric chain connection assisting their slithering and accordingly improving the facility of polymer chains mobility, so declines the firmness of the network, generating a less ordered film structure (Sothornvit and Krochta, 2005). The high Yung modulus values of the control sample at 74% RH could be attributed to hydrogen bonds between the starch chains. The decrease in Yung modulus with the increasing of glycerol content from 0% to 50% was caused by the plastic effect of water and magnification of pores and cracks causing by film swelling in higher moisture environment (Bertuzzi, Armada and Gottifredi, 2007). When the content of glycerol exceeded 20%, the decrease was obvious due to the defects in microstructure of films.

TABLE 2. Mechanical properties of OS films with different glycerol contents (% w/w) at 25 °C and 74% relative humidity.*

Sample	Glycerol (%)	Tensile strength (MPa)	Percent elongation at break (%)	Yung modolous (MPa)	Breaking factor (N/μm)
Oat Starch Film	0	14.89±0.38^{a}	4.66±0.10^{c}	3.40E2±195.87^{a}	74.44±1.89^{a}
	20	11.82±0.20^{b}	6.19±0.26^{c}	2.26E2±56.01^{b}	59.08±0.98^{b}
	30	4.61±0.94^{c}	11.24±0.26^{b}	99.13±27.17^{c}	23.03±4.69^{c}
	40	2.42±0.29^{d}	12.60±4.95^{b}	36.97±17.49^{d}	12.05±1.45^{d}
	50	1.93±0.07^{d}	16.29±0.56^{a}	32.34±8.35^{d}	9.65±0.36^{d}

* Values are the average of triplicates ± standard deviation. Means with different small letters are significantly different (P□0.05).

3.5 Color

The Hunt L, a, and b color values, Yellowness index (YI), total color difference (ΔE) and whiteness index (WI) of the films are shown in Table 3. The control sample was generally non-transparent and opaque (lowest L value) but films with 50% (w/w) glycerol showed the low negative a (redness) and positive b (yellowness) values (−3.67 and 6.00, respectively). There were considerable differences in Hunter "L" and "a" values among the OS films, but the major differences in color value were distinguished in the b values of the films because of difference of glycerol content. By increasing of glycerol content, the clarity intensified and the "a" values of the films increased. On the other hand, b values enhanced with incorporation of glycerol. However, the additional concentration of glycerol was capable to increase the lightness (L value) of the films. Addition of glycerol resulted in a significantly decrease (P□0.05) in ΔE (increase clearness). YI showed the different pattern as ΔE. Also, WI increased by growing of glycerol content.

TABLE 3. The Hunter color values (L, a and b), total color difference (ΔE), yellowness index (YI), and whiteness index (WI) of OS films as a function of glycerol content.*

Sample	Color value	0	20	30	40	50
OS film	L	52.67±0.58^{d}	55.67±0.58^{c}	57.33±0.58^{b}	58.33±0.58^{ba}	59.67±0.58^{a}
	a	-6.68±0.58^{c}	-5.33±0.58^{b}	-4.67±0.58^{ba}	-4.33±0.58^{ba}	-3.67±0.58^{a}
	b	13.00±1.00^{a}	10.00±1.00^{b}	8.67±0.58^{cb}	7.33±0.58^{dc}	6.00±1.00^{d}
	YI	35.25±2.39^{a}	25.65±2.35^{b}	21.61±1.65^{c}	17.97±1.51^{dc}	14.43±1.28^{d}
	ΔE	46.04±0.30^{a}	42.31±0.40^{b}	40.36±0.63^{c}	39.14±0.64^{d}	37.93±0.53^{e}
	WI	50.45±0.29^{e}	54.23±0.38^{d}	56.21±0.65^{c}	57.47±0.65^{b}	58.72±0.49^{a}

* Values are the average of triplicates ± standard deviation. Different letters in each rows shows the significant statistical difference between the samples (P<0.05).

4. COCLUSION

The film forming capability of an underutilized source of starch and some traits for its potential industrial applications were exhibited. The presence of lipids in higher contents on oat starch in comparison with other starches could perform as a water vapor barrier, resulting in a smaller variation of the equilibrium moisture contents in all film formulations under diverse relative humidity. The addition of plasticizer was compulsory to improve film characteristics, particularly the mechanical ones. As plasticizer content of films enhanced

Young's modulus and tensile strength decreased with simultaneous increase in percent elongation at break. It appears that inclusion of relatively low concentrations of glycerol (for example 20% and 30% w/w) in the film solution can develop the mechanical properties of the OS film. Additionally, glycerol in concentrations of 20% and 30% (W/W) in filmogenic suspension was ideal plasticizer concentration permitting to decrease WVP (6.66±0.04 and $4.35\pm0.38\times10^{-12}$g/h m Pa, respectively). By increasing of glycerol content, optical properties of the OS films became better conspicuously.

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EFFECT OF CHEMICAL MODIFICATION OF HYDROXYPROPYLATION ON THE PHYSICAL AND MECHANICAL PROPERTIES OF BIODEGRADABLE EDIBLE FILMS FROM WHEAT STARCH

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ABSTRACT:

The films made from native wheat starch are breakable and hard to handle. The main aims of this study were to modify wheat starch in order to determine the possibility of using modified starches in preparation of edible films. Therefore, wheat starch was hydroxypropylated by addition of 30 mL propylene oxide at 40 ^{o}C for 24 hours and edible films were produced using modified starches with different concentrations of glycerol (0, 20, 30, 40 and 50% starch dry weight basis) by casting method. The effects of modification on the some physical properties of the resulted films were investigated. The films produced from hydroxypropylated starch without glycerol had lower water vapor permeability than native starch. On the other hand, water vapor permeability of modified starch increased by all glycerol levels. Determination of the mechanical properties of the films at 25 ^{o} C and different relative humidity (11, 22, 38, 68, 74 and 84%) revealed that increasing the relative humidity and the presence of glycerol and hydroxypropyl groups improved the mechanical properties of the films.

Keywords: Hydroxypropylated wheat starch, Edible films, Water vapor permeability, Mechanical properties

1. INTRODUCTION

Ecological apprehensions have induced a great attention in natural and compostable materials, and thus subjects such as biodegradability and environmental safety are becoming essential. It is the explanation why material components such as natural fibers, biodegradable polymers can be considered as interesting – environmentally safe – alternatives for the improvement of new biodegradable compounds. Starch is one of the most abundant natural plant polysaccharides and, thanks to its cheapness, renewability, and biodegradability, it can be used as a raw material for the elaboration of biologically degradable materials and, thus, promises an environmental solution to the plastic and petroleum waste issues. Several studies have been done to analyze the properties of starch-based films (Arvanitoyannis, Billiaderis, Ogawa and Kawasaki, 1998; Garcia, Martino and Zaritzky, 2000; Mali and Grossmann, 2003; Mali, Grossmann, Garcı´a, Martino and Zaritzky, 2002, 2004, 2005). Even so, starch shows several drawbacks such as a strong hydrophilic character (water sensitivity) and poor mechanical properties compared to usual synthetic polymers, which create it unacceptable for some applications such as packaging uses (John and Thomas, 2008). In the main, many approaches are recommended to alleviate these limitations. One approach is the modification of starch. Chemical modification e.g. cross-linking has long been studied as a way to decrease these troubles and to create low water sensitive and high strength materials (Kim and Lee, 2002). Also, High viscosity of the starch pastes can be diminished by acid hydrolysis of the starch molecules. In that way, hydrolyzed starch could be dissolved in water at higher concentration than native starch. Modification of starch molecules at the granular state by hydroxypropylation is also predominantly attractive. Such chemical modification relating to the introduction of hydrophilic groups into starch molecules ameliorates the solubility of starch and the functional properties of starch pastes, such as shelf life, freeze/thaw stability, cold storage stability, cold water swelling, and yields reduced gelatinization temperature, as well as retarded retrogradation. Due to these properties and their toxicology safety, hydroxypropylated starches have an extensive variety of applications (Sing, Kaur and McCarthy, 2007). Films of hydroxypropylated starch can be prepared easily thanks to a lower solubilization temperature. Advantageous properties for use as packaging materials, especially transparency and flexibility have been evidenced (Vorwerg, Dijksterhuis, Borghuis, Radosta and Kroger, 2004; Lafargue, Lourdin and Doublier, 2007).

This work aims at using hydroxypropylated wheat starch (HPWS) and native wheat starch (WS) with different glycerol contents to produce films for casting application. Influence of

hydroxypropyl groups and glycerol on physical and mechanical properties of edible film were thoroughly studied.

2. MATERIALS AND METHODS

2.1 Materials

Starch extracted from wheat (*Triticum aestivum*) with a moisture content of 9.24 ± 0.50% was purchased from Fars-Glucosin, Co. Marvdasht, Iran. Glycerol and Propylene oxide, sodium hydroxide and sodium sulphate, barium chloride (all analytical grades) were obtained from Merck Schuchardt OHG, Hohenbrunn, Germany.

The wheat starch (WS) contained 9.24 ± 0.32% moisture; 0.14 ± 0.01% protein; 0.27 ± 0.10% ash and 0.66 ± 0.01% lipid as determined by the Approved AACC procedures (AACC, 2003). The amylose content of the starch was 26.35 ± 0.21% that was determined after de-fatting of starch with 80% methanol using the iodine method as described by Williams, Kuzina and Hlynka (1970).

2.2 Hydroxypropylation of starch

Etherification of wheat starch (WS) with propylene oxide was carried out in the presence of NaOH and Na_2SO_4 at pH 11.3 by slightly modifying the procedure outlined by Kaur, Singh and Singh (2004). The method described by the Joint FAO/WHO Expert Committee on Food Additives (1997) was used for the determination of the hydroxypropyl groups.

2.3 Film preparation

Hydroxyporpylated wheat starch (HPWS) and wheat starch (WS) films were prepared as follows. Starch slurry was first prepared from a dispersion of 5 g each starch in 100 mL distilled water containing (0, 20, 30, 40 and 50% w/w) of plasticizer-Glycerol at room temperature (25 ºC) for 5 min. This suspension was transferred to a water bath at 98 ºC for 30 min, with frequent magnetic agitation (500 rpm). Twenty-five grams of the gelatinized suspension were immediately poured onto a Petri dish (10 cm in diameter) and the samples were further dried at 40 ºC for 48 h and placed in a desiccator with concentrated sulfuric acid at 25 ºC to reach constant weight.

2.4 Mechanical properties

HPWS and WS films were equilibrated at different relative humidities (11, 22, 38, 64, 74 and 84%) for 72 h at 25 °C before being tested and the thickness of the samples was measured with a digital micrometer before test. Measurements were taken at five different positions for each sample and the average value of these determinations was calculated. This average value was used to calculate the cross-sectional area of the samples (the area is equal to the thickness multiplied by the width of each samples). The mechanical properties were determined using a TA.TX2i Stable Micro Systems texture analyzer according to the ASTM D882-91 (1996) method. The samples were clamped between pneumatic gripsso force (N) and deformation (mm) could be recorded using aextension rate of 1 mms^{-1}, with initial distance between the grips equal 40 mm. The parameters calculated were: percent elongation at break (deformation divided by initial probe length and multiplying by 100, %) and tensile strength (by dividing maximum force by film cross-section (thickness_width), MPa) were obtained from force vs. deformation curves. Eight film specimens (40 mm×15 mm) of each formulation were used in the analysis.

2.5 Water vapor permeability (WVP)

WVP tests were determined using the ASTM method E96-95 (1995) based on the method outlined by Mali, Grossmann, Garcia, Martino, and Zaritzky (2006). Special cups, with an average diameter of 6 cm and a depth of 4.5cm were utilized to determine WVP of films. Films were cut into discs with a diameter slightly larger than the diameter of the cup. After placing 3 g of anhydrous calcium cloride in each cup, they were covered with edible films of varying composition. RH=0% was maintained using anhydrous calcium cloride in the cup. Each cup was placed in a desiccators containing saturated NaCl solution in a small beaker at the bottom. A small amount of solid NaCl was left at the bottom of the saturated solution to ensure that the solution remained saturated at all times. Saturated NaCl solution in the desiccator provides a constant RH of 75% at 25 ◦C. The desiccator was kept in an incubator at 25.0±0.1 ◦C. After steady state conditions were reached (about 2 h), eight weight measurements were made over 24 h and water vapor transport was determined by the weight gain of the cup. Changes in the weight of the cup were recorded as a function of time. Slopes were calculated by linear regression (weight change vs. time). The water vapor transmission rate (WVTR) was defined as the slope (g/h) divided by the transfer area (m^2). WVP (g. Pa^{-1} $h^{-1}m^{-1}$) was calculated as:

$WVP=[WVTR/P(R_1-R_2)]d$ Equation 1

where P is the saturation vapor pressure of water (Pa) at the test temperature (25 ◦C), R1 is the RH in the desiccator, R2, the RH in the cup and d is the film thickness (m). Under these conditions, the driving force is 1753.55 Pa. All measurements were performed in three replicates.

2.6 Statistical analysis

The experiments were performed in a totally randomized design. Analysis of variance (ANOVA) was carried out and the results were separated using the Multiple Ranges Duncan's test ($P<0.05$) using statistical software of Statistical Package for Social Science (SPSS) 16 (SPSS, Inc., New Jersey, USA).

3. RESULTS AND DISCUSSION

3.1 Mechanical properties

The extent of molar substitution (MS) of HPWS at 30 mL of propylene oxide was 0.12 which is within the limit allowed for the use of hydroxypropylated starches in food applications by the US Food and Drug Administration (FDA) (Dias, Tekchandani and Mehta, 1997), which stipulates that hydroxypropyl group of the modified starch should not be more than 7.0 g/100 g i.e. MS= 0.2.

Results of the mechanical tests are shown in Table 1. Investigation of mechanical properties of films showed that unplasticized WS film had the typical pattern of brittle materials, since it exhibited high values of tensile strength at break and low values of deformation at break in comparison with HPWS film without glycerol. In both films enhancement of %RH and/or polyol had a strong plasticizing action, tensile strength diminished from 7.98 to 0.20 MPa and 23.42 to 1.49 MPa and the maximum elongation changed from 0.44% to 1.24E2% and 0.53% to 21.43% for HPWS and WS films, respectively. In the case of HPWS film, however, at low relative humidities (RH□38%) and low concentration of glycerol content (20 and 30%) tensile strength increased. This could be attributed to the small quantity of glycerol and %RH. As well as, hydroxypropyl groups can probably hydrolyze branched chains of starch molecule that induce formation of highly linear structure which in turn allow forming more hydrogen bonds between the starch chains, small molecules of glycerol and water increasing the tensile strength and breaking factors in the resulted films in these conditions. Generally, the plasticizer molecules interfered with starch packing, decreasing intermolecular attraction and increasing polymer mobility. This involved

an increase in elongation and a decrease in tensile strength as glycerol content and %RH rose in film formulations. Similar results have been previously reported for hydroxypropylated starch films (Arvanitoyannis, Billiaderis, Ogawa and Kawasaki, 1998), potato and barley starch films (Koskinen, Suortti, Autio, Myllarinen and Poutanen, 1996) and for oat starch films (Galdeano, Mali, Grossmann, Yamashita and Garcia, 2009).

3.2 Water vapor permeability (WVP)

The WVTR and WVP values of the different films with varying glycerol contents are given in Table 2. As can be seen there, the WVP of the films was affected by glycerol. The films without plasticizer (glycerol) had the lowest WVP and HPWS film have slightly lower water vapour permeability, compared to the wheat starch film (9.68±0.01 $gh^{-1}m^{-1}Pa^{-1}$), high WVP of native wheat starch film without plasticizer could be originated by micro cracks in film. WVTR and WVP increased significantly by addition of glycerol ($P<0.05$). An enhancement in the inter chain space caused by inclusion of glycerol molecules between the polymer chains may promote water vapor diffusivity through the film and therefore, hasten the water vapor transmission. The high hydrophilicity of glycerol molecules, which helps the adsorption of water molecules, could also contribute to the increase in the WVP of the film (Gontard, Guilbert and Cuq, 1993). Similar results have been reported for cassava starch (Alves, Mali, Beleia and Grossmann, 2007), oat starch (Galdeano, Mali, Grossmann, Yamashita and Garcia, 2009).

TABLE 1. Mechanical properties of HPWS and WS films with different glycerol contents (% w/w) at 25 °C and different relative

		RH (%)	Glycerol (%) 0	20	30	40	50
Tensile strength (MPa)	**HPWS Film**	11	7.98±0.31^{Id}	10.94±0.44^{Fc}	13.72±0.34^{Eb}	20.84±0.15^{Aa}	21.68±1.75^{Aa}
		22	10.58±0.60^{Gc}	14.77±0.93^{Eb}	27.17±0.84^{Aa}	11.01±0.73^{Cc}	6.49±0.18^{Cd}
		38	28.75±0.42^{Aa}	25.05±1.52^{Ab}	9.12±0.57^{Fc}	1.96±0.45^{Ed}	0.57±0.05^{GH,e}
		64	21.36±0.62^{Ca}	4.063±4.49^{Ib}	1.06±0.08^{Ic}	0.73±0.07^{F,dc}	0.43±0.09^{Hd}
		74	9.12±0.87^{Ha}	1.77±0.28^{Jb}	0.91±0.08^{Ic}	0.69±0.08^{Fc}	0.32±0.07^{Hc}
		84	5.16±0.15^{Ja}	1.03±0.28^{Jb}	0.62±0.07^{Ic}	0.42±0.06^{F,dc}	0.20±0.06^{Hd}
	WS Film	11	23.42±0.12^{Ba}	21.77±0.01^{Bb}	19.25±0.48^{Bc}	17.48±0.49^{Bd}	12.27±0.13^{Be}
		22	21.43±0.25^{Ca}	18.70±0.84^{Cb}	17.41±0.50^{Cc}	16.75±0.52^{Bc}	4.77±0.09^{Dd}
		38	19.52±0.23^{Da}	17.47±00.50^{Db}	16.10±0.31^{Dc}	4.53±0.23^{Dd}	2.87±0.16^{Ee}
		64	13.40±0.17^{Ea}	11.95±2.61^{Fb}	5.62±0.39^{Gc}	2.05±0.13^{Ed}	1.85±0.08^{Fd}
		74	11.78±0.17^{Fa}	9.36±0.24^{Gb}	3.89±0.21^{Hc}	2.20±0.10^{Ed}	1.65±0.06^{Fe}
		84	10.19±0.20^{Ga}	7.26±0.27^{Hb}	3.42±0.35^{Hc}	1.63±0.12^{Ed}	1.49±0.14^{FG,d}
Percent elongation at break (%)	**HPWS Film**	11	0.44±0.19^{Id}	0.76±0.10^{Fd}	2.80±0.24^{FG,c}	4.31±0.33^{Fb}	13.99±0.72^{Ha}
		22	0.56±0.13^{Id}	0.88±0.17^{Fd}	3.80±0.40^{FG,c}	8.17±0.34^{Fb}	36.13±2.42^{Ea}
		38	1.91±0.12^{GH,d}	3.09±0.87^{Fd}	5.99±0.70^{Ec}	30.44±3.16^{Db}	51.58±2.03^{Da}
		64	9.18±0.33^{De}	16.16±0.79^{Cd}	41.40±3.17^{Bc}	60.23±3.91^{Cb}	70.13±4.60^{Ca}
		74	15.89±0.37^{Be}	22.13±2.31^{Bd}	42.06±4.12^{Bc}	72.07±1.35^{Bb}	78.95±3.41^{Ba}
		84	26.47±0.21^{Ae}	35.79±3.80^{Ad}	49.88±3.44^{Ac}	97.49±9.77^{Ab}	1.24E2±6.64^{Aa}
	WS Film	11	0.53±0.07^{Ie}	1.05±0.57^{Fd}	1.89±0.23^{Gc}	5.20±0.05^{Fb}	5.95±0.07^{Ia}
		22	1.81±0.14^{He}	3.17±0.10^{Fd}	3.82±0.14^{FG,c}	8.32±0.16^{Fb}	14.24±0.14H^{Ea}
		38	2.15±0.30^{Ge}	3.53±0.23^{Fd}	4.61±0.06^{FG,c}	15.07±0.44^{Eb}	16.69±0.23^{GH,a}
		64	4.62±0.16^{Fe}	6.75±0.13^{Ed}	10.76±0.09^{Dc}	16.16±0.06^{Eb}	19.18±0.01^{FG,a}
		74	5.66±0.14^{Ee}	7.98±0.08^{Ed}	11.85±0.05^{CD,c}	16.91±0.06^{Eb}	21.43±0.10^{Fa}
		84	11.31±0.15^{Ce}	12.45±0.63^{Dd}	14.16±0.07^{Cc}	19.10±0.60^{Eb}	21.43±0.06^{Fa}

* Values are the average of triplicates ± standard deviation. Means at same line with different small letters are significantly different (p□0.05); means at same column with different capital letters are significantly different (P□0.05).

TABLE 2. Water vapor permeability (WVP) of HPWS and WS films as function of glycerol concentration at 25 °C and 75% RH.*

		Glycerol (%w/w)				
		0	20	30	40	50
HPWS Film	Thickness $\times 10^{-3}$ (m)	0.18±0.02	0.17±0.04	0.16±0.08	0.17±0.06	0.18±0.05
	WVTR $\times 10^{-5}$ ($gh^{-1}m^{-2}$)	4.51±0.12^{e}	6.03±0.47^{d}	8.52±0.41^{c}	12.58±0.59^{b}	14.99±1.23^{a}
	WVP $\times 10^{-12}$ ($gh^{-1}m^{-1}Pa^{-1}$)	4.63±0.04^{e}	5.85±0.05^{d}	7.77±0.23^{c}	12.20±0.13^{b}	15.38±0.51^{a}
WS Film	Thickness $\times 10^{-3}$ (m)	0.17±0.04	0.15±0.03	0.16±0.03	0.17±0.03	0.19±0.05
	WVTR $\times 10^{-5}$ ($gh^{-1}m^{-2}$)	9.98±0.09^{b}	3.34±0.27^{d}	5.99±0.26^{c}	9.23±0.69^{b}	12.49±0.18^{a}
	WVP $\times 10^{-12}$ ($gh^{-1}m^{-1}Pa^{-1}$)	9.68±0.13^{b}	2.86±0.07^{d}	5.47±0.05^{c}	8.95±0.28^{b}	13.53±0.21^{a}

* Values are the average of triplicates ± standard deviation. Means at same line with different capital letters are significantly different (P□0.05).

4. CONCLUSION

The film forming capacity of hydroxypropylated wheat starch was evaluated. HPWS and WS films plasticized with various glycerol content were prepared by a casting method. HPWS was a good matrix-forming material that allowed the manufacture of edible films even without plasticizer with the stability and cohesion needed for their surface, permeability, and mechanical characterization. Such films were easy to handle, flexible, no crack and they were not sticky. However, hydroxypropylation was found to affect physical and mechanical properties of WS films. Water vapor permeability increased with the present of hydroxypropyl groups. Modification brought about a reduction in tensile strength with concurrent increase in elongation at break. It seems that incorporation of a relatively low concentration of glycerol (for example 20% and 30% w/w) in the both film solutions can improve the mechanical and physical properties of films.

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STUDIES ON MOISTURE SORPTION ISOTHERMS OF HYDROXYPROPYLATED-GELATINIZED WHEAT STARCH

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ABSTRACT:

The main aim of this study was to evaluate the effects of hydroxyporpylation on the sorption isotherm of gelatinized wheat starch. Therefore, wheat starch was hydroxypropylated by addition of 30 ml propylene oxide at 40°C for 24 hours. Then, gelatinized starch was produced using 5 g native and modified starch with 100 ml distilled water by casting method. The moisture sorption isotherm of starches were studied at various relative humidities (11, 22, 38, 65, 74 and 84% RH), at 5, 15, 25 and 40 °C. The equilibrium moisture content of the gelatinized starch increased dramatically above a_w=0.4. Guggenheim-Anderson-deBoer (GAB), Brunauer-Emmett-Teller (BET) and Peleg sorption models were fitted to the experimental data. The results showed that hydroxypropylation and reduction of the temperature enhanced the equilibrium moisture content and monolayer water (M_0) of the gelatinized wheat starch. The GAB and Peleg models were found to be the best-fit models for gelatinized native and hydroxypropylated starches, respectively.

Keywords: Sorption isotherm, Hydroxypropylation, Wheat starch, Modeling.

1. INTRODUCTION

Starch as one of the most important polymeric carbohydrates imparts to products in various industries. Even though starch consumption is restricted in some industrial food applications because of its low shear resistance, thermal resistance, disintegration and high affinity towards retrogradation (Cousidine, 1982). These defects can be overcome by starch modification using chemical, physical and enzymatic methods or a combination of them.

Amongst different chemically modified starches, hydroxypropylation plays a significant role in food products. Hydroxypropylation of starch is done by etherification process with propylene oxide as the etherifying reagent. This process causes the introduction of hydroxypropyl groups onto the polymeric chain of starch. Hydroxypropylation of starches impart extended shelf-life, freeze-thaw stability and cold storage stability to starch-based food products.

One of the types of physically modified starch is gelatinized starch, which is sometimes called instant starch. To produce it, the native starch is gelatinized and then come in the form of dry powder. Gelatinized starch is used as thickener in products with the lowest thermal process.

Polymers such as starch are usually found in foods with high hydrophilic capability. As with other foodstuffs, stability of starch and starchy products significantly depends on their compound, the environment relative humidity and temperature (Haque and Roos, 2004). These variables are associated with the water present within the food matrix and will ascertain its physical characteristics, as well as its chemical and microbial stability. In this way, food water sorption characteristics acquired at various temperatures should be investigated, so that a better knowledge of food storage circumstances which assurance for a longer food shelf life can be attained. The association between the moisture uptake in food and the relative humidity of the air with which the food is in equilibrium at a constant temperature can be illustrated by sorption isotherm in food science (Lagoudaki, Demertzis and Kontominas, 1993). Numerous mathematical models exist to explain water sorption isotherms of food materials, which can be divided into several categories; kinetic models based on a mono-layer (Mod-BET model), kinetic models based on a multi-layer and condensed film (GAB model), semi-empirical (Ferro-Fontan, Henderson and Halsey models) and empirical models (Smith, Peleg and Oswin models). The monolayer water content supplies information about the minimal water content giving food stability, which can be achieved from isothermal data calculated from BET and GAB models. The criteria used to select the most proper sorption model are the degree of fitting to experimental data and the physical meaning of the model (Ansari, Farahnaky and Majzoobi, 2011).

The objectives of this study were (a) to determine the sorption isotherms of gelatinized and hydroxypropylated-gelatinized wheat starch at 5, 15, 25 and 40 °C, (b) to assess several sorption models (including kinetic, semi-empirical and empirical models) for their ability to describe the experimental sorption data.

2. MATERIALS AND METHODS

2.1 Materials

Pure wheat starch (WS) was purchased from Fars-Glucosin, Co. Shiraz, Iran. Propylene oxide, sodium hydroxide and sodium sulphate, barium chloride (all analytical grades) were obtained from Merck Schuchardt OHG, Hohenbrunn, Germany.

The wheat starch contained 9.24 ± 0.32% moisture; 0.14 ± 0.01% protein; 0.27 ± 0.10% ash and 0.66 ± 0.01% lipid as determined by the Approved AACC procedures (AACC, 2003). The amylose content of the starch was 26.35 ± 0.21% that was determined after de-fatting of starch with 80% methanol using the iodine method as described by Williams, Kuzina and Hlynka (1970).

2.2 Hydroxypropylation of starch

Etherification of wheat starch with propylene oxide was carried out by the procedure outlined by Kaur, Singh and Singh (2004).

The method described by the Joint FAO/WHO Expert Committee on Food Additives (2001) was used for the determination of the hydroxypropyl groups. The extents of molar substitution of hydroxypropylated wheat starch (HPWS) at 30 ml of propylene oxide were 0.12. The hydroxypropylated wheat starch conform to the FDA guideline for maximum permissible level of hydroxypropyl substitution (hydroxypropyl groups not more than 7.0%; molar substitution (MS) 0.2).

2.3 Preparation of gelatinized starch

Native and modified wheat starches (5 g) were blended at 25 °C for 5 min with 100 ml distilled water. These suspensions were stirred using magnetic stirrer (500 rpm) for 30 min in water bath at 90 °C. Dispersions were afterward cooled at 40 °C and mixed quietly for 20 min to release all air bubbles. Then, about twenty-five grams of the gelatinized suspension were poured into a Petri dish (10 cm diameter) and the samples were further dried at 40 ºC for 24 h and placed in a desiccator with concentrated sulfuric acid at 25 ºC. Dried starch was milled using a laboratory mill (model national, Japan) and sieved to obtain particle size in the range of 70-120μm.

2.4 Modeling of sorption isotherms

Sorption isotherms were determined according to the gravimetric method (Ansari, Farahnaky and Majzoobi, 2011). In this study the recognized BET (Eq. (1)), GAB (Eq. (2)) and Peleg (Eq. (3)) equations were employed to fit the experimental data and to calculate the monolayer moisture values of the samples which is an important parameter in food deteriorations. These models are explained and rearranged as given below:

$$M = \frac{M_0.C.a_w}{(1-a_w)(1+C.a_w-a_w)} \quad (1)$$

$$M = \frac{M_0.C.K'.a_w}{(1-K'.a_w)(1-K'.a_w+C.K'.a_w)} \quad (2)$$

$$M = K_1 a_w^{n1} + K_2 a_w^{n2} \quad (3)$$

Where M is the equilibrium moisture content (% db); M_0 is the monolayer moisture content; a_w is the water activity; C, K', K_1, K_2, n_1 and n_2 are model constants. Fitting of experimental data into the above equations was done using the "Solver" in Excel program (Microsoft Office, 2007).

The suitability of the equations was estimated and compared using the statistical parameter according to Ansari, Farahnaky and Majzoobi (2011): the mean relative percentage deviation modulus (M_e), was determined to present a measure of the proportion of variability characterized to the model.

$$M_e = \frac{100}{n}\sum_{i=1}^{n}\frac{\left|M_{i,\exp}-M_{i,pre}\right|}{M_{i,\exp}} \quad (4)$$

Where $M_{i,exp}$ is the experimental value, $M_{i,pre}$ is the predicted value, n is the population of experimental data.

2.5 Statistical analysis

The experiments were performed in a totally randomized design. Analysis of variance (ANOVA) was carried out and the results were separated using the Multiple Ranges Duncan's test ($p<0.05$) using statistical software of Statistical Package for Social Science (SPSS) 16 (SPSS, Inc., New Jersey, USA).

3. RESULTS AND DISCUSSION

3.1 Sorption isotherm

The equilibrium moisture sorption of the samples at 5, 15, 25 and 40 ˚C is shown in Table 1. The equilibrium moisture content of starches enhance drastically with increasing a_w

(relative humidity) of the ambient environment, at a constant temperature. Furthermore, the sample containing propylene oxide included more moisture content in comparison with the related control at the same temperature. This may be associated with the hydrophilic nature of the hydroxypropyl groups used in this study. It can be obviously seen from Table 1 that the moisture content of the starch powders decreased with reducing temperature from 5 to 40 ºC. At high temperatures, the attractive forces between molecules reduce owing to an increase in kinetic energy of water molecules. Thus, water molecules with slow motion at low temperatures are bound more easily to proper binding sites on surface (Ansari, Farahnaky and Majzoobi, 2011). The equilibrium moisture sorption isotherms of dried starch powders at 5 and 40 ˚C are presented in Figures 1. The sorption isotherms exhibit an increase in equilibrium moisture content with increasing water activity, at a constant temperature. The shape of the isotherm is manifested in the form of a sigmoidal shaped curve, indicating a Type II isotherm according to Brunauer's classification, (Brunauer, Deming, Deming and Troller, 1940), showing a slowly increasing sorption pattern at low water activities (a_w<0.65) as it enhanced noticeably at higher water activities (a_w>0.65).

TABLE 1. Equilibrium moisture content (EMC, g water/ g dry matter), of different starches at 5, 15, 25 and 40°C. GWS is gelatinized wheat starch; G-HPWS is gelatinized-

a_w at 5°C	**0.113**	**0.291**	**0.424**	**0.732**	**0.785**	**0.876**
GWS	0.017± 0.003^{Fb}	0.045± 0.004^{Eb}	0.072± 0.005^{Db}	0.142± 0.005^{Cb}	0.232± 0.005^{Bb}	0.335± 0.007^{Ab}
G-HPWS	0.024± 0.004^{Fa}	0.058± 0.005^{Ea}	0.098± 0.005^{Da}	0.196± 0.007^{Ca}	0.343± 0.007^{Ba}	0.592± 0.006^{Aa}
a_w at 15°C	**0.113**	**0.234**	**0.409**	**0.693**	**0.765**	**0.859**
GWS	0.014± 0.002^{Fb}	0.024± 0.006^{Eb}	0.045± 0.006^{Db}	0.123± 0.006^{Cb}	0.198± 0.011^{Bd}	0.302± 0.008^{Ab}
G-HPWS	0.019± 0.007^{Fa}	0.037± 0.006^{Ea}	0.066± 0.010^{Da}	0.178± 0.009^{Ca}	0.286± 0.011^{Ba}	0.508± 0.008^{Aa}
a_w at 25°C	**0.113**	**0.225**	**0.382**	**0.654**	**0.742**	**0.843**
GWS	0.011± 0.002^{Fb}	0.025± 0.007^{Eb}	0.033± 0.008^{Db}	0.074± 0.006^{Cb}	0.127± 0.006^{Bb}	0.244± 0.006^{Ab}
G-HPWS	0.015± 0.004^{Fa}	0.034± 0.005^{Ea}	0.054± 0.009^{Da}	0.133± 0.006^{Ca}	0.229± 0.006^{Ba}	0.411± 0.006^{Aa}
a_w at 40°C	**0.112**	**0.216**	**0.328**	**0.628**	**0.713**	**0.823**
GWS	0.007± 0.002^{Fb}	0.012± 0.004^{Eb}	0.017± 0.003^{Db}	0.054± 0.009^{Cb}	0.117± 0.006^{Bb}	0.157± 0.009^{Ab}
G-HPWS	0.011± 0.002^{Fa}	0.019± 0.005^{Ea}	0.035± 0.007^{Da}	0.112± 0.004^{Ca}	0.154± 0.006^{Ba}	0.243±0 .005^{Aa}

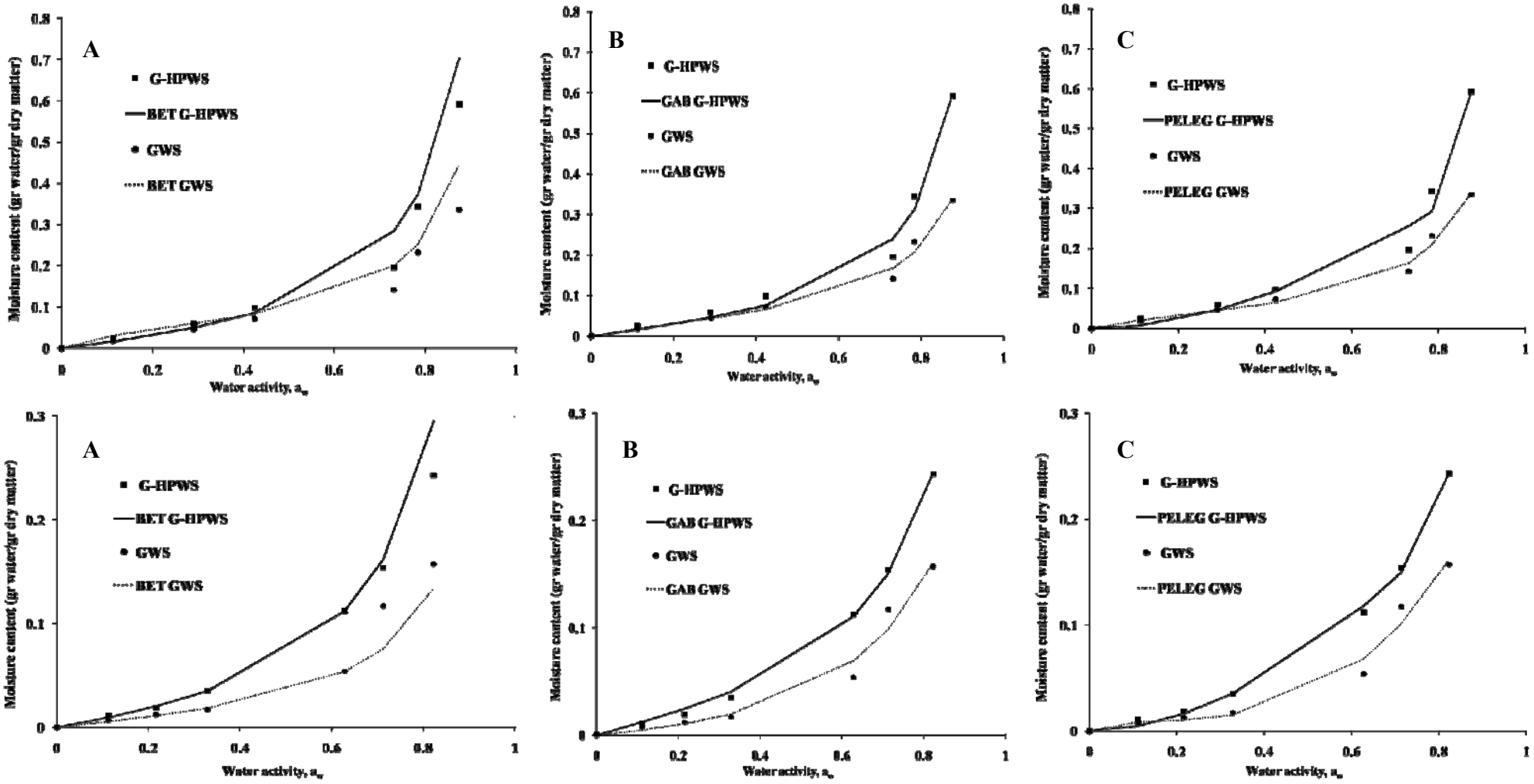

Figure 1. Equilibrium moisture sorption isotherm of different starches at 5 °C (first row) 40 °C (second row). The symbols are experimental data and the lines are from the equations obtained by fitting the experimental data to BET (A), GAB (B) and PELEG (C) equations. GWS is gelatinized wheat starch; G-HPWS is gelatinized-hydroxypropylated wheat starch.

3.2 Modeling of sorption isotherms

The parameters of the sorption isotherm models for dried starch powders are shown in Table 2 (Peleg, GAB and BET). The gelatinized-hydroxypropylated wheat starch showed the higher BET monolayer value (m_0) compared to gelatinized wheat starch over the range from 5 to 40°C. Similar findings were obtained from GAB, where (m_0) was also significantly higher ($p<0.05$) for gelatinized-hydroxypropylated wheat starch compared to its counterpart. This may be due to hydrophilic nature of hydroxypropyl groups that increased the moisture content and the (m_0) values.

Furthermore, the (m_0) values for two materials represent an affinity to reduce with an increase in temperature. This decrease in monolayer moisture content can be described by considering the structural changes in starch polymers at high temperature. The degree of hydrogen bonding in such polymers is reduced with increasing temperature, thus decreasing the accessibility of active sites for water binding and thus, the monolayer moisture content (Westgate, Lee and Ladisch, 1992). In the case of the Peleg equation, increases in K_2 and n_2 are related to the enhancement in the sorption behavior for the gelatinized-hydroxypropylated wheat starch.

Evaluation of the quality of model fittings was carried out through the determination of the mean relative percentage deviation modulus (M_e) and the corresponding values are also shown in Table 2. As shown in Table 2, the average M_e (not shown) for gelatinized wheat starch at different temperatures is much lower in GAB model than Peleg or BET, i.e. this model better fits the experimental data.

The GAB model gives M_e values ranging from 6.93% to 5.74% (with average value of 5.23%) for gelatinized wheat starch, comparing with average M_e values of 6.17% for the BET model, and average M_e values of 5.84% for the Peleg model. However, the Peleg model presents a good fit with the experimental sorption isotherms of gelatinized-hydroxypropylated wheat starch with M_e average values of 2.29%.

TABLE 2. Estimated BET, GAB and Peleg constants and monolayer moisture levels for different starches at different temperatures. GWS is gelatinized wheat starch, G-HPWS is gelatinized-hydroxypropylated wheat starch.

			Temperature °C			
Model constants			**5**	**15**	**25**	**40**
Peleg	**GWS**	k_1	0.130 ± 0.039^{Aa}	0.055 ± 0.003^{Bb}	0.063 ± 0.002^{Bb}	0.011 ± 0.004^{Cb}
		k_2	0.556 ± 0.058^{Bb}	0.543 ± 0.049^{Bb}	0.657 ± 0.053^{Ab}	0.301 ± 0.012^{Cb}
		n_1	0.844 ± 0.028^{Aa}	0.507 ± 0.071^{Cb}	0.700 ± 0.064^{Bb}	0.096 ± 0.004^{Db}
		n_2	6.909 ± 0.977^{Ab}	5.022 ± 0.835^{Bb}	7.314 ± 0.459^{Aa}	3.541 ± 0.553^{Cb}
		M_e (%)	8.57	5.21	5.88	3.70
	G-HPWS	k_1	0.159 ± 0.053^{Ba}	0.129 ± 0.036^{Ba}	0.110 ± 0.011^{Ba}	0.223 ± 0.017^{Aa}
		k_2	1.354 ± 0.395^{Aa}	1.128 ± 0.265^{Aa}	0.957 ± 0.053^{Aa}	0.435 ± 0.028^{Ba}
		n_1	0.772 ± 0.064^{Ba}	0.848 ± 0.099^{Ba}	0.825 ± 0.072^{Ba}	1.614 ± 0.119^{Aa}
		n_2	8.281 ± 0.358^{Aa}	6.889 ± 0.691^{Ba}	6.479 ± 0.572^{Bb}	8.658 ± 0.518^{Aa}
		M_e (%)	3.93	2.78	1.37	1.06
GAB	**GWS**	m_0 (%)	9.516 ± 0.478^{Ab}	8.753 ± 0.332^{Bb}	6.727 ± 0.065^{Cb}	6.292 ± 0.205^{Cb}
		C	6.556 ± 0.310^{Aa}	5.905 ± 0.517^{Aa}	5.032 ± 0.018^{Ba}	4.469 ± 0.064^{Ba}
		K	0.957 ± 0.006^{Ab}	0.931 ± 0.008^{Bb}	0.860 ± 0.012^{Cb}	0.744 ± 0.032^{Da}
		M_e (%)	6.93	2.12	6.12	5.74
	G-HPWS	m_0 (%)	11.921 ± 0.581^{Aa}	11.249 ± 0.784^{Aa}	10.173 ± 0.299^{Ba}	8.499 ± 0.470^{Ca}
		C	6.122 ± 0.010^{Ab}	$5.794\pm0.578^{BA,a}$	$5.256\pm0.054^{BC,a}$	4.794 ± 0.069^{Ca}
		K	0.986 ± 0.015^{Aa}	$0.959\pm0.037^{BA,a}$	$0.917\pm0.010^{B,ba}$	0.625 ± 0.016^{Cb}
		M_e (%)	4.35	2.14	6.39	3.39
BET	**GWS**	m_0 (%)	9.541 ± 0.608^{Ab}	8.493 ± 0.209^{Bb}	6.814 ± 0.099^{Cb}	6.658 ± 0.124^{Cb}
		C	6.745 ± 0.120^{Ab}	$6.289\pm0.339^{BA,b}$	$5.880\pm0.596^{CB,b}$	5.302 ± 0.066^{Ca}
		M_e (%)	0.24	9.71	6.79	7.81
	G-HPWS	m_0 (%)	12.234 ± 0.895^{Aa}	10.549 ± 0.798^{Ba}	9.800 ± 0.477^{Ba}	8.000 ± 0.527^{Ca}
		C	$7.414\pm0.449^{A,ba}$	7.190 ± 0.240^{Aa}	6.085 ± 0.015^{Ba}	5.372 ± 0.631^{Ba}
		M_e (%)	3.34	2.04	5.27	4.61

* Values are the average of triplicates ± standard deviation. Means at same row with different capital letters are significantly different (p□0.05); means at same column with different small letters are significantly different for each model constant (p□0.05).

4. CONCLUSION

The moisture sorption isotherms of gelatinized wheat starch and gelatinized-hydroxypropylated wheat starch have been determined at 5, 15, 25 and 40 ◦C by the static gravimetric method. A sigmoidal shape, characteristic of Type II isotherms, was found for different starches in the range of temperature (5-40 °C). The equilibrium moisture content increased with decreasing temperature at a constant water activity. The experimental data of sorption isotherms were successfully fitted using GAB and Peleg models in the range of investigated temperatures and water activities, according to the values of M_e. The monolayer water content, whether determined by BET or GAB models, was found to be inversely related to temperature. These results provide a sound basis for future application of modified wheat starch in different industries.

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EFFECT OF TEMPERATURE AND SHEAR RATE ON RHEOLOGICAL CHARACTERISTICS OF CUTTING OIL EMULSION

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Abstract

The effect of temperature and shear rate on rheological behavior of cutting oil emulsion was investigated in this work. The model of Herschel-Bulkley was used to fit the shear stress dependence of the shear rate for temperatures between 20°C and 100°C. The temperature increase induced not only a decrease in the yield stress and the consistency index of cutting oil emulsion but also an increase of the flow index of cutting oil emulsion. The temperature dependence of limit viscosity at high shear rate of the cutting oil emulsion was fitted by an Arrhenius equation. For constant shear rate applied on the cutting oil emulsion at 20°C a thixotropic behavior was observed and analyzed using a modified model of Herschel-Bulkley in which a structural parameter λ was included in order to account for time dependent effect.

***Key words*:** Yield stress; structural parameter; thixotropy, temperature; Viscosity.

1.Introduction

During mechanical operations of cutting and machining metals, a considerable quantity of heat is generated, mainly due the high plastic deformation in the primary shear zone, and to the frication of the chip on the tool rake face (Bensadok et al, 2008). Cutting oil emulsions are use to reduce the detrimental effects of heat and friction on both tool and work -piece. The cutting oil emulsions produces three positive effects in the process heat elimination, on the chip tool interface and chip removal (Pottirayil et al, 2011). The cutting oil emulsions are as well used for lubrication of drilling tools. The cutting process is generally lubricated by oil in

water emulsions (Liu et al, 2007). In general the cutting oil emulsions behave as non Newtonian fluids characterized by a generalized behavior at low oil concentration, a plastic behavior at medium oil concentration and a viscoelastic behavior at high oil concentration (Benali, 1993).

Cutting oil emulsions are defined as materials that exhibit a colloidal and thixotropic behaviour (Stachowiak and Batchelor, 2001).The thixotropy is generally defined as a progressive decrease in viscosity over time under constant applied shear stress, followed by gradual recovery when this stress is removed (Hammadi et al, 2012). Mujumdar, 2002 describe the thixotropy as reversible breakdown of the particulate structure under shear, the structure formed at rest is often (but not necessarily) associated with a flow yield. In this case, the variable viscosity is explicitly associated with a variable microstructure, characterized by a structure parameter the kinetics of which governs the time-dependent behavior (Dullaert and Mewis, 2005). In this work the influences of temperature and shear rate on the cutting oil emulsions rheological properties were evaluated, and a modified model of Herschel-Bulkley was employed to describe the thixotropic behavior of cutting oil emulsions.

2. Materials and methods

2.1. Sample preparation

Cutting oil emulsion is a mixture of drinking water and cutting oil. This cutting oil is made of mineral oil to which is added around sulfonate surfactant, and which is called TASFLOUT 22-B; it is usually available. Homogenization was obtained by continuous mechanical agitation for 2 h at ambient temperature

2.2. Experimental set up

The rheological measurements were performed by using a torque controlled rheometer (RS600 from Thermo-Fischer), equipped with a cone- and-plate geometry (diameter: 60 mm; angle: 2 degree; gap: 105 μm). In order to prevent changes in composition during measurements due to water evaporation, a solvent trap was placed around the measuring device.

2.3.1. Effect of temperature in rheological behavior of cutting emulsions

In order to avoid any memory effect, the sample was pre-sheared at 200 s^{-1} for 60 s after loading in the measuring device. The sample was then kept at rest for 600 s prior to measurements in order to allow the material to recover, at least partially, its initial structure.

The imposed shear rate range depends on the volume concentration of cutting oil emulsions. Consequently, for the following experiments, a continuous ramp of shear rate ranging from 0.001 to 200 s^{-1}, has been applied on each sample during 600 s. The experiments were performed at different temperature between 20°C and 100°C. A new fresh sample was used for each temperature in order to avoid any irreversible evolution of the cutting oil emulsion. In order to investigate the reproducibility of results, two replicates were made for most of the experiments.

2.2. Apparent viscosity evolution under constant shear rate

After a rest time of 600 s, the samples were sheared during 120 s at different constant shear rates (50 s^{-1}, 100 s^{-1}, 150 s^{-1}, 200 s^{-1} and 250 s^{-1}) at a constant temperature of 20°C. A new fresh sample was used for each applied shear rate in order to avoid any irreversible evolution of the cutting oil emulsion.

3. Results and discussion

3.1. Effect of the temperature on rheological behavior of cutting oil emulsion

3.1.1. Typical flow curve of the cutting oil emulsion

The variation of the shear stress τ as a function of the shear rate γ' at different temperatures (20°C to 100°C) for the 60 % Vol of cutting oil emulsion (Fig. 1) clearly shows a Non-Newtonian behavior after a yield stress. Therefore experimental data were fitted to the classical model of Herschel-Bulkley:

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

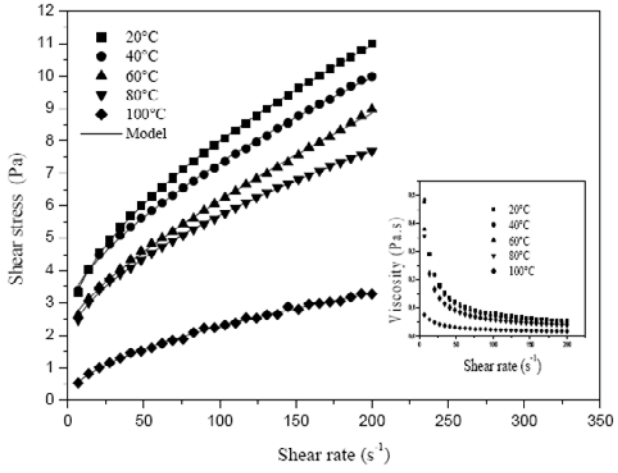

Fig.1. Shear stress as a function of shear rate at different temperatures (Voulme

concentration of cutting oil emulsion 60 % Vol). The solid lines correspond to the curve fitting to Eq.1. The shear rate dependence of the viscosity is presented in the inset.

The fitted parameters of the model of Herschel-Bukley as a function of temperature are presented in Figs 2 and 3. It appears that yield stress and consistency index decreases with increasing temperature but the flow index, increases with temperature increase.

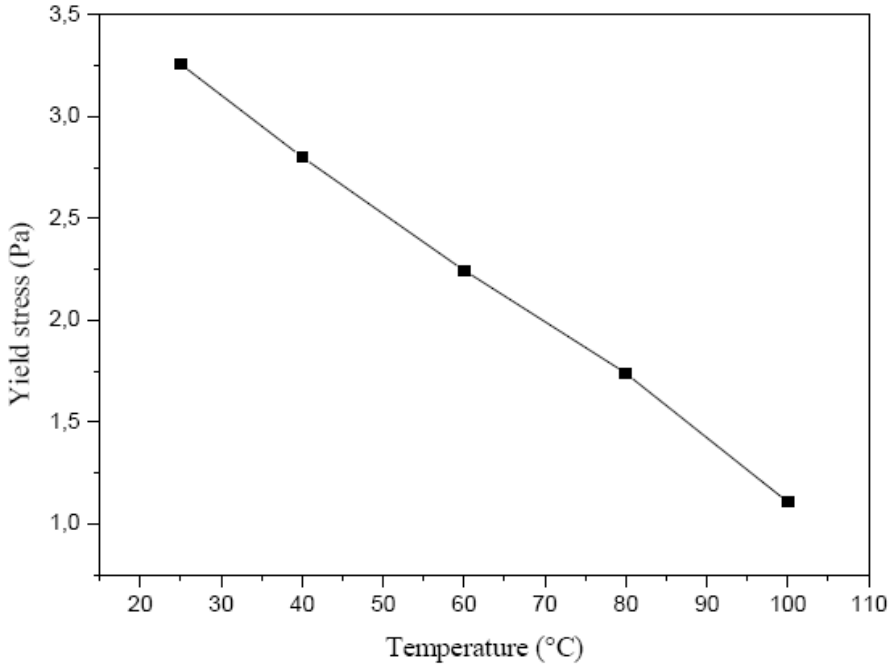

Fig.2. Variation of yield stress as a function of temperature
(60% Vol of cutting oil emulsion)

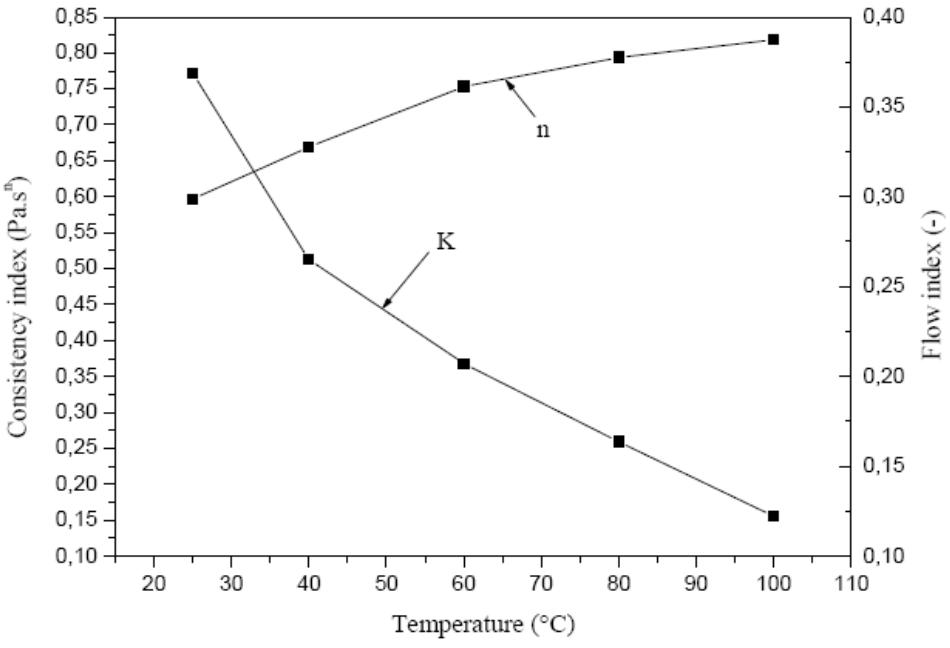

Fig.3. Variation of consistency index and flow index as a function of temperature (60% Vol of cutting oil emulsion)

3.1.2. Effect of temperature on limit viscosity of cutting oil emulsion

It is also clearly seen on the insets of Figs. 1 that the apparent viscosity steeply decreases and evolves toward a constant value at high shear rate, which was called as the limit viscosity n_{Lim}. Fig. 4 shows that the limit viscosity of the cutting oil emulsion decreased from 0.069 to 0.036 Pa.s for the volume concentration of 60 % Vol as the temperature was increased from 293 to 373 °K. A likely explanation is a weakening of the network strength between particles of the dispersions due to thermal motion. This temperature dependence of the limiting viscosity can be described by an Arrhenius type equation involving the absolute temperature (T), universal gas constant (R), energy of activation for viscosity (Ea) and a constant A:

$$\eta_{Lim} = A\, exp(\frac{E_a}{RT}) \qquad (2)$$

Witch A=0.0041 and E_a=0.72 J/mol

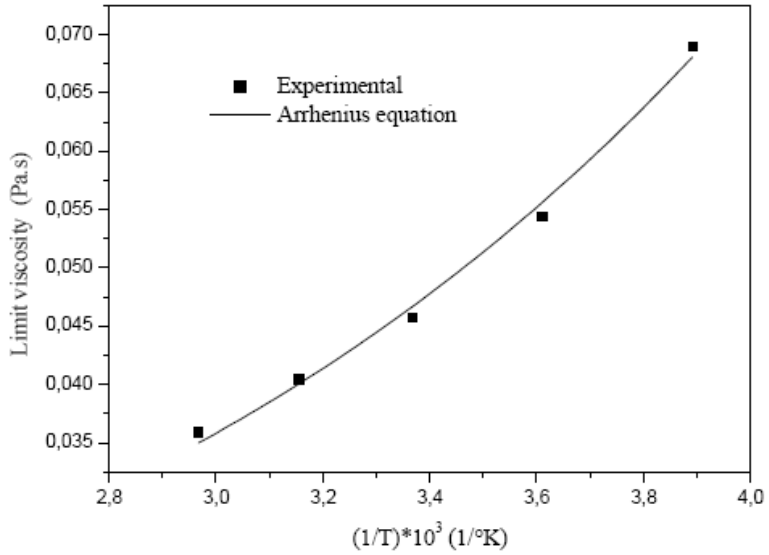

Fig.4. Limit viscosity as a function of 1/T at volume concentration 60 % of cutting oil emulsion. The solid lines correspond to the curve fitting to Eq.2.

3.2. Apparent viscosity evolution under constant shear rate and determination the Structural parameter

Fig.5 shows the apparent viscosity evolution as a function of time for different for different shear rates varying from 50 to 250 s^{-1}. For all values of shear rates, the viscosity decreases significantly with time and tends to constant values.

In order to analyse the structural evaluation of the emulsion, we applied the phenomenological model of Tiu and Boger (1974) based on an approach initially suggested by Moore (1959) and refined by Cheng and Evans. This choice was motivated by the simplicity (minimum number of parameters) and efficacy of this model. Cheng and Evans argued that the stress-strain equation depends on the fluid model and must be completed with a kinetic equation of structural parameter λ changing with time. This parameter describes the current state of agglomeration. As pointed out before, the flow curves of cutting oil emulsion were found to be well fitted by the model of Herschel-Bulkley, with satisfactory agreement (see Fig. 1). Following the model proposed by Tiu and Boger (1974), the Herschel–Bulkley model was then modified to take into account the structural changes by introducing a structural decay parameter λ. Thus, the equation of state is assumed to be:

$$\tau(t) = \lambda(\tau_{y0} + + K_1 \dot{\gamma}^{n_1}) \tag{3}$$

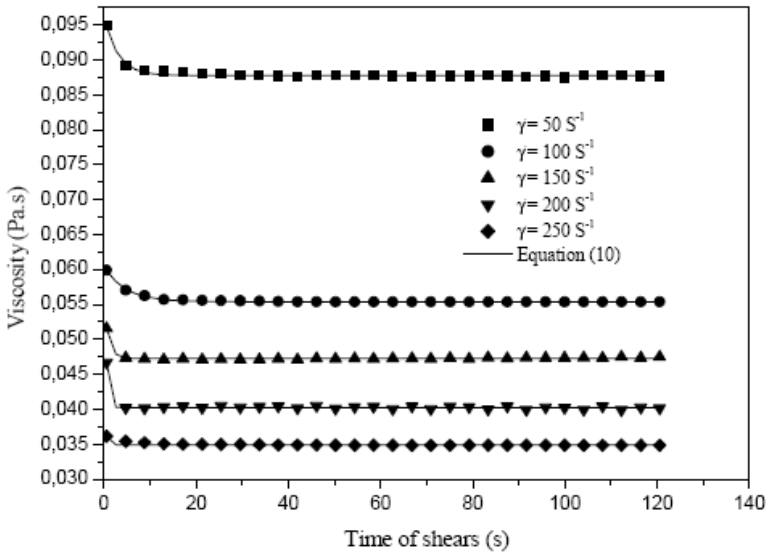

Fig.5. Viscosity of cutting oil emulsion as a function of time shears at 20°C for constant shear. The solid line corresponds to curve fitting to eq.10.

The structural parameter ranges from an initial value of unity at zero shear time (agglomerated structure at rest). For their rate equation Tiu and Boger, 1974 employed a second-order kinetic equation:

$$\frac{d\lambda}{dt} = -k_2(\lambda - \lambda_e)^2 \text{ for } \lambda > \lambda_e \tag{4}$$

where k_2 is rate constant that is a function of shear rate to be determined experimentally and can be considered as a measure of the rate of structure breakdown, i.e., the degree of thixoropy (Butler,1995). Then entire time-dependent behavior of our cutting oil emulsion might be completely described by Eqn. (3) and (4) with the parameters t_{y0} , K1 , n1 and k2

evaluated from experimental data. The rheological constants t_{y0} , K1 , n1 are determined with initial shearing conditions (λ=1 and t=0), and Eq.(4) reduces to the classical Herschel-Bulkley equation. In other words, they are determined from the initial shear stress in the material, observe the beginning of a test, for each shear rate considered. Fig.6 shows the zero shear stress t_{y0} at a function of shear rate for volume concentration of cutting oil emulsion equal 60%Vol. The curve were fitted to the model of Herschel-Bulkley and the apparent yield stress value obtained by extrapolation to γ = 0. The resulting equation was:

$$\tau_{Zero} = 0.547 + 0.6\dot{\gamma}^{(0.49)} \text{ , } R^2 = 0.994 \quad (5)$$

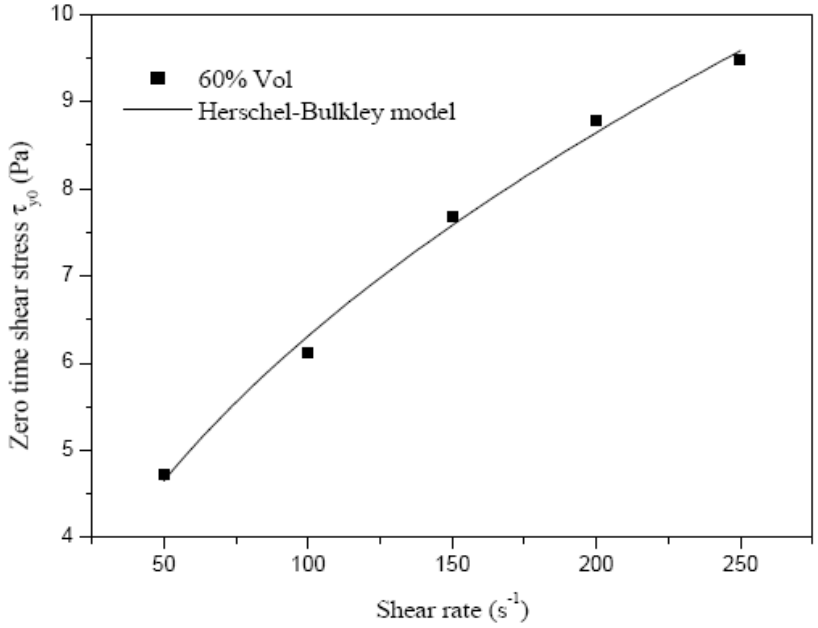

Fig.6. Zero time shear stress as a function of shear rate for 60% Vol cutting oil emulsion and comparison with the Hershel bulkley model (temperature 20°C).

The structural parameters λ and λe are expressed in terms of the apparent viscosity n = τ/γ to find k_2. Equating the rheological model Eq. (3) to the definition of the apparent viscosity which, in this case, is a function of both shear and time, it is found:

$$\lambda = \frac{\eta\dot{\gamma}}{\tau_{y0}+K_1\dot{\gamma}^{n_1}} \quad (6)$$

Equation (6) is valid for all values of λ including λ_e at η_e, the equilibrium of the apparent viscosity. Differentiating Eq. (6), with respect to time at a constant shear rate and combining the resulting equation with Eq. (4), yields:

$$\frac{d\eta}{dt'} = -a_1(\eta - \eta_e)^2 \tag{7}$$

where

$$a_1 = \frac{k_2\dot{\gamma}}{\tau_{y0}+K_1\dot{\gamma}^{n_1}} \tag{8}$$

Integrating Eq.(7) at constant shear rate from $\eta = \eta_0$ at $t' = 0$ at $t' = t'$ yields:

$$\frac{1}{\eta-\eta_e} = \frac{1}{\eta_0-\eta_e} + a_1 t' \tag{9}$$

The equilibrium viscosity n_e was found by fitting the time dependence of the viscosity (Fig. 5) by an exponential decay curve (Roussel, 2006; Coussot,2002) of the form:

$$\eta = \eta_e + (\eta_0 - \eta_e)\exp(-\beta t) \tag{10}$$

where β is the rate constant in (s^{-1})
Fig.7 shows the variation of initial viscosity η_o and equilibrium viscosity η_e as a function of shear rate.

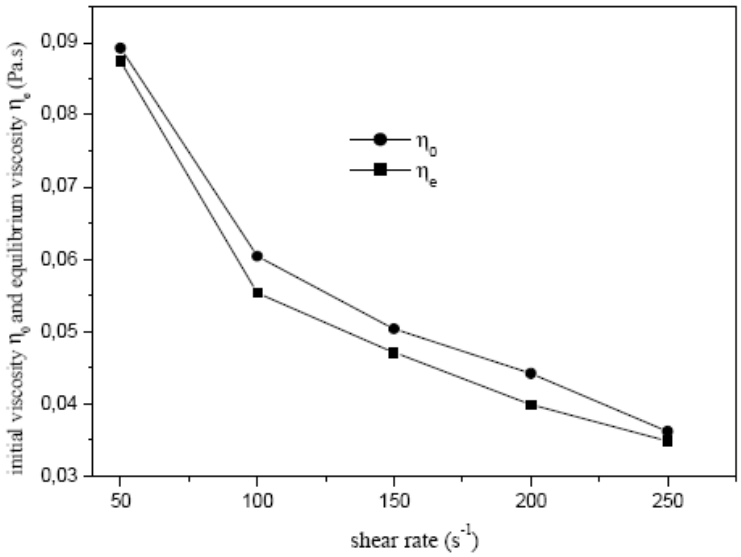

Fig.7. Initial viscosity and equilibrium viscosity determined by equation (10) as a function of shear rate (60% Vol of cutting oil emulsion).

Using Eq.(9), a plot of $\frac{1}{\eta-\eta_e}$ versus t', at constant shear rate, should yield a straight line with slope equal to a_1. The same procedure is repeated at different shear rate. Fig.8 shows the data for 60 % Vol cutting oil emulsion.

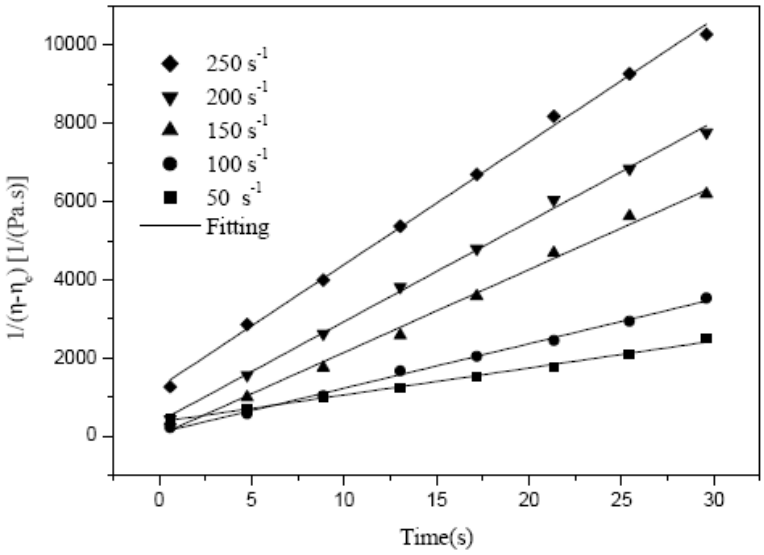

Fig.8. Linear relationship between $\frac{1}{\eta-\eta_e}$ and time different shear rates (60% Vol cutting oil emulsion) at temperature 20 °C. The solid line corresponds to a fitting of data by linear regression.

Fig.9 shows the evolution of the parameters a1 and k2 as a function of shear rate for 60% Vol cutting oil emulsion. It was found that both a1 and k2 can be described by a power function of shear rate (Hammadi et al, 2012):

$$a_1 = \alpha_1 \dot{\gamma}^{\beta_1} \tag{11}$$

$$k_2 = \alpha_2 \dot{\gamma}^{\beta_2} \tag{12}$$

With

$$\alpha_1 = 1.567 \pm 0.334;\ \beta_1 = 0.961 \pm 0.0390,\ R^2 = 0.996$$

$$\alpha_2 = 0.965 \pm 0.190;\ \beta_2 = 0.460 \pm 0.039,\ R^2 = 0.982$$

The value of the structural parameter λ_e was determined at each shear rate by using Eq. (13).

$$\lambda_e = \frac{\eta_e}{\eta_0} \tag{13}$$

Where η_0 are the initial viscosity and η_e the equilibrium viscosity demined by equation (10).

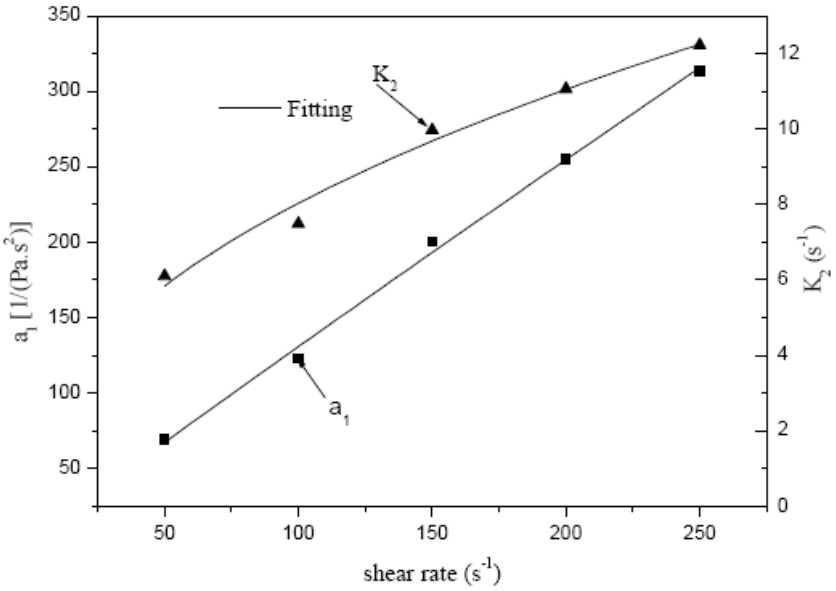

Fig.9. Parameters a_1 and k_2 of Tiu and Boger model as a function of shear rate for 60 % Vol cutting oil emulsion.

Fig.10 shows the evolution of equilibrium structural parameter as a function of shear rate. A model of Herschel-Bulkley was found to describe relationship between equilibrium structural parameter and shear rate:

$$\lambda_e = 0.934 + 0.0142\dot{\gamma}^{(0.256)}\ , R^2 = 0.999 \qquad (14)$$

As the rate equation (Eq.(4)), cannot be integrated analytically for varying shear rate conditions, further progress is still necessary to predict shear stress at given constant shear rate. In order to derive an equation that would predict the structural parameter,λ for a constant shear rate after a given time, Eq. (6) was rearranged to yield

$$\lambda = \lambda_e + \frac{1}{\frac{1}{(1-\lambda_e)} + K_2 t} \qquad (15)$$

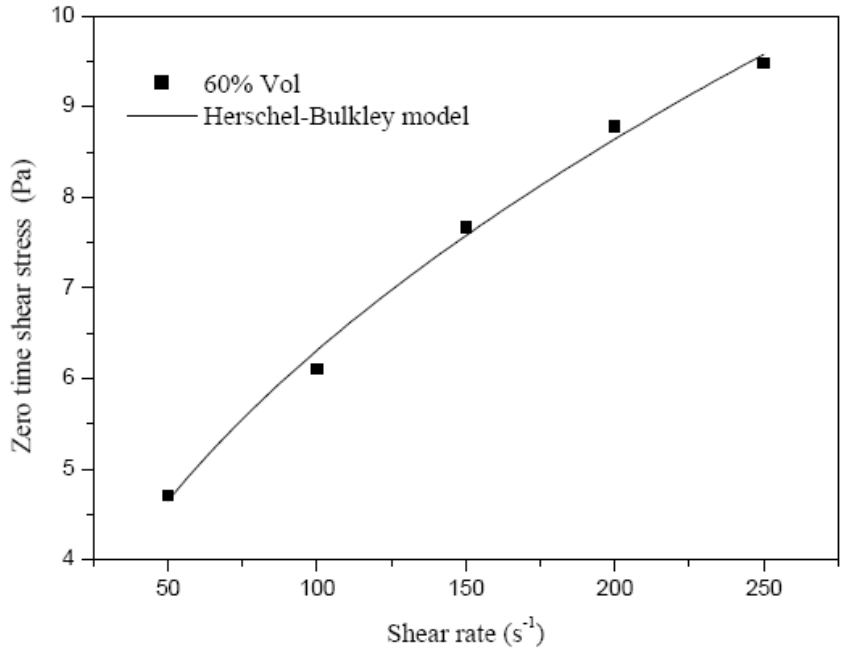

Fig.10. Equilibrium structural parameter versus shear rate at 20°C together with Herschel- bulkley model (60 % Vol of cutting oil emulsion)

Fig.11 shows the evolution of structural parameter as a function of time for each values of shear rate. As shown, structural parameter changes are most significant during the initial shear period, after which they maintain a nearly constant value.

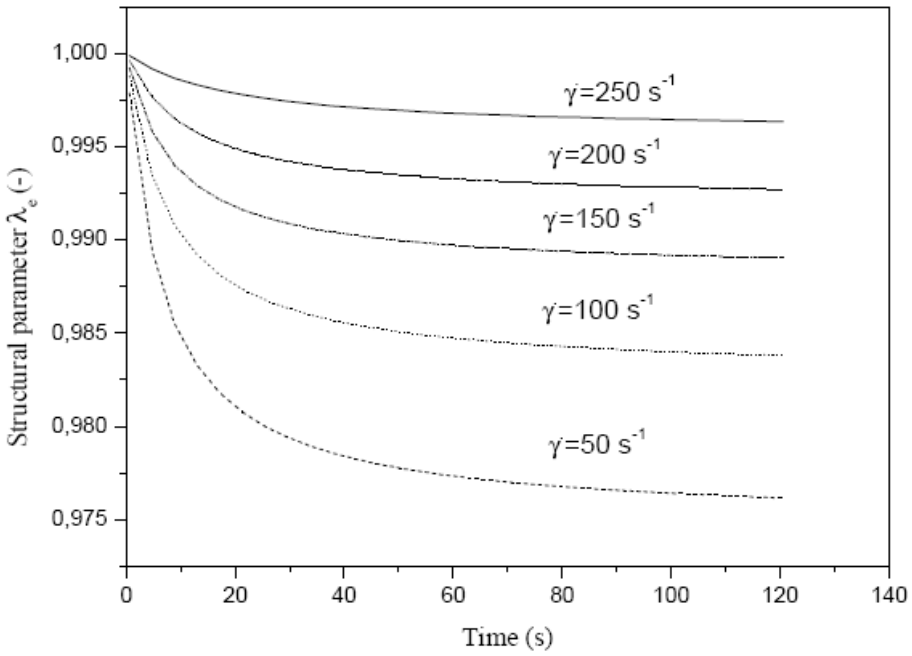

Fig.10. Structural parameter as a function of time at 20° (60 % Vol of cutting oil emulsion)

4. Conclusion

In this study the effect of temperature on the rheological behavior of cutting oil emulsion and the effect of shear rate on structure buildup and breakdown of cutting oil emulsion have been performed.

The model of Herschel-Bulkley, whose parameters are temperature dependent, was found to be well correlated with the behavior of cutting oil emulsion. The yield stress and the consistency index decreased with temperature while the flow index decreased with increase temperature. The limit viscosity of the cutting oil emulsion decreased with increasing temperature, and their relationship could be modeled by the Arrhenius equation.

The time dependant viscosity decreased rapidly with shearing time and reached an equilibrium stage. In order to take into account the structural evolution of the cutting oil emulsion, the model of Herschel-Bulkley was modified by the introduction of a structural parameter which allows a good description of the thixotropic behavior of the cutting oil emulsion.

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UV-LED TECHNOLOGY FOR NON THERMAL TREATMENT OF LIQUID FOODS

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ABSTRACT:

UV-C irradiation performs effective sterilization excluding the use of heat to inactivate microorganisms. It has been used to disinfect water, air, surfaces and foods such as fruit juices, liquid egg, milk and fresh produce. Generally of UV disinfection systems operate with low- or medium-pressure mercury lamps which are large in size and fragile, require high amounts of energy to operate and have a short lifespan of approximately 4000- 10000 h. Moreover they contain mercury which is an important environmental contaminant. Therefore, a safer, less expensive and more durable UV disinfection devices are required. The new UV-LED technology has been launched as an alternative to traditional UV irradiation systems. Advantageous UV-LEDs are known to be shock resistant, more efficiently use electricity, and have a lifetime exceeding 100000 h. Additionally, having a very compact design make them available for the sterilization of small or narrow spaces. Most importantly, UV-LEDs do not contain toxic substances (mercury) or pollutants. Hence, these safer devices are now being investigated for water disinfection. Recent results indicated the same inactivation efficiency of UV-C LEDs with low-pressure mercury lamps. This work compares the available UV-C irradiation with the new UV-C LED technology and assess the applicability of this new technology in food industry.

Keywords: low-pressure mercury lamps, UV-LED technology, UV irradiation, disinfection

1. INTRODUCTION

Thermal pasteurization is the best known technique in order to remove pathogens, reduce the number of spoilage microorganisms and inactivate the enzymes which decrase the quality of the products. However, use of high temperatures may cause some quality problems such as

colour, taste and flavour defects (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso and Ortega-Rivas, 2007; Choi and Nielsen, 2004).

Increased trend towards fresh-like products forced the researchers to investigate alternative processing techniques (Tahiri, Makhlouf, Paquin and Fliss, 2006; Basaran-Akgul, Churey, Basaran and Worobo, 2009). One of the alternative method to thermal pasteurization is UV-C radiation. Inactivation mechanism of UV-C irradiation is based on the absorption of UV photons by the genetic material and the formation of dimers which inhibit the transcription and replication of the cell (Oguma, Katayama and Ohgaki, 2002; Bolton, Linden and Asce, 2003; Koutchma, 2009). Antimicrobial effect of UV-C light is very well known and this technique is used for disinfection of fruits surfaces, hospital equipments, water resources etc. (Begum, Hocking-Ailsa and Miskelly, 2009; Pan, Vicente, Martinez, Chaves and Civello 2004; Bintsis, Tzanetaki and Robinson, 2000; Nigro, Ippolito and Lima, 1998).

Generally low or medium pressure mercury vapor lamps at 254 nm wavelength are used for UV-C irradiation. However these lamps contain mercury which is known to be toxic for both environment and human body. Additionally UV reactors need to be designed according to the shape of the lamps. These lamps are mostly large in size and take up too much space. Moreover, UV lamps are not resistant to shock and have a low life span (approximately 4,000-10,000 hours). Therefore, it is necessary to design new sterilization equipments in various sizes which do not contain toxic substances and have low energy consumption rate. Use of UV-LEDs for sterilization and disinfection purposes is a new approach. They have a very compact, shock-resistant and robust design. They also can be used for sterlization of narrow spaces and allow saving space due to their small sizes. UV-LEDs do not need a warm-up time in contrast to traditional UV-C lamps. Hence they consume less energy. It was also reported that UV-LEDs have relatively longer life time exceeding 100,000 hours. Most importantly, they do not contain any toxic substances which are harmful for human health and the environment. They are capable of emitting UV light at multiple individual wavelengths. Besides, it is possible to use the combination of different UV-LEDs emitting light at different wavelengths. In literature it was indicated that UV-LEDs emitting light at 275 nm resulted in much higher microbial inactivation (Bowker, Sain, Shatalov and Ducoste, 2011). This is due to the fact that protein absoprtion spectrum reaches the maximum near 280 nm and thus enzymes become more sensitive to inactivation at these wavelengths. Furthermore it is known that UV light at UV-A wavelength (320-400 nm) inhibits the protein synthesis by damaging cell membranes and has better penetration property (Chevremont, Farnet, Coulomb and Boudenne, 2012a). Considering all these circumstances it is more effective to use UV-LED

systems which allow the use of LEDs at different wavelengths so as to enhance the enzyme inactivation.

2. UV-C IRRADIATION

2.1 Theoretical background

Ultraviolet light is a part of electromagnetic spectrum with ranges between 100-400 nm (Miller, Jeffrey, Mitchell and Elasri, 1999; Bintsis, Tzanetaki and Robinson, 2000; Begum, Hocking-Ailsa and Miskelly, 2009).

The spectrum can be divided into four groups based on wavelengths. The subgroups are;

- UV-A (320 to 400 nm)
- UV-B (280 to 315 nm)
- UV-C (200 to 280 nm)
- UV-V (100 to 200 nm)

The highest germicidal effect of UV light was reported to be observed between 250 and 270 nm (Tran and Farid, 2004; Guerrero-Beltran and Canovas, 2005). Koutchma (2009) and Oteiza, Giannuzzi and Zaritzky (2010) declared that the most efficient inactivation can be obtained at 253.7 nm due to the maximum absorption of UV photons by the genetic material at this specific wavelength.

Low or medium pressure mercury vapor lamps were reported to be used for many years in UV light treatment (Koutchma, 2009).

The mechanism of UV-C irradiation is based on the absorption of UV photons by DNA or RNA pyrimidine bases. Incident light causes the formation of dimers on the same DNA strand which inhibit the transcription and replication of the cell.

2.2 Applications

UV-C light treatment was reported to be effective on the inactivation of bacteria, protozoa, algea and viruses. This process has been used to disinfect water effluent, potable water and water for swimming pools for many years (Begum, Hocking-Ailsa and Miskelly, 2009). It is also used for surface disinfection of foods, decontamination in hospitals, pharmaceutical industry and public buildings (Begum, Hocking-Ailsa and Miskelly, 2009; Pan, Vicente, Martinez, Chaves and Civello, 2004; Nigro, Ippolito and Lima, 1998).

US Food and Drug Administration (US FDA) has approved the use of UV-C light as a germicidal agent for the disinfection of fruit juices (US FDA 2000). There are many

applications of UV-C irradiation on different fruit juices (Torkamani and Niakousari, 2011; Gabriel, 2012; Azhuvalappil, Fan, Geveke and Zhang, 2010; Koutchma, Keller, Chirtel and Parisi, 2004; Choudhary and Bandla, 2012).

2.3 Advantages and disadvantages

UV-C light processing is a physical treatment which does not leave chemical residues (EPA, 1999; Canitez, 2002). Also it is effective against most type of microorganisms (Bintsis, Tzanetaki and Robinson, 2000).

However, lack of penetration in highly absorptive media, reduced effectiveness in existence of organic matters and in presence of suspended particles are the disadvantages of the system. Moreover, it was reported that UV-C light treatment has no effect on the enzymes which influence the fruit juice quality, such as pectinmethylesterase (PME), polyphenoloxidase (PPO) and peroxidase (POD) (Tran and Farid 2004; Noci, Riener, Walkling-Ribeiro, Cronin, Morgan and Lyng, 2008).

3. UV-LED TECHNOLOGY

3.1 Theoretical background

Use of UV light emitting diodes (UV-LEDs) is a relatively new method to generate UV light. UV-LEDs are created by connecting p-type and n-type semiconductors that move electrons into positively charged holes between these two materials. Light is generated when the electrons and holes recombine at a junction. The wavelength of light depends on the type of material that is used for those semiconductors (i.e. indium gallium nitride for light in the visible range, and aluminium gallium nitride and aluminium nitride for UV range) (Bowker, Sain, Shatalov and Ducoste, 2011).

3.2 Applications

There are many applications of GaN-based blue, green, and white light emitting diodes (LEDs) like backlighting of mobile phones, laptops and TVs, street lighting, and car headlight (Techneau, 2010). UV-LEDs are also used for;

- Water disinfection (UV-C, UV-A)
- Sensing (UV-C, UV-A)
- UV curing (UV-B, UV-A)
- Medical treatment (e.g. treatment of psoriasis, UV-B)

- Tanning (UV-B)
- Lithography (UV-A)
- Non-line-of-sight communication (UV-C)
- Sterilization (e.g. medical, UV-C)

In literature there are limited numbers of studies related to the use of UV-LEDs for water disinfection purpose. Chatterley and Linden (2010) reported that most of those data were available for LEDs emitting light at UV-A range. However, UV-C LEDs were also indicated to be preferred for this purpose (Li, Hirota, Yumoto, Matsuo, Miyake and Ichikawa, 2010; Chevremont, Farnet, Coulomb and Boudenne, 2012a; Chevremont, Farnet, Sergent, Coulomb and Boudenne, 2012b; Bowker, Sain, Shatalov and Ducoste, 2011; Würtele et al, 2011; Hamamoto et al, 2007). Moreover combination of UV-A and UV-C LEDs was used in some studies (Aoyagi et al, 2011; Chevremont, Farnet, Coulomb and Boudenne, 2012a).

UV-A radiation mechanism is based on the inactivation of microorganisms by damaging proteins and producing hydroxil and oxygen radicals which destroy cell membrane and other cellular components. On the other hand UV-C radiation causes the formation of cyclobutane thymine dimers by directly affecting the microbial DNA and inactivates microorganisms without any intermediate steps (Chatterley and Linden, 2010). Although DNA damage caused by UV-C radiation can be repaired by the enzyme photolyase, there is no possibility to repair the damage to bacterial membranes by UV-A radiation.

Chevremont, Farnet, Coulomb and Boudenne (2012a) showed that coupling UV-A and UV-C could be paired by using the germicidal effect of UV-C and greater penetrating ability of UV-A. They also found that use of coupled wavelenghts 280/365 nm and 280/405 nm caused total dissappearance of fecal enterococci, total coliforms and fecal coliforms in the effluent (Chevremont, Farnet, Coulomb and Boudenne, 2012a). Besides lack of possibility to repair the damage in the bacterial membranes occured after UV-A exposure increased the efficiency of microbial inactivation (Chevremont, Farnet, Coulomb and Boudenne, 2012a).

3.3 Comparison of UV-LEDs and traditional UV lamps

Table 1 shows the main differences between UV-LEDs and traditional UV lamps. According to that the mercury-vapor lamps contain mercury, which is toxic to the environment as well as to the human body. On the other hand a UV-LED is an environment-friendly sterilizer. It does not contain any toxic substances so it does not have harmful effects (Mori et al, 2007). Additionally, traditional UV lamps are fragile, non-durable to shock, less energy efficient and large in size. Sterilizers using UV lamps must be designed according to the shape of the lamps

which take up a lot of space. It was also speculated that these lamps have a short life span of approximately 4000-10000 hours (Chevremont, Farnet, Coulomb and Boudenne, 2012a). Hence, the cost of a UV device equipped with mercury lamps were reported to be relatively higher (Hamamoto et al, 2007). Moreover low pressure mercury lamps are known to emit nearly monochromatic UV light at a wavelength of 254 nm whereas UV-LEDs offer the possibility to use preferred wavelenght (Crawford et al, 2005).

TABLE 1. Differences between UV-LEDs and traditional mercury vapor UV lamps

UV-LEDs	**Traditional Mercury Vapor UV Lamps**
Do not contain toxic substances	Contain mercury
Robust design	Fragile
Compact size	Take up a lot of space
Longer life time	Short life span
Use different light source emitting UV light at different wavelengths (UV-A, UV-B, UV-C)	Emit monochromatic UV light at 254 nm (UV-C)

In contrast to traditional UV lamps, UV-LEDs were shown to have a robust design, compact size, higher energy efficiency, reduced frequency of replacement due to long life time of nearly 100000 hours since they do not need a warm-up time (Chevremont, Farnet, Sergent, Coulomb and Boudenne, 2012b; Vilhunen, Rokhina and Virkutyte, 2010). Furthermore using UV-LEDs will provide the opportunity to develop small, space-saving sterilization equipments. UV-LED devices can sterilize small and narrow spaces due to their compact size (Mori et al, 2007). Therefore, design of a new sterilization equipment of low energy consumption which is in various shapes and sizes without using harmful substances is possible using UV-LEDs.

Another advantage of UV-LEDs can be the possibility to compose a UV-LED array at different wavelengths in order to enhance the germicidal effect. Such a system will hold for the potential to design sterilization units which target a wide range of pathogens or the main pathogens specific for the product of concern (Chatterley and Linden, 2010).

CONCLUSION

UV-LED technology is a promising method for nonthermal processing of liquid foods since it has many advantages over conventional UV-C lamps. It is first essential to develop a static or

bench top apparatus which is constructed by using UV-LEDs in order to compare the results of the studies in the literature performed by conventional UV-C lamps with those of the new system. However, it is known that power outputs of LEDs at the germicidal wavelengths (250-260 nm), where UV absoprtion of DNA reaches the maximum, are low. Despite the disinfectant property reduces with increasing wavelength, power output shows an increase. Therefore it is worth investigating whether an increase in the power output using UV-LEDs at higher wavelengths compensate for the reduction in the germicidal efficiency.

There are a few studies in the literature related to use of this new technology for disinfection purpose. The water disinfection is the only applied area among these limited studies. Hence, there is a gap in the literature in terms of disinfection of liquid foods. More needs to be done on this area.

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STUDY OF METHODS TO REDUCE CONTAMINATION OF FOOD FROM FOOD PACKAGING BASED ON PLASTICIZED PVC (ARGON PLASMA)

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ABSTRACT

In this work a surface treatment process using argon plasma to reduce the migration of the additives contained in the initial plastic package (PVC) stabilised with epoxidized sunflower oil was studied and the migrated substances were investigated.

One plasticized formulation containing 40 % of dioctyl phthalate (DOP) was considered. Migration tests were carried out at 40°C with agitation in crude olive oil.

The variation of the weight of the PVC samples with time was investigated. The migration phenomena were studied by using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM).

Keywords: PVC, migration, argon plasma, FTIR, SEM.

1. Introduction

Materials in contact with food must ensure its protection and conservation, without altering its organoleptic or physico-chemical properties: it is the principle of inertia [DE CHAMPS, 2004; Muncke, 2009; Kozlowski, 2005]. It is well known that contacting a plastic container with food induces inevitable contamination of the food and can lead to interference to give possibly unknown compounds called neoformed or send her a taste and odor undesirable or changes in the properties of the plastic material which will become unable to optimally protect the food [MESSADI & GHEID 1994]. In order to reduce these phenomena, different approaches have been developed:

1. Surface modification;
    a. Crosslinking surface;
    b. Changing the surface hydrophilic / lipophilic;
    c. Siding
    d. Extraction surface;
2. Permanent use of plasticizers;
3. Combination of the two su-cited;

In this work, the modification of the polymer surface by crosslinking using a cold plasma source with argon as the plasma gas was investigated.

This method aims to approximate the molecular chains of the polymer thus increasing cohesion and decreasing the free volume and create a three-dimensional network which will act as a barrier and additives contained in the package to migrate to the food and the same time, the food from entering the interior of the polymer.

The cold plasma treatment depends on four important parameters:

- The power of plasma;
- The exposure time of the plasma samples;
- The pressure inside the plasma reactor;
- The distance between the source and the sample.

2. Materials and methods

The phenomenon of migration is analyzed based on the change in mass of PVC pellets in contact with the environment simulator as well as using the Fourier transform infrared (FTIR), further characterization of the morphology of the pellets was carried out by scanning electron microscopy (SEM).

2.1. Preparation of PVC sheet to 40% plasticizer

Resin and additives, whose proportions are specified in Table 1 were mixed in methanol [BROUILLET & FUGIT, 2009], which is then removed by evaporation in an oven at 60 °C.

The mixture obtained is perfectly homogeneous then introduced into a mixer with two rotary cylinders (Lescuyer Blere), heated at 135 °C for 15 to 20 minutes. The gelled mixture is then placed between two plates (Table press Fontijne) at 170 ° C under a pressure of 300 KN for 5 minutes to obtain the desired thickness (2.0 ± 0.1) mm.

From the plates thus produced were cut using a steel mold disks for undergoing migration tests. These discs have a thickness of (2.0 ± 0.1) mm and a diameter of (22.0 ± 0.1) mm.

Table 1: Formulation of PVC sheets

Formulation	Content in grams
PVC resin	100
Zn and Ca stearates complex	2
Stearine	1
Epoxidized sunflower oil (ESO)	10
Dioctyl phthalate (DOP)	40

2.2. Argon plasma treatment

The device is composed of expériemental:

- A spherical enclosure stainless steel 1m in diameter. It is connected to a pumping system (primary and secondary) for discharging air to achieve a vacuum of the order of 10^{-6} mbar.

- An inductive source. The power control is done by a RF radio frequency generator (13.56 MHz) via an impedance.

In our study, we followed three parameters of power, pressure and time of exposure, the distance was set at 1 cm from the source. To this end, we conducted a sweep of power between 60 and 120W, exposure times ranging from 1 to 3 minutes and the pressure-2mbar 7.5.10, 10-1.1.10-1 mbar and 1 mbar. (See Table 2).

Table 2: Working conditions Argon Plasma Reactor

Indice	Conditions
CN 1 a	P= 120W, t= 3mn, D= 20sccm, d= 1cm, p= $7,5.10^{-2}$mbar
CN 1 b	P= 120W, t= 1mn, D= 20sccm, d= 1cm, p= $7,5.10^{-2}$mbar
CN 2 a	P= 100W, t= 3mn, D= 20sccm, d= 1cm, p= $7,5.10^{-2}$mbar
CN 2 b	P= 100W, t= 1mn, D= 20sccm, d= 1cm, p= $7,5.10^{-2}$mbar
CN 3 a	P= 80W, t= 5mn, D= 20sccm, d= 1cm, p= $7,5.10^{-2}$mbar
CN 3 b	P= 80W, t= 3mn, D= 20sccm, d = 1cm, p= $7,5.10^{-2}$mbar
CN 3 c	P= 80W, t= 1mn, D= 20sccm, d = 1cm, p= $7,5.10^{-2}$mbar
CN 3 d	P= 80W, t= 2mn, D= 30sccm, d = 1cm, p= $1,0.10^{-1}$mbar
CN 3 e	P= 80W, t= 2mn, D= 40sccm, d = 1cm, p= $1,1.10^{-1}$mbar
CN 4 a	P= 60W, t= 3mn, D= 20sccm, d = 1cm, p= $7,5.10^{-2}$mbar
CN 4 b	P= 60W, t= 1mn, D= 20sccm, d = 1cm, p= $7,5.10^{-2}$mbar

2.3. Test migration

The kinetics were followed by isothermal for 10 days at 40 ° C in 50 ml of liquid simulator controlled agitation continues. A sample is taken every 48 hours (PVC disc 10 mL crude olive oil) to keep the ratio (oil volume) / (number of disks) [MESSADI & Vergnaud, 1997] . To identify the nature of the phenomenon occurring during contact with PVC pellets mid simulator foods (olive oil raw) and know the overall migration rate, the evolution of rate of change of mass of pellets depending on the contact time in hours was followed with [BOUSSOUM et al, 2006]: $\tau\,(\%) = \frac{m_t - m_0}{m_0} \times 100$

Where: m_t: mass of the pellet collected on day d after wiping, still weighing until the weight becomes constant (about 5 or 6 days).

m_0: mass of the pellet before initial immersion in the simulator.

The decrease of the ratio means the passage of a certain quantity of the polymer additive in the middle simulator and conversely increase the penetration means that there simulator environment in the specimen [ATEK & al, 2005].

2.4. FTIR spectroscopy analysis

The PVC samples were dissolved in tetrahydrofuran. After evaporation of the solvent, a polymeric film was recovered and analyzed with a Jasco FTIR- 430 spectrophotometer. The resolution was 2 cm^{-1}.

2.5. SEM characterisation

The PVC samples were analysed after metallisation by a scanning electron microscope PHILIPS type ESEM XL.

3. Results and discussion

3.1. Migration testing

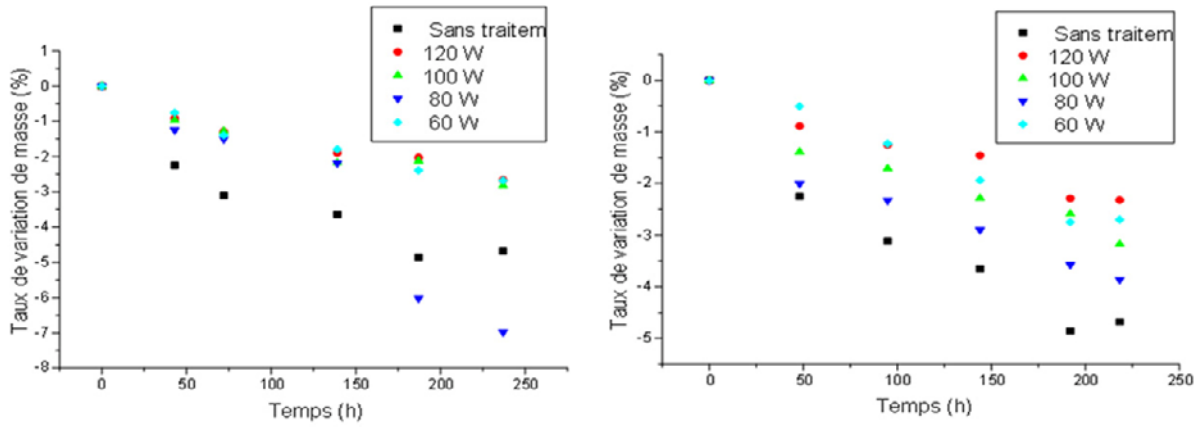

Figure 7: Effect of power on the rate of change of mass for a fixed pressure 7,5.10^{-2} mbar and a fixed exposure time: 3min A-, B-1min.

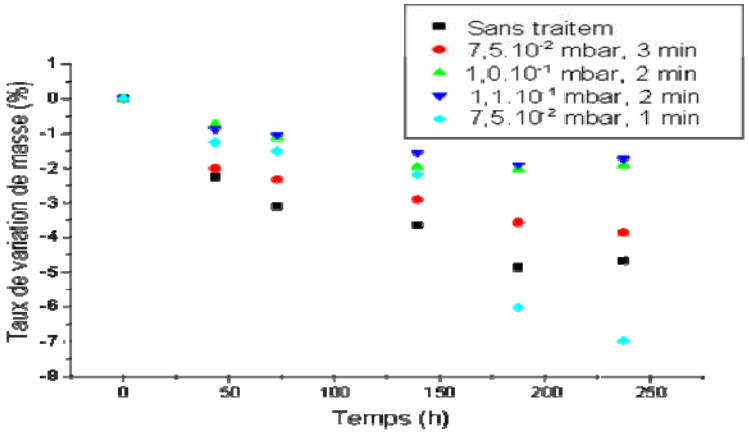

Figure 8: Effect of pressure on the rate of change of mass power set at 80 W and an exposure time set to 2min

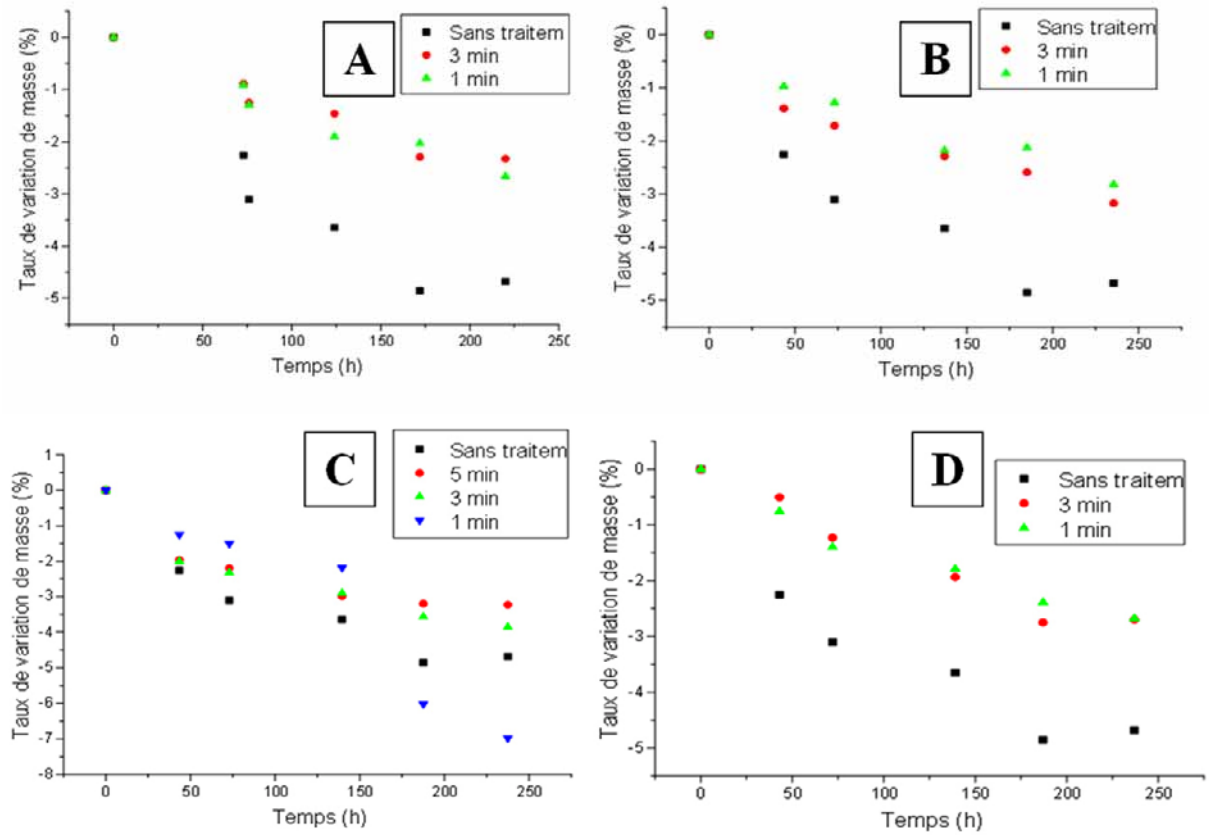

Figure 9: Effect of exposure time on the rate of change of mass in a fixed pressure $7{,}5.10^{-2}$ mbar and a power set at: A-120 W, 100 W-B, C-80 W-60 W D

We note that in general the curves is decreasing, which indicates a decrease in the rate of change of mass as a function of time and therefore a migration of certain additives took place. In addition, the kinetics of decrease is much more marked in the case of the untreated plastic formulation compared to that has been processed. This shows that the argon plasma treatment of the latter decreased the migration.

DOP plasticizer incorporated into the polymer partially destroyed the interactions between PVC chains are responsible for the cohesion of the material and helps to change the properties of PVC. Cohesion reduced and the existence of a concentration gradient induced diffusion of additives in PVC pellet (concentrated medium) mid simulator.

We note from Figures 1 and 2 both power and pressure settings have a positive effect on the reduction of the phenomenon of migration.

From the Figure 3, we see that after 1 min of treatment, there was a decrease in the migration phenomenon quite large in comparison with the untreated material. In addition, although the difference is not significant, the pellets that were treated for 3 min have been the least transfer phenomenon that those who were treated for 1 min.

Therefore, the greater the pressure, the more we will charged species inside the reactor and thus the probability that these species collide with the material increases. In the same spirit, the greater the power, the greater the intensity of UV radiation and visible increase and charged species will have a greater speed, and the clash between the reactive species and the polymer surface will be greater.

	Conditions						
	Without treatment	CN1a	CN1b	CN2a	CN2b	CN4a	CN4b
Overall migration ($mg.dm^{-2}$)	4,94	2,91	2,95	3,38	3,02	2,29	2,78
	Conditions						
	Without treatment	CN3a	CN3b	CN3c	CN3d	CN3e	
Overall migration ($mg.dm^{-2}$)	4,94	2,26	4,13	5,44	2,12	1,83	

All values of global migration plans are lower overall migration established by the EEC for plastic packaging is 10 $mg.dm^{-2}$, which means that the migration did not affect the quality of the environment simulator (food) used.

In addition, the values of global migrations smaller are obtained in the case of Argon plasma treated pellets, confirming the effectiveness of this approach in reducing the overall migration of additives. In addition, there was a decrease in overall migration of more than 50% for the treated pellets under the conditions CN CN 3d and 3e for which we had changed pressure.

3.2. SEM morphological analysis:

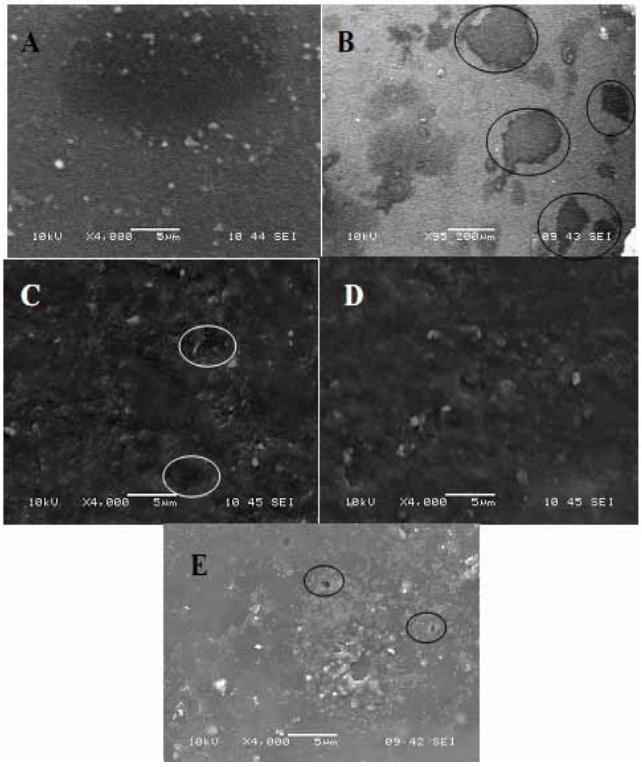

Figure 4: SEM analysis PVC pellets:
(A)- pellet-witness, witness after pellet B-10j contact with HOB, (C)-treated wafer (under the conditions CN 1a) after contact with 10j HOB, (D)-treated wafer (under the conditions CN 2a) after contact with 10j HOB, (E)-processed wafer (under the conditions CN 2b) after contact with 10j HOB.

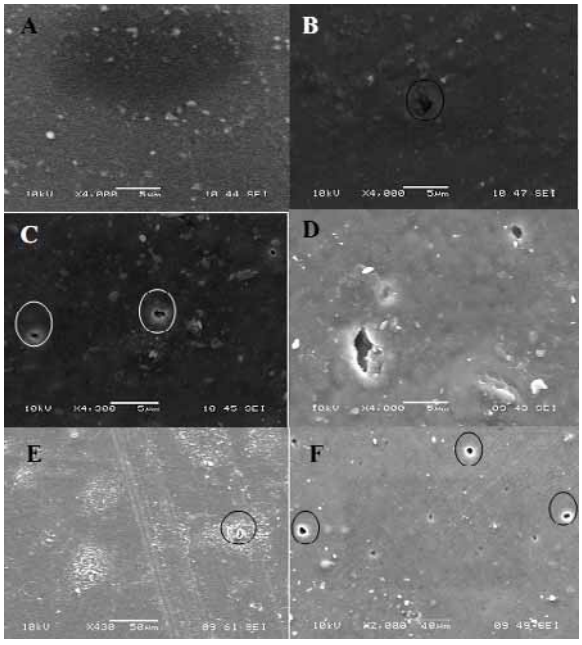

Figure 5: SEM analysis PVC pellets:
(A)- pellet-witness, (B)-treated wafer (under the conditions CN 3a) after contact with 10j HOB, (C)-treated wafer (under the conditions CN 3b) after contact with 10j HOB, (D)-treated wafer (under the conditions CN 3c) after 10 h of contact with the HOB, (E)-treated wafer (under the conditions CN 4a) after contact with 10j HOB, (F)-treated wafer (under the conditions CN 4b) after 10 h of contact with the HOB.

By comparing the images of the control sample and those having undergone migration testing for 10 days, we observe:

- The appearance of dark areas (holes) indicating that there was migration of additives mid simulator;
- The surfaces of the pellets have undergone migration tests are rough in comparison with the control, which has a much smoother surfaces;
- Holes observed are more important in the case of PVC untreated samples;
- The PVC pellets treated with argon plasma are less dark areas indicating that the treatment was effective.

3.3. Infrared spectroscopic analysis

In the presence of a mixture of polymer and additives, the spectrum represents the total sum of the spectra of the individual components. The interpretation is done by comparison with reference spectra of known products based on tape, however, may have characteristics that the sample to be studied [ATEK et al, 2005].

The band at 1432 cm^{-1} present in the spectra of the studied formulation as well as the PVC resin is the CH_2 bond in PVC [Marcilla et al., 2008]. It is taken as a reference for the calculation of the ratios of the absorbance for semi-quantitative estimation of the migration of additives.

A 1725 / A 1432: Study of the migration of DOP and HTE.
A 1577 / A 1432: Study of the migration of the complex Ca, Zn.
A 1460 / A 1432: Study of the migration of DOP, the HTE complex Ca, Zn and stearic acid.
A 1121 / A 1432: Study of the migration of DOP.

Overall, we note that the absorbance ratios are lower in the case of argon plasma treated pellets than those who were, indicating that migration is more important for them. The crosslinking argon plasma increase benefit cohesion within the PVC molecule, and therefore the migration of additives is less important.

We see also that in the case of almost all curves in Figures VII. 7. to VII. 10., Reports absorbance increased up to 48 hours, then gradually decreased. This case has already been observed in other studies [GHEID et al., 1990]. It indicates an initial penetration of the liquid in the tubes calculator PVC, which has the effect of causing or enabling the migration of the additives present.

4. Conclusion

In conclusion, the surface treatment to destroy the main routes connecting the chlorine in carbon chains of PVC, has helped bring the molecular chains of the latter increasing the cohesion between the chains thus reducing the free volume and create a three-dimensional network which will act as a barrier to the transfer phenomenon. This barrier will be unfavorable to diffusion and therefore result in a decrease in migration. The plasma consists of atoms and molecules in the excited state and emits more charged species such as ions and electrons, radiation visible to the naked eye. These reactive species of the plasma induced radical formation on the surface of PVC, which can lead to cuts in chains, to transfer reactions or recombination leading to crosslinking ie formation of a three-dimensional network of the polymer surface .

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THE STUDY OF INTERACTIONS COTENANT/CONTAINER: CASE OF FOOD PACKAGING FOR FAT PRODUCTS

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ABSTRACT

This aim of this study the interactions between polypropylene (PP) food packaging and food stimulant. For that purpose two different temperatures (20°C and 40°C) and two stimulants (olive oil and sunflower oil) were considered.

The interaction phenomena were investigated according to the rate mass variation of PP samples with time and by using Fourier transform infrared spectroscopy (FTIR), Fourier Transform Infrared Spectroscopy (FTIR).

The results show the presences of the two phenomena, migration of additives and sorption of the simulant.

Keywords: PP, food packaging, migration, sorption, AAS, FTIR.

INTRODUCTION:

Today, 70% of the processed food are in contact with plastic packaging. Because these materials contain various additives (antioxidants, plasticizers, dyes, catalysts, fillers, antistatic agents, lubricants ...) and because these substances are not covalently bonded to the polymer chains, they may a risk of food contamination. The risk is now even greater as most foods are in contact with these materials [1-5].

Part of migrants can either modify the organoleptic characteristics of the food, or share with toxicological [6,7].Among the polymers used in food packaging, there is polypropylene. Which is used in the packaging of fatty products, packaging dairy products. Its essential properties are high resistance to heat and most chemicals agents, which makes it an interesting polymer [8].This work is to study the interactions likely to occur in the case of margarine trays based on PP, and to determine the effect of food stimulant (sunflower oil and crude olive oil) and the effect temperature (20 ° C and 40 °) on the rate of mass variation.

The chosen liquids for testing migration simulate fatty foods like olive oil or sunflower oil as recommended guidelines directives 82/711/EEC and 97/48/EC (Official Journal of the European Communities' No L 222/10).

To conduct this study the rate of mass variation with time was investigated. FTIR spectroscopy was used to perform a qualitative and semi-quantitative analysis of migration phenomenon.

2. EXPERIMENTAL

2.1 Migration testing

Migration tests were made by using circular PP samples (22 mm of diameter) cut with a steel mold. The pellets were cut and a diameter of 22mm. Migration tests covered a period of 12 days with stirring at a temperature of 20 °C and 40 °C.

Cell migration glass contains 12 samples placed in a stainless steel rod rings separated by glass and immersed in 120ml of stimulant (sunflower or olive oil):

- sunflower oil
- olive oil

A PP sample of a pad and 10 ml of food stimulant were taken off every day. Each sample was wiped and weighed using a KERN analytical balance with 0.0001 mg precision;

The rate of the mass variation (τ, %) was calculated according the following relation:

$$\tau\ (\%) = [(m_t - m_0) / m_0] * 100$$

m_t: mass of the sample at time t after wiping (weighing is continued until the stabilization of the mass).

m_0: mass of the sample prior to immersion in the food simulant.

2.2 FTIR analysis

The FTIR spectra of the PP films were recorded using a Perkin Elmer infrared spectrophotometer in ATR mode with a resolution of 2 cm^{-1} .

3- RESULTS AND DISCUSSION

3.1 Rate of mass variation

Figures 1 and 2 show the evolution of the rate of mass variation in the case of the two considered simulators (olive oil and sunflower oil) (20 °C and 40 °C).

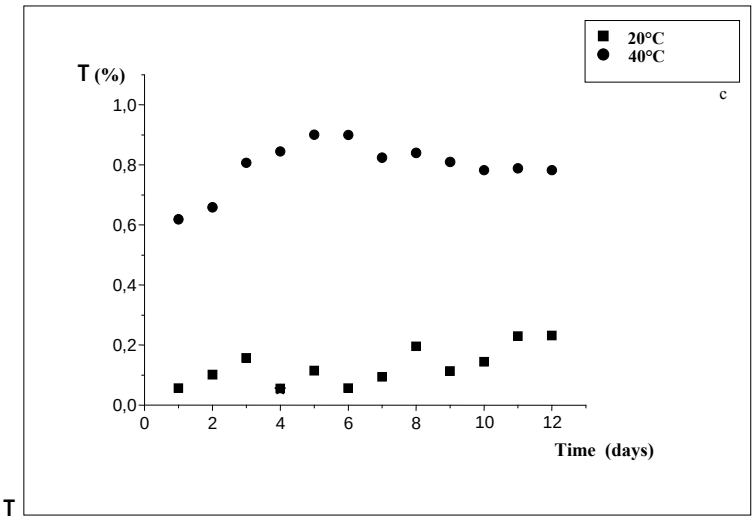

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Fig.1: Effect of temperature on the rate of mass variation in olive oil.

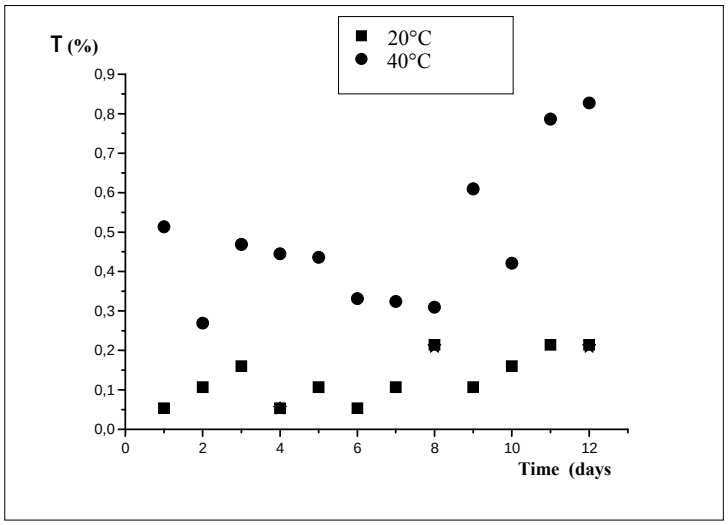

Fig.2: Effect of temperature on the rate of mass variation in sunflower oil .

For the two food simulants, it can be noted that the highest rate of mass variation occurred at greater than 40 °C.

This is due to the fact that temperature promotes the mobility of molecules, which favours their migration in the food stimulant.

Figure 3 shows the influence of the food simulant on the rate o mass variation at40 °C.

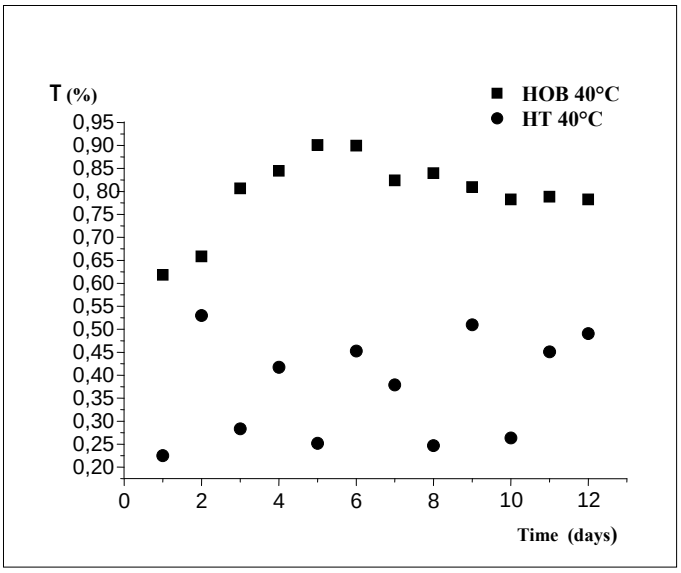

Fig.3: Effect of the nature of the food simulant on the rate of mass variation at 40 ° C.

The rate of mass variation is higher in the case of olive oil, which means that the PP has more affinity for olive oil in comparison with sunflower oil.

3.2 FTIR analysis

The purpose of this analysis is to perform a qualitative and semi-quantitative investigation on the migration phenomenon to in identify the nature of migrant species.

In the presence of a mixture of polymer and additives, the infrared spectrum represent the sum of all components, when the formulation and packaging are not known; the analysis is limited to the identification of functional groups of additives and their evolution over time.

Analysis of the infrared spectrum of light.

The infrared spectrum of the dye patch without witness (who suffered no migration test) is shown in fig.4.

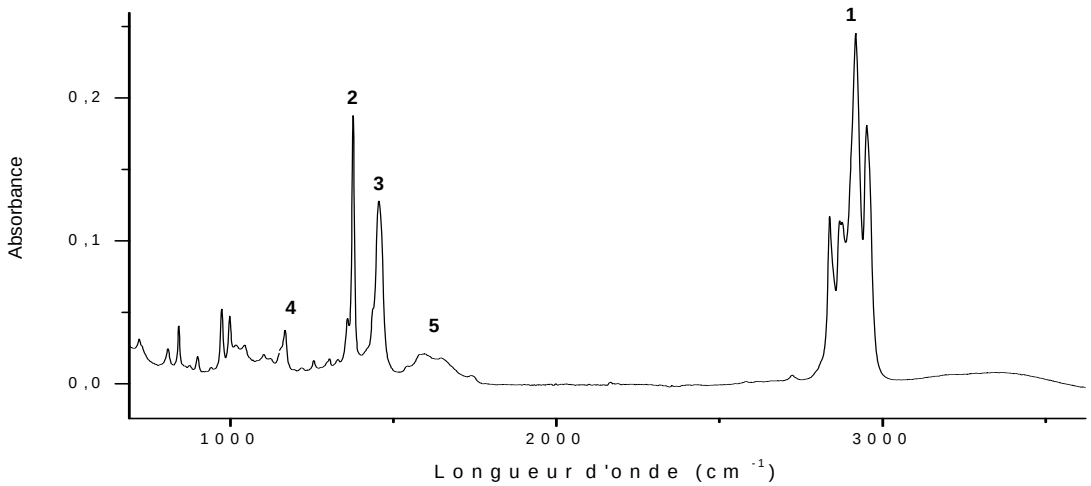

Fig.4: Infrared spectrum of the pellet PP

Table 1 summarizes the main bands appear in the infrared spectrum of the pellet.

Table 1: Main characteristic bands

Number	Wave number (cm^{-1})	Vibrate Mode	Group
1	2918	Elongation	-CH (PP)
3	1374	Déformation	$-CH_3$ (PP)
2	1450	Déformation	-CH, $-CH_2$ (PP)
4	1166	Elongation	-C-O
5	1600	Elongation	-C=O

The presence of the bands corresponding to the stretching vibration (-C-O) and (-C = O) indicates the presence of an ether and a carbonyl function, which would be present in certain components of the packaging. The other bands are due to PP.

3.2.1 Analysis of infrared spectra of polypropylene pellets after contact with olive oil

The intensity of the bands corresponding to the functional groups is related to the diffusion phenomena. If the intensity of the bands decreases, this means that migration occurred. If the opposite happens then sorption has taken place and there will be an increase in the intensity of the bands.

The infrared spectra of polypropylene pellets after contact with olive oil at 40 ° C at different times are shown in fig.5.

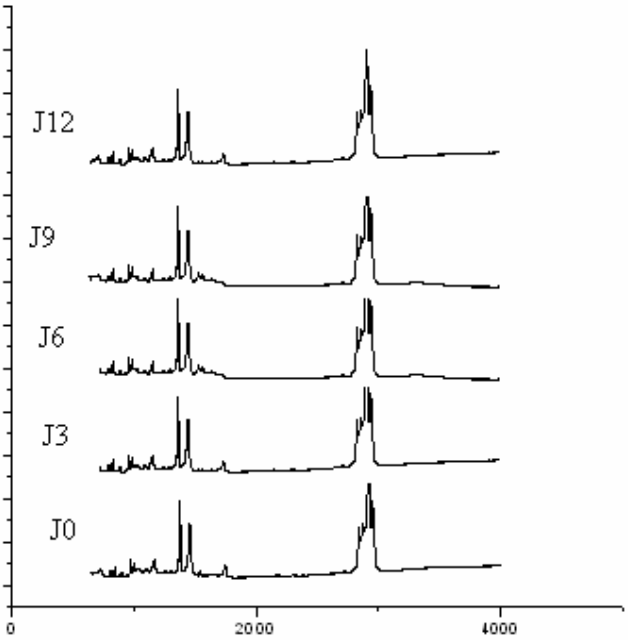

Fig.5: Infrared spectra of PP pellets have been in contact with olive oil at 40 ° C for different times in days (D)

From the spectra, the evolution of ether and carbonyl functions cannot be observed as additives or degradation products of the polymer are present at very low concentrations compared to polypropylene.

For a semi-quantitative estimate of absorbance ratios were estimated. The calculation of the latter is made with respect to a fixed band, in our case we have considered the band corresponding to the bending vibration of-CH 3 group (1374 cm^{-1}).

To monitor carbonyl and ether groups will be calculated following reports:

- 1600/1374 : compounds with carbonyl group migration
- 1166/1374 : compounds with ether group (C-O-) migration.

The evolution of these relationships over time of contact with the olive oil is shown in FIGS fig.6, fig.7.

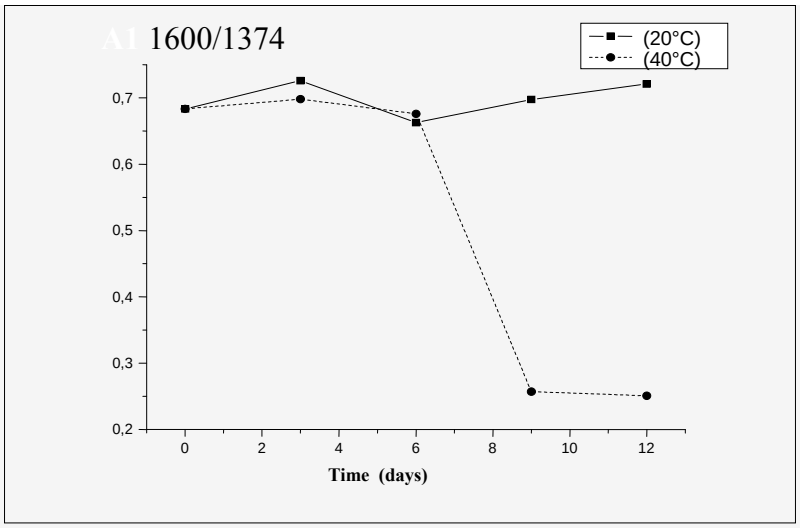

Fig.6: Variation of absorbance ratio as function of time in the case of pellets having been in contact with olive oil at 40 ° and 20 ° C

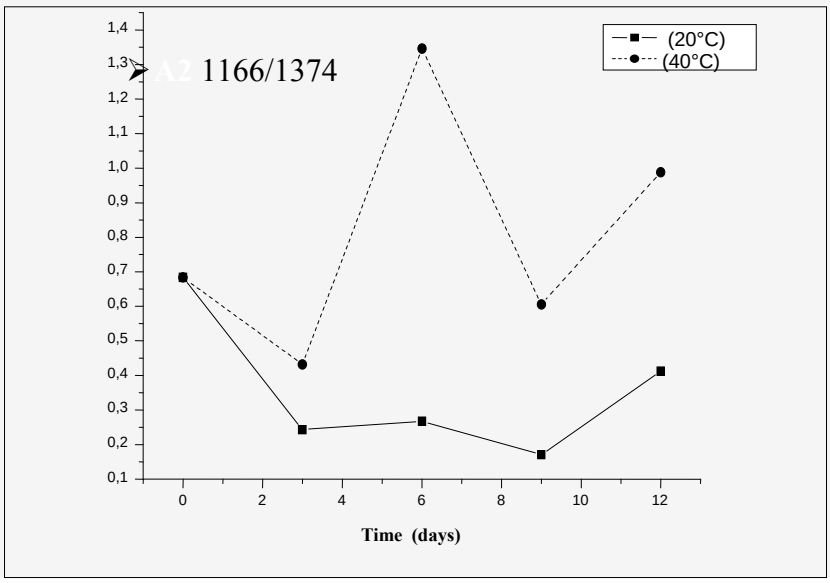

Fig.7: Variation of absorbance ratio in the case of pellets have been in contact with sunflower oil at 40 ° and 20 ° C.

The shape of these curves indicates the presence of two diffusion processes because in some cases the ratio of absorbance increases and, in other cases, it decreases, and therefore the substance (s) containing ether groups and carbonyl move in both directions (sorption and migration). This confirms the results obtained in the estimation of the rate of change of mass. apart from the case of group-C = O in olive oil (Fig.6) or it has been observed possible migration of substances containing the grouping.

As among the most widely used additives in the PP are the Irganox 1010, Irganox 1076 in [9].

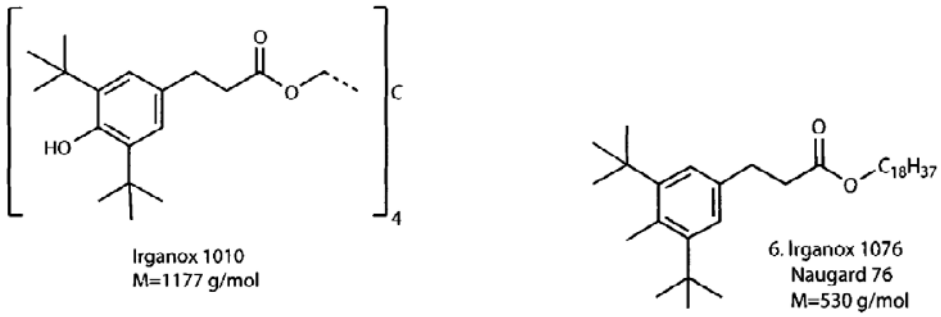

Fig.8 Chemical structures of Irganox 1010, and Irganox 1076.

By comparing the FT-IR spectrum of PP, we note the absence of a strong band at 3400 cm^{-1} which corresponds to an alcohol related to an aromatic ring. So that we would probably additive Irganox 1076.

To see a manner it would be preferable to work with hexane, which is a simulant that has no C = O band that could interfere with the additive [10].

4. CONCLUSION:

The results obtained from the mass variation tests showed the presence of two interaction phenomena which are migration and sorption and showed that these phenomena are even more important with the temperature.

Infrared analysis of our pellets helped prove the existence of certain additives or degradation compounds containing carbonyl and / or ether groups in their molecular chain. Monitoring reports absorbances has highlighted the migration and / or sorption of substances with these groups.

FTIR analysis has allowed us to define the qualitative analysis by identifying the additif, which could be Irganox 1076, antioxidant which is widely used in the PP.

To see a more effective this migration, it would be preferable to work with hexane, which is a stimulant that has no C = O band that could interfere with the additive.

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SPIRULINA MICROALGA. A FOOD FOR FUTURE

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ABSTRACT

Spirulina is a microscopic alga, known also as blue-green alga. It belongs to the family of cyanophyceae. These beings are original and very enigmatic: they feed themselves by photosynthesis like plants but their cells have no cellulosic membrane like bacteria (which explains their very high digestibility, about 83%). They appeared very early on Earth, more than 3 billion years ago. By their intense production of oxygen they considerably modified the atmosphere on Earth so it became possible for organic life to emerge.

Furthermore this microscopic organism possesses many properties: it is the most proteic product known on Earth (almost 70% of its dried mass). It has also many therapeutic properties: it is efficient against several diseases linked to malnutrition and other diseases like thyroid cancer, diseases linked to digestive tube, pancreas and many others (see Yacoub, 2010).

The biotechnology (production) of this product is very simple and it can be performed by any peasant which is very interesting for developing countries.

Key words: microalgae, Spirulina, protein, malnutrition, thermophysical characteristics, biotechnology, therapeutic properties.

1. INTRODUCTION.

Developing countries face today, major problems on all domains: economic, political, financial, etc. Most countries of the African continent experience armed rebellions which imply political instability and economic stagnation.

Diseases, malnutrition and other corollaries of underdevelopment are visible everywhere. The infant mortality due to food deficiencies in the world is 33000 children from 0 to 5 years who die every day because of malnutrition (most of them in Africa). This means, more than 12

million children die per annum (Documents of Intergovernmental Spirulina Project and CISRI, *Fox R*. and *Manin*, 2000). KWASHIORKOR is the disease most frequently met. This disease is due to deficiencies in proteins, vitamins and oligo-elements.

The hygiene problems arise at each stage of food production. It was discovered that the majority of problems appears at the stage of raw materials production. Such raw materials containing a lot of impurities, do not meet standards of quality required by the international market.

In the countries of the South, many farmers miss means and knowledge to apply various technologies necessary to the development of their exploitations; technologies which they badly understand and have difficulties to integrate in their usual practices.

The processing industries in the countries of the South (Developing countries) did not yet adopt a system of hygiene and environment management in conformity with the international standards very often because they are under-informed on the standards and relevant codes of good practice required by importers.

In addition, in the countries of the South exist several institutions of products quality control but, in the majority of the cases, they suffer an obvious lack of coordination and co-operation.

The quality control requires a checking of the results at laboratory. In the countries of the South, there are not sufficient laboratories and those existing do not have the necessary capacity to carry out certain tests, which disqualifies them in the eyes of Western importers.

To answer to the challenge of food supply in the South, it is interesting to develop the production of Spirulina which is a food of first choice in the struggle against malnutrition. The first section presents the product Spirulina (Description and biochemistry) and the methods of artificial culture, thermophysical characterization (determination of thermal conductivity, thermal diffusivity, thermal capacity), sorption isotherms at different temperatures. The second section is devoted to results and discussions and the third comprises a detailed list of references.

2. MATERIAL AND METHODS

2.1. Material

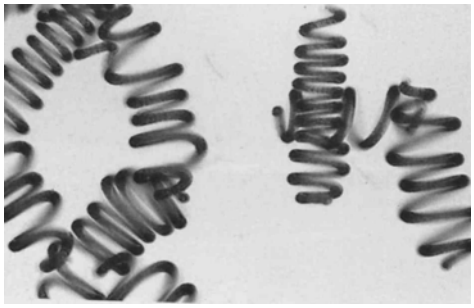

Fig. 1: Image of Spirulina trichomes seen under microscope (x40, source Fox, 1999)

The first observations of the blue alga, known today as Spirulina, go up to the 16th century at the time of southern America Spanish conquest (*Sironval C*., 1993) and the first scientific studies go up to the 19th century (*Ripley D. Fox*, 1999).

Since then, and until our days, the interest of Man for the alga does not cease growing from day to day. Today, there is more than 3000 quotations of Spirulina in the specialized scientific litterature and Internet. This passion for the blue-green alga is explained by the world food crisis on one hand, and the consumers mistrust towards genetically modified organisms, on the other hand. In addition, being an inexhaustible source of proteins, it constitutes an effective weapon in the fight against malnutrition (*Sironval C*., 1993), the diseases due to food deficiencies, as much of other diseases. Certain studies show that Spirulina is effective against certain cancers (cancer of thyroid for example) and many other immune system deseases (*Gustafson R. K*. et *al*, 1989). Moreover, the microalgae produces a blue pigment-phycocyanin- which is not only an excellent substitute in the industry of the blue dye, but has remarkable therapeutic properties. Spirulina can hide much more other not yet explored "treasures". It has been, moreover, the subject of a very significant number of works. Spirulina did not deliver all its secrects yet, considering the last medical applications in the treatment of various deseases, as well as the use of Spirulina as basic food for long duration space missions (*Morist A*. et *al*, 2001). Furthermore, Spirulina can be used to modify other planets so that Man can live in, what is called “terra forming”. The list would be long, if one starts to count down the alga new applications. But initially let us see how the alga appeared on our planet.

"Just for a moment, let us return in the past at a very hot day, perhaps 3,5 billion years, certainly more than 2,8 billion years ago, when something arrived to a rather large bacterium,

occupied digesting carbon dioxide of volcanic origin, sulphur, phosphorus and some metallic ions varied and tasty from the doubtful water of the puddle pool where it was. At zenith, a pale sun was shining through a yellowish atmosphere of methane, hydrogen, nitrogen and carbon dioxide. Something occurred! Most probably, a package of ultraviolet photons, after having avoided collusion with gas molecules while crossing this atmosphere, strike our bacterium in a significant point of its desoxyribonucleic acid (DNA) chain; this acid commanded the cell to grow by extracting carbon, hydrogen, water oxygen and carbon dioxide with the assistance of solar energy. The oxygen atoms in excess were gathered per pairs and were driven out through the membrane of the cell. Our bacterium was diverting itself seing the small oxygen bubbles escaping, bursting the surface of water with a "plop" and an iridescent sun flash ".(*Fox R. D.*, 1999)

However, the blue algae are very different from the other algae whereas they have much in common with bacteria. Spirulina is neither a bacterium, since it nourishes itself by photosynthesis like a plant, nor a plant since it does not have definite cytoplasm. It is to some extent an intermediate being between vegetable and animal world.

2.1.1. Description

Arthrospira platensis: trichomes of 6-12 μ of diameter, rolled up in spiral, narrowed a little in the articulations; whorls of 15-40 μ diameter with a distance between them of 10-60 μ. In 1973, *Fott* and *Karim* (*Fott* and *Karim*, 1973), concluded that the African material should rather be attached to the species *Spirulina geitleri*, in particular because the diameter of the whorls decreases regularly towards the ends of the trichomes. However, in a double of *oscillatoria platensis*, whorls whose diameter decreases towards the ends of the trichome, were observed. The material of Chad has the trichomes narrowed a little on the level of the articulations, which corresponds to the original description of *S. platensis* and not to that of *S. geitleri*. This species, very abundant in the lakes of Kanem only, was very seldom observed elsewhere (*Iltis*, 1974, 1975).

It should be also noticed that the spiral form is not universal since there is Spirulina in the shape of "sticks", observed in the farm of *J.P. Jourdan* (MIALET, Southern France). This type of alga lives in the surface layers of water and is completely absent in depth. We suppose whereas the right form is the original form. *The spiral is formed following a mechanical work needed for the search for food and light or by stresses due to the medium and the environment.*

2.1.3. Biochemical composition

Proteins: The proteins form the major part of the dry mass (of 60 à.70%) with a spectrum of acid similar to that of other micro-organisms. Phycocyanin is a chromoprotein that one meets in the alga in two forms (the c-phycocyanin and the allophycocyanin), which are characterized by their respective molecular weights. Their contents vary from 15 to 20% of the alga dry weight. Moreover, acting like a light collecting antenna, phycocyanin seems to be used as storage material for nitrogen. The ribulose 1-5 diphosphate carboxylase makes approximately 12% of the dry weight.

***Carbohydrates*:** The carbohydrates form approximately 14% of the alga dry weight.
Lipids: when they are extracted using methanol and chloroform as a mixture, true lipids form from 6% to 7% of the dry weight. The fatty acids and the insaponifiables respectively account for 83,1% and 16,9% of the total quantity of lipids. The insaponifiables fraction consists in sterols and triterpenic alcohols. Sterols represent 1% to 5% of insaponifiables, cholesterol being the essential component. triterpenic alcohols account for 5% to 10% of the insaponifiables, made up mainly of alpha-amyrin.

***Nucleic acids*:** The nucleic acids are present within the limits from 2,83 to 4,5% of the dry weight. The ribonucleic acid represents the most significant part of the nucleic acids (2,2 to 3,5% of the dry weight). The desoxyribonucleic acid is present within the limits from 0,63 to 1% of the dry weight.

***Ashes*:** The fraction of ash lies between 6,4 and 9% of the dry weight and its composition depend on the conditions of culture. The analysis of ashes often shows the presence of potassium, phosphorus, chlorine, magnesium, calcium, iron and other elements. It is interesting to notice that the chemical concentrations of elements in the dry biomass do not reflect at all their concentrations in the culture medium. In the table below, is represented the amino acids content of Spirulina obtained by Chromatography, for the nitrogen based method is not exact enough.

Table 1: Chromatography of the amino acids of spirulina from two sites in Chad.

Composition in essential amino acids	**Spirulina Mbodou (%)**	**Spirulina Touffou (%)**
Threonin	4,47	4,52
Valin	7,94	8,24
Methionin	0,08	0,08
Isoleucin	5,71	5,73
Leucin	9,92	10,17
Lysin	1,47	1,53
Phenylalin	3,54	3,65
Histidin	4,25	4,54
Arginin	5,50	5,52
Composition in nonessential amino acids		
Aspartic acid	9,42	9,91
Serine	4,55	4,66
Glutamic acid	15,14	13,77
Glycin	9,47	9,64
Alamin	12,56	12,67
Cystin	0,07	0,04
Tyrosin	1,18	1,14
Homoserin	0,40	0,80
Prolin	4,33	4,39

2.1.4. Spirulina as a food

Usually the algal proteins are slightly used when mono gastric organisms animals or human, are fed with intact cells. The blue-green algae in general, and Spirulina in particular, are unique in this situation because they are highly digestible. According to *Becker & Venkataraman* (1982), only small differences are observed between the digestibility of fresh Spirulina (82%), sun dried Spirulina and that dried by congelation which have a digestibility of 65% and 70% respectively; differences in digestibility even smaller between various forms of Spirulina (fresh or dried) were uncovered by *Hernandez & Shimada* (1978).

Spirulina was tested in experiments of animals feeding. The simplest method to evaluate proteins in tests of animals food, consists in determining the proteins effectiveness ratio (PER). *Becker et al* (1976), compared the PER of *Scenedesmus* with that of Spirulina using various methods. As one expected it, the values of the PER of sun dried Spirulina are higher than those of sun dried *Scenedesmus*. The values of the PER of sun dried Spirulina are lower than those of the PER of *Scenedesmus* dried in a drum drier, thus showing the value of this method of drying. Of course, the PER of Spirulina dried in a drum drier is higher than that of freeze-dried Spirulina. The value of the PER can appreciably vary in the case of Spirulina produced for commercial goals. A study shows that the PER of Spirulina coming from Mexico is 2,20 whereas that of a sample coming from a different source is only 1,86 (*Bourges et al.*, 1971). The biological value (BV) of Spirulina, defined as the ratio of the quantity of absorbed nitrogen on the total quantity of provided nitrogen, is as high as 79,5% for sun dried Spirulina, to which methionine is added, compared with 87,7% for casein (*Becker & Venkataraman*, 1982).

There is another method to test the nutritional value of a source of proteins: it is the cycle of depletion-repletion, in which guinea-pigs (for example of the rats) are initially underfed and then one provides them with sufficient quantities of food are tested. In an experiment, the regeneration of the enzymatic activity is highest by using a mode rich in casein, but the group fed by methionine enriched Spirulina, shows almost approxcimately the same results (*Becker & Venkataraman*, 1982).

Spirulina is very well appropriate as a dietetic supplement (*Narshima et al.*, 1982).

In short, it was shown that Spirulina is a satisfactory source of proteins for much of animals. It is particularly useful as food supplement and has a lowering effect of cholesterol level.

Furst (*Furst*, 1978) mentionned the report of *Fray Toribio de Benavente*, which reached the valley of Mexico in 1524 and described the harvest of "*tecuitlatl*", a certain time of the year, when it (Spirulina) is densest, the autochtones living along the Mexico City Lake, use a basket finely braided to gather a kind of fine mud spread out on the surface of water". They practise a cavity on the shore which they cover with fine sand and put harvest above this sand bed to make it dry under the sun. The paste which at the beginning measures 3-4 cm thickness measures nothing any more but 2-3 mm thickness after a drying of few days. According to the report, "the Indians eat this product, appreciate it much and sell it in the market. It plays the role of cheese in our meals". But identification of *tecuitlatl* did not come from Mexico but from Africa. In the Fifties, a research at the planetary level, led the researchers to study the possibilities of an algal culture on a large scale. In 1963, the French Petrolium Institute had

been interested in news about "*dihé*" local name of Spirulina, which the populations living in the area of Lake Chad in central Africa (Republic of Chad) consume. One decade later, *Jean Léonard*, a Belgian botanist crossing the Sahara, discovered independently *dihé* and brought back the techniques used by Kanembou for the production of this alga (*Furst*, 1978). For Kanembou, *dihé* is frequently consumed. According to season, one finds it in almost ten different meals. *Dihé* is consumed fresh only by pregnant women who believe that its dark color protects the future baby from 'evil eye'. Generally speaking, *dihé* is consumed as seasoning with a certain number of sauces which accompany cereals paste. The dried *dihé* is crushed in a mortar and the powder obtained is pulp in water. One adds to sauce, salt, pepper, beans, meat (if available) or fish. In a meal, a person roughly consumes 10 to 12 grams of *dihé*, which makes 8% of the requirements in calories and more than 10% of the requirements in proteins (*Furst*, 1978). *Dihé* is consumed in abundance only in period of food shortage (*Ciferri*, 1983).

The food value of Spirulina is amplified by the fact that it has a rather low percentage of nucleic acids (~4%) compared to that of bacteria. This percentage is extremely high in vitamin B_{12}, the cellular wall is easy to digest, contrary to the cellulose walls met in the other food algae. This cellular wall is entirely not poisonous and the lipids are made of unsaturated fatty acids which do not form bad cholesterol. This make of Spirulina a potential food component for people suffering coronary diseases and obesity, as it suggested by *Durand - Chastel & Santillan* (*Durand - Chastel & Santillan*, 1976).

The prospect to prepare fermented food like cheese, yoghourt and others, containing Spirulina, opens new possibilities for it, without taking into acount the potentialities of various extracts (*Switzer*, 1980). The slimming effect of Spirulina was tested recently in Germany and the results obtained are spectacular (*Richmond*, 1986).

More recent studies show that Spirulina is very effective in feeding fish (*Nandeesha M. C. et al.*, 2001).

It is significant to stress that the European Space Agency recently launched a draft study of the possibilities of making Spirulina a food of choice for space flights of long duration (the famous MELISSA project).

2.1.5. Spirulina as a medicine

It was reported that Spirulina has varied therapeutic properties and according to *A. Jassby* (*Richmond*, 1986), some of these properties are:

1) The pharmaceutical compounds, containing Spirulina as active ingredient, accelerate wounds cicatrization (*Clément, Rebeller & Zarrouk*, 1967). The effect was observed with whole Spirulina juice and extracts. The treatment was carried out with creams, balsams, solutions and suspensions. A separate study showed that Spirulina and its enzymatic hydrolysates, can promote skin metabolism and prevent keratinization (*Yoshida*, 1977).

2) Interesting results were obtained in giving by oral way phycocyanin (the blue pigment extracted from Spirulina), to laboratory mice to which cancerous cells of liver were injected (*Ijima et al.*, 1982). The amounr of survivals in the group having undergo the treatment, was appreciably higher than that of the control group. Moreover, it was noticed, in later studies, that the lymphocytes activity of the treated group was higher than that of the group of control like that of normal mice. According to *Ijima et al.* (1982), phycocyanin can, generally speaking, stimulate immune system, thus ensuring a protection against varied diseases.

3) Epidemiologic studies suggest that a regime highly rich in vitamin A, lowers the risks of cancer. More recent studies show that taking provitamin A (β–carotene) and not preformed vitamin A of animal origin is in correlation with a fall of the risk of cancer (*Shekelle et al.*, 1981). More recent studies still, show that the extracts of Spirulina are highly effective against thyroid cancer and HIV-1 responsible for AIDS disease (*Gustafson R. K*. et al, 1989)

4) It was reported that γ-linoleic (GLA) acid stimulates the synthesis of prostaglandin E_1 (PGE_1), which is in charge in the organism, of blood pressure regulation, the synthesis of cholesterol, ignition and cellular proliferation (*Tudge*, 1981). There are indications that deficiencies in GLA and PGE_1 could result in several degenerative diseases: The clinical studies show that the consumption of GLA helps against arthritis (*Kunkel et al.*, 1982), heart diseases, obesity (*Vaddai & Horrobin*, 1979) and lack of zinc (*Huang et al.*, 1982). Alcoholism, maniac depression, certain old age symptoms and schizophrenia are partially caused by the deficiency in GLA (*Horrobin*, 1981 ; *Horrobin & Huang*, 1983).

5) Last, the extracts of Spirulina rehabilitate a sufficient part of cholinesterase activity in human erythrocytes, which is inhibited by pesticides containing organic phosphates (*Matsueda, Nagaki & Shimpo*, 1976).

One can add to these purely scientific facts other virtues which certain consumers of Spirulina in Chad report like aphrodisiacs effects, the fall of premenstrual stresses, effects against night blindness, gastric problems, etc.

2.2. Methods

2.2.1. Spirulina culture at laboratory.

Was carried out Spirulina culture in the following way:

We used a sample of algae, coming from the LAGEP (Laboratory of Automatic and Process Engineering) of Lyon1 University (France). They are indeed a few 800 ml of cultures. It is *Spirulina Lonar* variety mixed with *Spirulina Paracas* variety in the shape of more or less right "sticks". To launch the culture, a modified and simplified Zarrouk medium (Natron +urea+ Iron perchloride) was prepared. The sample is put in the medium, after this remained a few days (2-3 days) in a refrigerator. The whole was mixed in a transparent plastic material container. We installed in the container, an immersion heater and a bubbles producing device. Above hangs a fluorescent tube of 100 W in order to provide the cultures in light even in the night. We take from time to time, a sample to observe under the microscope. At the end of 10 days, an increase in the concentration of cultures was observed.

Each day the external conditions are measured (temperature, air relative humidity), as well as the density of the cultures using a Secchi disc.

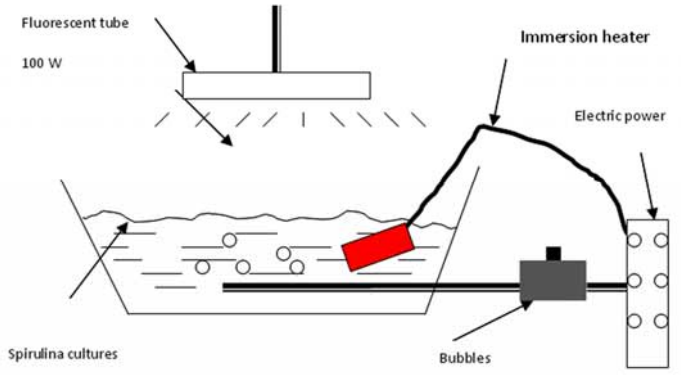

Fig.2: Spirulina culture experimental device

2.2.2. Measure thermal conductivity

• This method requires rather short operational times (four hours maximum to reach steady state) and the precision is comparable to other methods. It consists in putting the sample in a box which contains a heating film. Below, the sample is put in contact with a cryostat whose temperature is of 0°C. Thermocouples are put above and below the sample. When the difference between ambient air temperature T_a and that of the box T_b i.e.: $T_a - T_b < 0,1$ then, thermal losses are minimal and the regime can be considered stationary. The thermal loss

coefficient is given. One calculates thermal conductivity then, thanks to the equation which gives the energy balance of the system:

$$V^2 / R = C(T_b - T_a) + \lambda / e(T_c - T_f).S \qquad (1)$$

V is the tension which feeds the heating film, **R** is the resistance of the heating film; **C** is the thermal loss coefficient; **e** is thickness of the sample; $\mathbf{T_c}$ - temperature at the top of the sample, $\mathbf{T_f}$ is the temperature below the sample, and **S** the sample's surface.

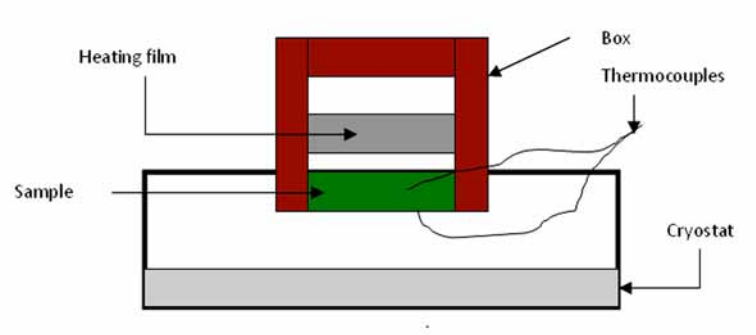

Fig3. Thermal conductivity measurement device: "boxes method"

2.2.3. Measurement of thermal diffusivity.

- **"Short" signal technique**: this method consists in stripping the thermogram of the sample's given point of which one of the faces was subjected to a short duration thermal shock (impulse of flow or temperature)..

It was shown whereas for reduced exchange coefficients lower than 1 (what is often the case in practice), thermal diffusivity can be obtained by one of the following formulas:

$$a=\frac{e^2}{t_{5/6}}\left[1{,}15-1{,}25.t_{2/3}/t_{5/6}\right] ; \; a=\frac{e^2}{t_{5/6}}\left[0{,}761-0{,}926.t_{1/2}/t_{5/6}\right] a=\frac{e^2}{t_{5/6}}\left[0{,}617-0{,}862.t_{1/3}/t_{5/6}\right] \qquad (2)$$

Where e is the thickness of the sample; t_ρ is the time between the start of the excitation until the moment when the temperature of the free (not irradiated) face of the sample rises ρ time of its maximum level during the test.

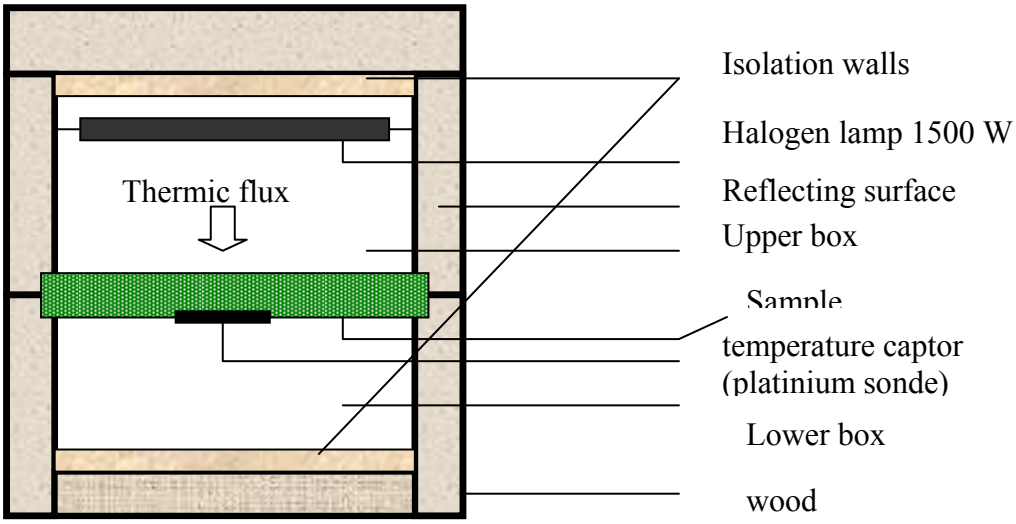

Fig.4: General diagram of thermal diffusivity measurement the device

2.2.4. Measurements of the material water activity

To determine the isotherms of sorption, a sample of dried Spirulina paste, was spread out in a sufficiently homogeneous thin film over an aluminum foil. The prepared sample is placed in a drying oven at adjustable temperatures. The mass of the product is measured all the ten or fifteen minutes. Then a second sample is subjected to the same treatment for a longer time (from 30 to 40 minutes). A third sample is handled the same manner as previously but for a longer period of time going from one hour and half to two hours. The water content is thus determined according to equation:

$$X_{eq} = \frac{m_H - m_s}{m_s} \qquad \textbf{(3)}$$

Where, m_H is the wet mass of the product m_S is its dry mass.

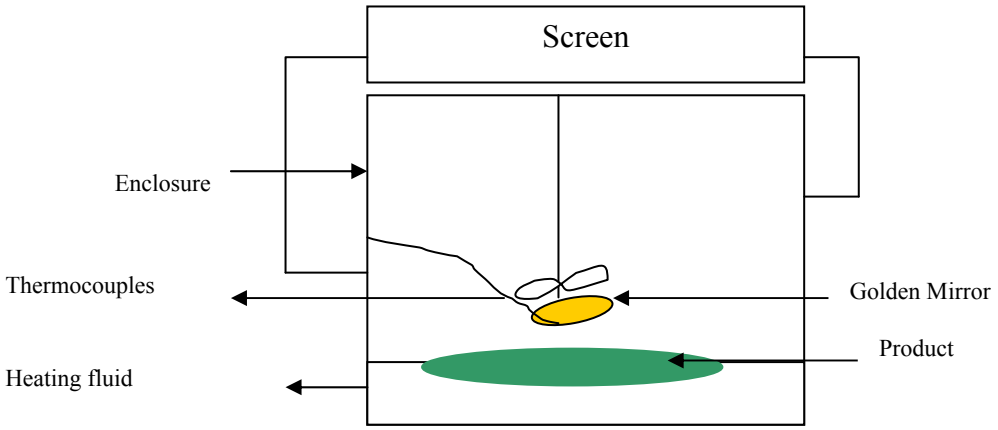

Fig. 5: *General diagram of the Activity meter*

Measurements with an Activity meter FAST (Food Analysis-Science and Technology) to measure A_W (the water activity) of the product. This apparatus is used to measure the water activity of agro food, chemical, medicinal and cosmetic products.

Its technology is based on the measure of air relative humidity above the product, by measurement of the dew point. A gold mirror, is heated then cooled, then a mist is formed on its surface. An optical sensor, located above the mirror measures the temperature of this mist T_m. This temperature is equal to the temperature of dew point. An IR thermometer measures the temperature of the sample surface T_S,

3. RESULTS AND DISCUSSIONS

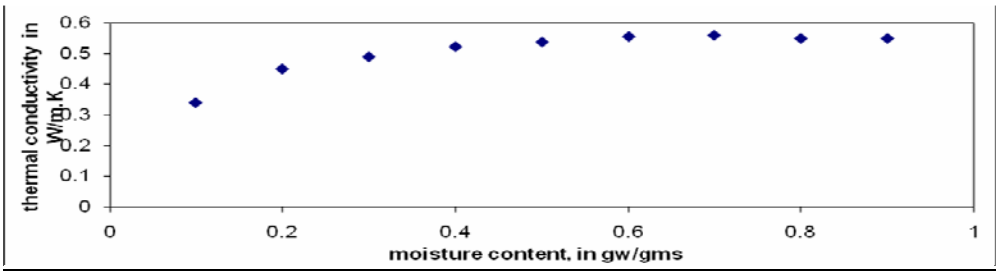

Fig.6: *Thermal conductivity versus moisture*

Specific heat values calculated from experimental data on thermal diffusivity are: $C_{P1} = 5{,}45$ kJ.kg $^{-1}$ K $^{-1}$; $C_{P2} = 5{,}84$; $C_{P3} = 6{,}10$; $C_{P4} = 6{,}36$; $C_{P5} = 6{,}65$; $C_{P6} = 6{,}89$; $C_{P7} = 7{,}17$: $C_{P8} = 7{,}44$; $C_{P9} = 7{,}68$; $C_{P10} = 7{,}92$. Calculations give for an average value of diffusivity: $\overline{a} = 1,\ 54.10^{-7}\ m^2\ s^{-1}$

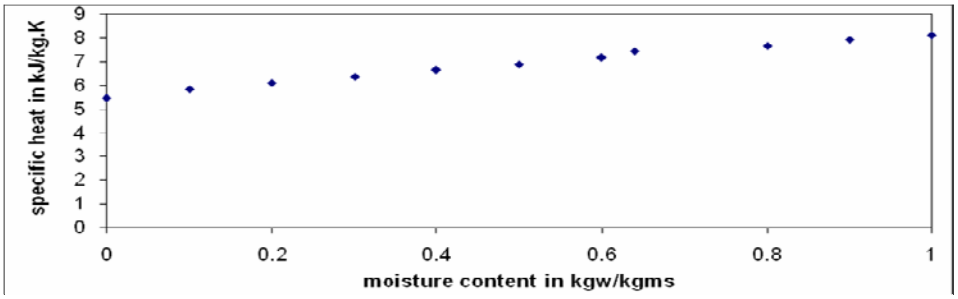

Fig.7: *Variation of specific heat with respect to water content*

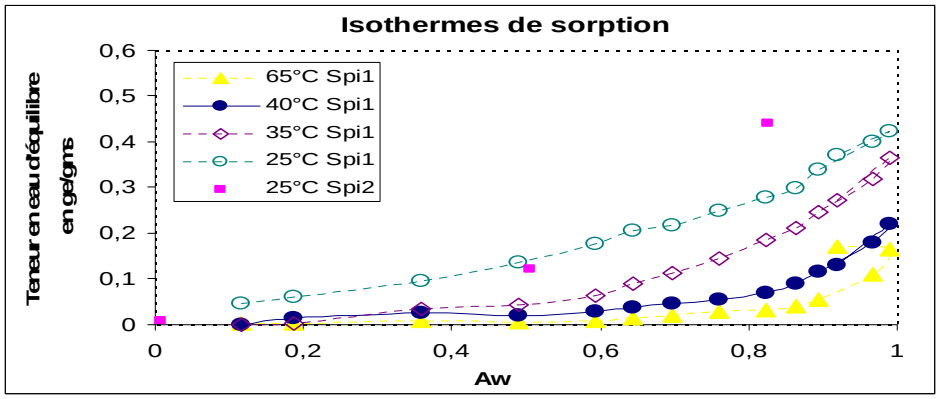

Fig.8*: Sorption isotherms of Spirulina paste at various temperatures*

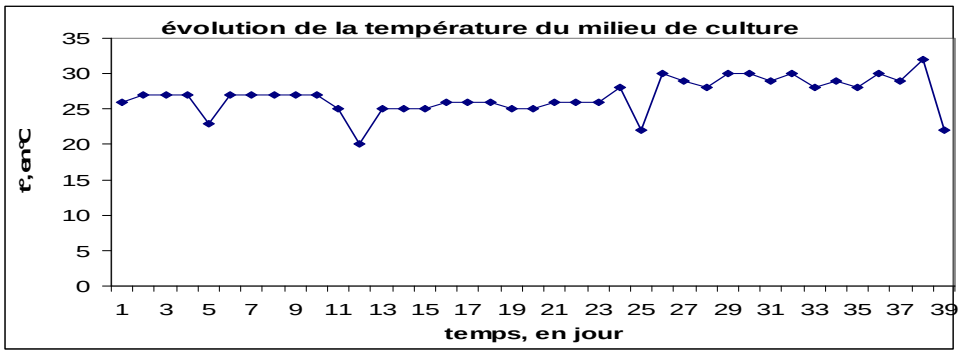

Fig.9 Evolution of culture medium temperature

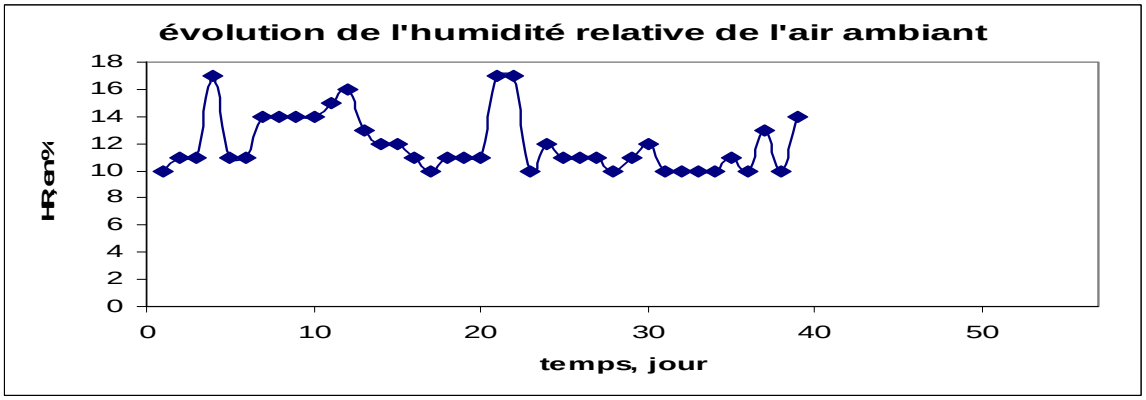

Fig.10: Evolution of ambient air relative humidity

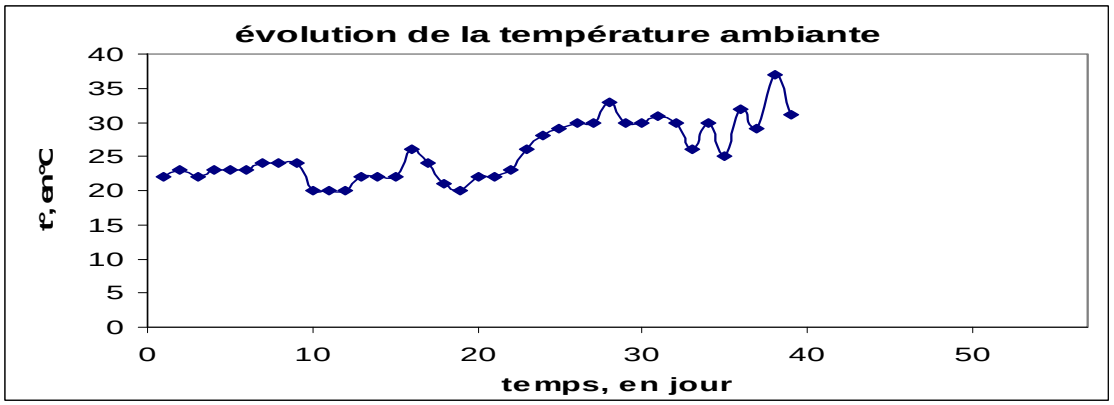

Fig.11: Quantities of water added to culture's medium

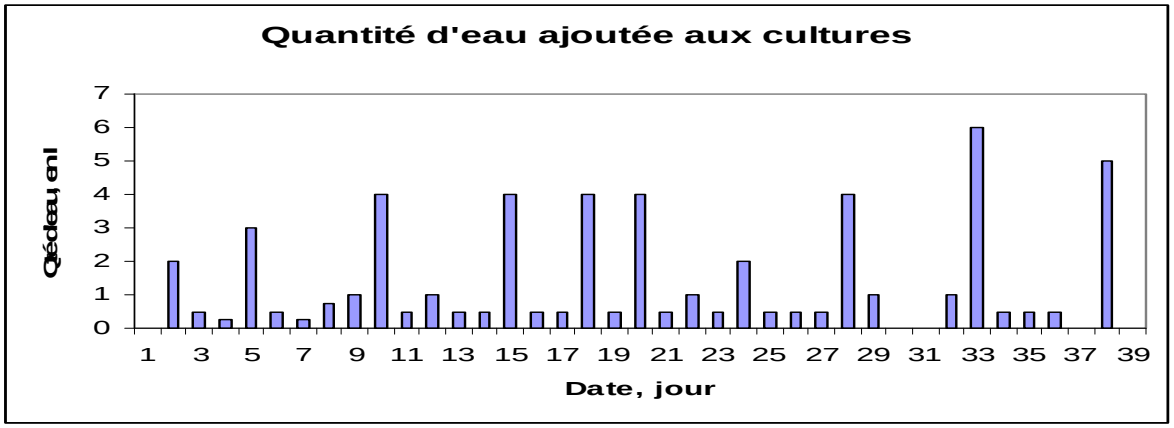

Fig.12: Evolution of ambient air temperature

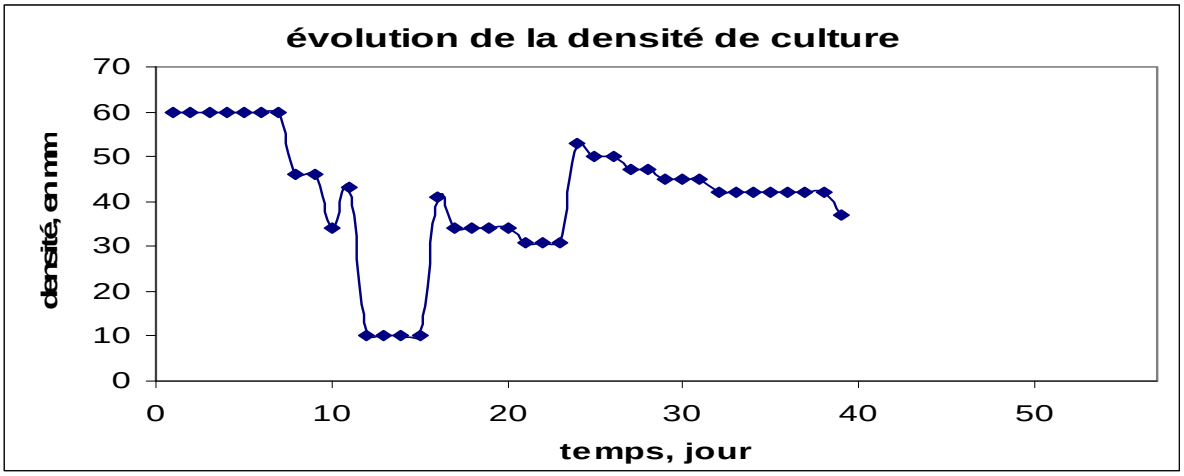

Fig.13: Evolution of Spirulina culture density

4. CONCLUSIONS

The experimental results show that the paste of Spirulina is a bad heat conductor, what could explain its great resistance to very high temperatures of the Kanem desert (until 48°C in the shade). But Spirulina is a micro-organism which also resists the low temperatures (until 10°C in the farm of Mr. *J P Jourdan*). Thus Spirulina is not appreciably affected by rather significant variations of temperature. Let us note however, that beyond certain temperatures (higher than 70°C for example), the trichomes open and enter in hibernation; what could lead them to death if the temperature continues to increase.

In addition, with the increase in the water content, thermal conductivity grows and reaches a limit value close to that of water's thermal conductivity; what in our opinion is explained by the fact that at a certain limit value of the water content, the properties of the alga are inhibited with regard to its "microscopic" character and the "macroscopic" character of measurements.

Moreover, we notice that the specific heat of Spirulina paste depends in a linear way with regard to the water content.

Furthermore, the method of the boxes that we used is effective only starting from a minimal product surface. Indeed studies carried on the spot at N'Djamena Faculty of Science in Chad; show that if the surface of the sample is lower than 100 cm^2, the results of measurements do not correspond any more to reality. Thus it is essential to use sufficiently large samples, to avoid data distortion.

On figure 8 are plotted the experimental sorption isotherms obtained in N'Djamena. The experimental points corresponding to spi2 were carried out at the University College of *Bourg En Bresse* (about 100 km east of Lyon (France)) with the same method. Apart the points obtained with water activity (A_w) close to 1, the values agree with expectations. The sorption isotherms are close to that of tropical products dried in sahelian zone, such as onions or mangos. During the drying of Spirulina in Chad, the average moisture of the air can vary from 10% in February to 55% in August. If the air temperature is higher than 35°C, these values

correspond to equilibrium water content lower than **0,05gwater per gram of dry matter**, i.e. **5%**. However, to ensure its conservation, water content must be lower than 8% in dry Spirulina (according to standards). These figures thus show that under the conditions of N'djamena's hygroscopy and average temperature, the equilibrium water content reached is sufficient to preserve the product.

Moreover, as the theory envisages it, the curves corresponding to high temperatures are lower than those of low temperatures.

The experimental sorption isotherms obtained better fit the Henderson model. In a forthcoming article, we will calculate the parameters of this model using experimental data.

It interesting to notice that the "Bell" shaped curve (BONIN, 1992) giving the evolution of Spirulina density could be seen in our results (Figure 13)

We see then that Spirulina has no viable concurrent as source of food supply. It doesn't need special skills to perform Spirulina culture at the semi-industrial level. Furthermore the production of Spirulina is more economic than that of conventional food as meat, corn, maize or sorghum (it necessitates less water, less space and less time for harvest).

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EFFECT OF *SATUREJA KHUZESTANICA* ESSENTIAL OIL, ULTRASOUND TREATMENT AND ULTRAVIOLET RADIATION ON THE OXIDATION STABILITY OF RAPESEED OIL

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ABSTRACT

The effects of ultrasound treatment (30 kHz, 100 W, 30 s), Ultraviolet (UV) irradiation (30 min), and *Satureja khuzestanica* essential oil (0.08%) extracted by ohmic ultrasonic extractor on oxidation of rapeseed oil were studied. GC-MS analyses of *Satureja khuzestanica* essential oil (EO) revealed that carvacrol (88.6%) was the major component of EO. Peroxide value (PV), and Thiobarbituric acid (TBA) value were measured in rapeseed oil and showed that EO had an antioxidant effect in comparison to BHT in sample oils treated or no treated by ultrasound and UV. The results indicate that ultrasound and UV treatment increase oil oxidation but EO can act as antioxidant and increase oil stability. Therefore, EO could be used as a natural antioxidant in oils for food uses.

Keyword: *Satureja khuzestanica*essential oil/ ohmic ultrasonic extractor /Ultrasound treatment / UV treatment/Lipid oxidation

1. INTRODUCTION

Lipid oxidation is a challenging problem in the production, processing and storage of vegetable oil. It causes problems such as Unpleasant tastes, odors and color of products and decrease in nutritional quality and safety by the formation of secondary toxic compounds.

Their oxidative stability depends on many factors, including the unsaturation of their fatty acids, composition of minor components, use of antioxidants and environment conditions like temperature, UV and exist of free radicals (Keszler et al., 2000; Hashemi et al., 2011a).Ultrasound and UV radiation are a type of alternative non-thermal technology for pasteurization and sterilization processing in food industry. Ultrasound is defined as mechanical waves with frequencies higher than threshold for human. It's simplest method for peeling, acceleration or inactivation enzyme reaction, extraction of essential oil and bioactive principles from herbs (Hashemi et al., 2011b, Dolatowski et al., 2007).

Satureja khuzestanica Jamzad, a member of Laminaceae family, a plant native to Iran. It is a small shrub, branched stem ± 30 cm high, densely leafy, broadly ovaiate-orbicular covered with short white hairs. Extracts and essential oil of this spice plant have been shown to have antiseptic, anti-inflammatory, anti-nociceptive and antimicrobial properties (Hadian et al., 2011; Hashemi et al., 2012).

In this paper, effects of ultrasound, UV treatment and EO on oxidation of canola oil during storage were investigated.

2. MATERIALS & METHODS

2.1 Plant Materials & oil

Aerial parts of *Satureja khuzestanica* were collected from the wild growing plants at the full flowering stage in September to October 2011.

Voucher specimen of the species (MPH-1582) was deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran. Commercial canola oil with no additives was obtained from Narges company.

2.2 Extraction of EO

Extraction of essential oil from *Satureja khuzestanica* was performed with the newly designed ohmic ultrasonic distillation. The extractor unit consisted of a cylindrical chamber (0.07 m internal diameter and 0.25 m length) made with Teflon. It was equipped with two Titanium electrodes.The system was fully automated for which the voltage (0–300 V) and current (0–16 A) and temperature could be controlled, monitored and recorded to a data sheet through out the experiment. A Hielscher ultrasonic device (UP100H, 100 W, 30 kHz) with a titanium sonotrode (tip diameter10 mm) was used to sonicate the sample containing the plant materials. The extraction unit was also equipped with an all-glass clevenger-type

apparatus.For each experimental run, 19 g (9% moisture) of the plant material was charged in to the chamber together with 500 mL brine (NaCl) solution (0.3% w/v). (Sodium chloride will provide sufficient electrical conductivity between two electrodes for the heat up process to be swift.) Prior to heating process, the plant materials which were fully immersed in brine solution was sonicated for 3 min in order to improve the EO release from the cell. The ohmic system was then switched on. A constant voltage of 150 V was applied between the two electrodes to increase the solution temperature from initial value of 20.8°C right up to boiling. The temperature rise was recorded at about 19.8°C/min. The extraction of EO was continued for 18 min. The EO was collected, dried under anhydrous sodiumsulphate and stored in sealed vials at 4 °C until used.

2.3 EO analysis

The EO was analyzed by GC-MS. The analysis was carried out on a Thermoquest- Finnigan Trace GC-MS instrument equipped with a DB-5 fused silica column (60 m ×0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed to increase from 60 to 250 °C at a rate of 4 °C/min and finally held for 10 min; transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35–465 amu with an ionising voltage of 70 eV and an ionisation current of 150 mA.

GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the continuous flow of 1.1mL/min; the split ratio was the same as for GC-MS. The oven temperature was raised from 60 to 250 °C at a rate of 4 °C/min and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. Semi quantitative data were obtained from FID area percentages without the use of correction factors.

Retention indices (RI) were calculated by using retention times of n-alkanes (C6–C24) that were injected after the oil at the same temperature and conditions. Compounds were identified by comparison of their RI with those reported in the literature (Adams, 2007) and their mass spectrum was compared with the Wiley Library (Wiley 7.0).

2.4 Oil sample preparation

Samples (control, rapeseed oil with EO at 0.08%, and rapeseed oil with BHT at 0.02%) are divided into two groups; the first group treated with ultrasound and UV treatment while the

second group prepared with no ultrasound or UV treatment. Then the PET bottles were poured with oils up to 250 mL volume of container, and stored in a laboratory oven under dark condition at 25 ± 1°C during 60 days. The temperature were controlled and the data recorded by data logger (LASCAR, England).

2.5 Ultrasonic and UV treatment

The canola oil (control, with EO or BHT) was treated without heating using a fixed Hielscher ultrasonic device (UP100H, 100 W, 30 kHz) with a titanium sonotrode (tip diameter10 mm) in a cooled jacket flask (250 mL). Approximately 100 mL of oil was placed in the flask and treated for 30 s with ultrasonic treatment. In addition, oil samples irradiated with UV rays for 30 min (1.8 kW UV lamp, EMA, Sverdlovsk, Russia).

2.6 Chemical tests

PV was measured by treating a solution of oil (5 ± 0.05 g) in 30 mL acetic acid–chloroform with 0.5 mL saturated potassium iodide solution and titration with 0.1 N sodium thiosulfate. Measurement of TBA value was done by heating a 5 mL aliquot of a solution of sample (50–200 mg) in 25 mL 1-butanol with 5 mL TBA reagent at 95 °C for 120 min and reading the absorbance at 530 nm using distilled water in the reference cuvette (AOCS, 1998).

2.7 Statistical analysis

Experiments on each of samples were performed at three times. Statistical analyses were calculated at each time, so were analyzed by SPSS (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA) and Minitab ver. 11.12 (Minitab Inc., USA).

TABLE 1. GC-MS of*Saturejakhuzestanica*essntial oil

Component	R_I	Percentage
α-Pinene	939	0.06
Myrcene	983	0.04
β-Pinena	978	0.04
p-Cymene	1018	0.4
Limonene	1026	0.7
γ-Terpinene	1063	0.06
trans-sabinene hydrate	1075	0.3
Linalool	1099	0.8
Borneol	1163	0.5
Terpine-4-ol	1164	0.6
Cumin aldeheyde	1218	0.1
Carvacrol methyl ether	1242	0.3
Thymol	1292	1.7
Carvacrol	1302	88.6
β-Caryophyllene	1432	0.4
β-Bisabolene	1528	0.06
Total		97

3. RESULTS AND DISCUSSION

3.1 EO composition

Amount of the essential oil extracted from *Satureja khuzestanica* using ohmic ultrasonic extractor was 16.41 % (v/w). GC-MS analysis of the EO resulted in the identifications of 16 compounds. The percentages of each component of EO were quantified by peak area using the FID detector. Percentages of components of the EO are summarized in Table 1. Main components of the EO include: carvacrol (88.6%), thymol (1.7%), and linalool (0.8%).

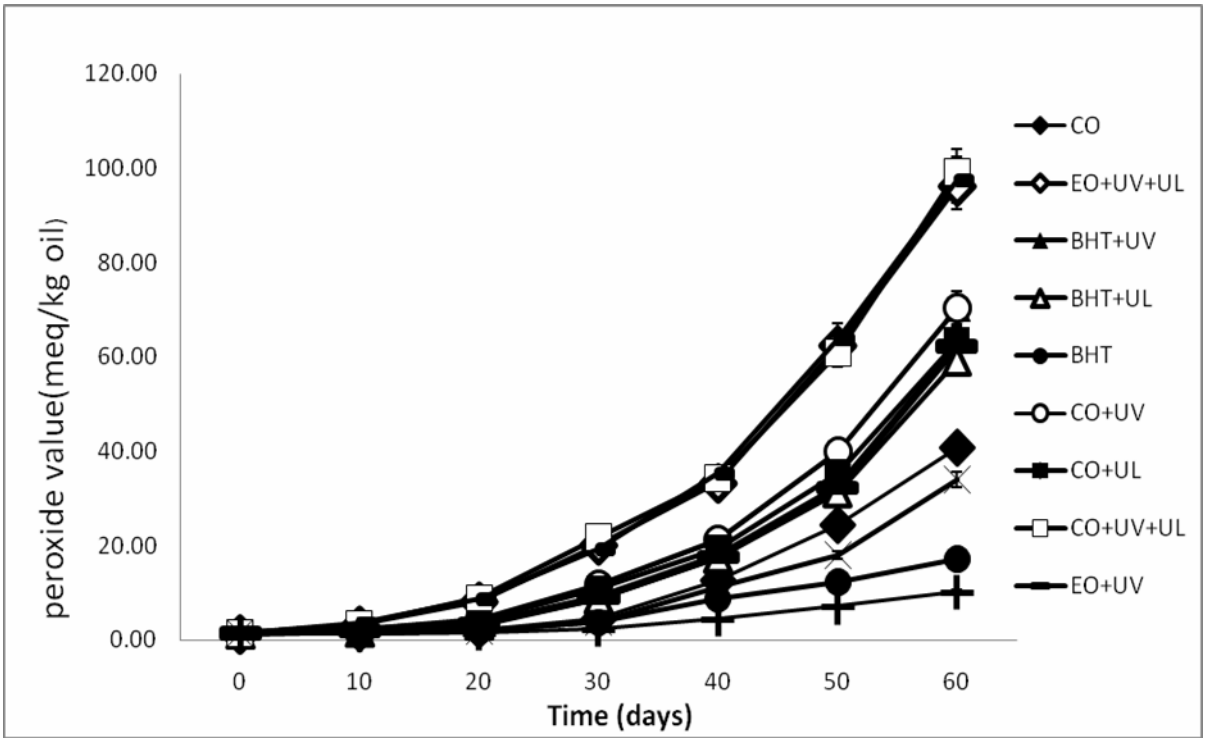

Figure. 1. PV of canola oil samples.

3.2 Effect of EO, UV and ultrasound treatment on the canola oil oxidation

The PV test is used for detection the initiation stage of the oxidation process (Hashemi et al., 2011b). As shown in Fig. 1 oil samples were prepared with EO show better efficacy against oxidative reactions in comparison with BHT. UV and ultrasound treatments both have negative effect on oxidative stability of all samples especially in oil without any additives. UV treatment resulted in higher peroxide formation than ultrasound treatment. The findings also confirm that ultrasound treatment combined with UV strongly increased oxidation of the samples so that antioxidant effects of EO and BHT was practically disappeared.

The TBA test is an easy and quick method widely used for the assessment of secondary product of oxidation in which malonaldehyde is derivatized (Hashemi et al., 2011b). The effects of EO and synthetic antioxidants (BHT) on TBA values of canola oil at 25 °C for 60 days are shown in Fig. 2. As it shows, TBA values were considerably higher in the samples with UV and ultrasound treatments, whereas UV was more destructive than ultrasound. However combination of ultrasound and UV had a large impact on the production of secondary oxidation but comparison of samples were prepared with EO to samples with BHT showed that TBA values in oil with EO were less than oil with BHT.

Result of GC-MS shows that main compound of EO is carvacrol and antioxidant activity of it depends on this component. This phenolic monoterpene compound of EO has a hydrogen donating ability which can act as chain breaking antioxidants in free radical chain reactions, converting lipid radicals to more stable products, thus extending the shelf life of canola oil (Hashemi et al., 2011a, Hashemi et al., 2012).

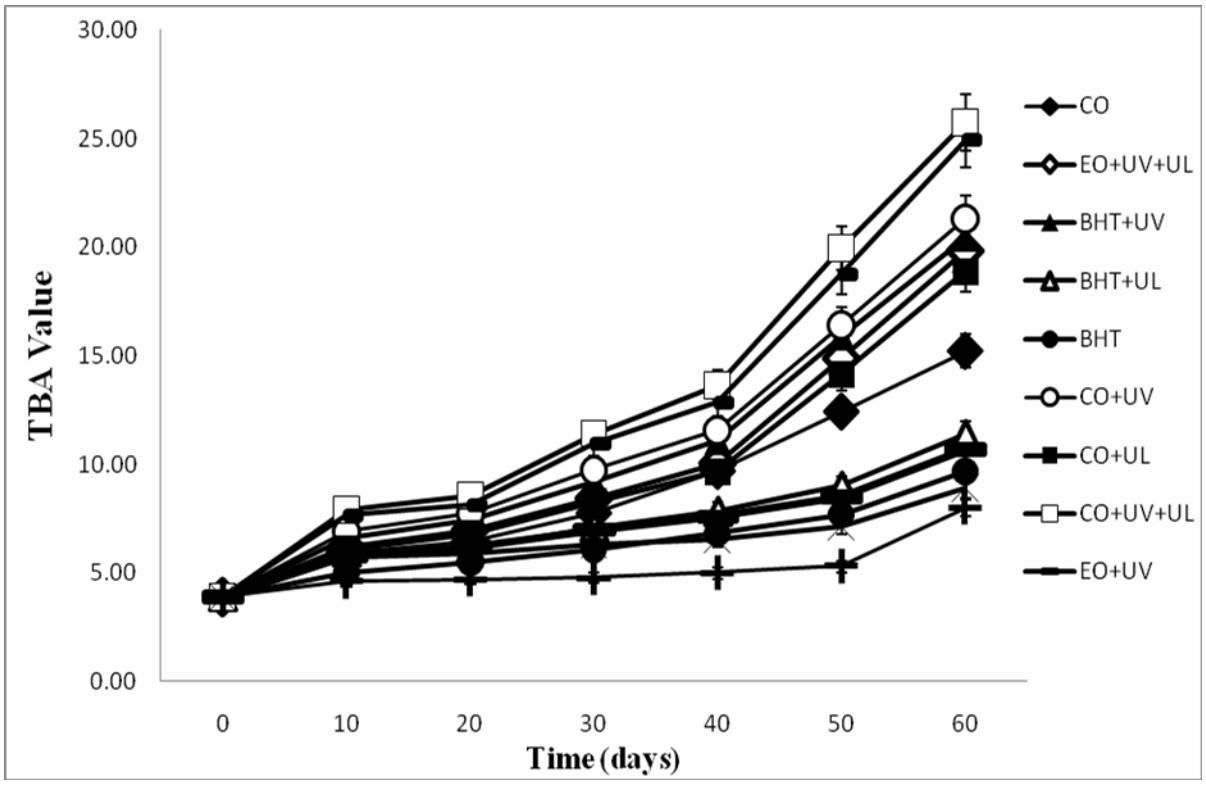

Figure. 2. TBA value of canola oil samples**.**

4. CONCLUSIONS

In this study we clearly demonstrate that EO is a suitable antioxidant for preserving rapeseed oil against oxidation. The EO offered better activity than those provided by synthetic antioxidant such as BHT particularly under forced conditions. In addition, ultrasound and UV treatment of food lipid can increase oxidation process.

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COMPARATIVE INVESTIGATION OF THE EFFECT OF DEHYDRATION PROCESSES ON COLOR, TOTAL PHENOLS, AND WATER AND OIL RETENTION CAPACITIES KINETICS OF LEMON (*CITRUS LIMON*. V. LUNARI) PEELS

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ABSTRACT

The aim of this work is to establish a comparative investigation of the effects of microwave dehydration (100, 180, 300, 450, 600 W), air drying (40, 50 and 60 °C) and infrared drying (40, 50 and 60 °C) on drying kinetics and on color, total phenols, water and oil retention capacities kinetics of lemon (*Citrus limon*. v. lunari) peels. Lemon peels were dried until reaching a final water content of 0.1 kg/kg DM. Drying time sharply decreased with increasing drying temperature or microwave power level. Microwave drying showed the short drying time. The maximal phenolic contents were preserved for microwave drying at 450 W (1.855 ± 0.016 g caffeic acid/100g DM). However, air drying and infrared drying (40 - 60°C) preserved total phenolic contents about 1.164 ± 0.063 et 1.231 ± 0.1 g caffeic acid/100g DM. Total color difference, ΔE, value increased progressively with increasing drying temperature or microwave power level. The microwave drying at 100 W and the infrared drying at 40 °C led to the lowest total color difference and preserved as well the yellowish initial color of fresh lemon peels. The infrared (40, 50 and 60 °C) and microwave (300, 450W) drying were in favour of the best water retention capacities.

Keywords: *lemon peels, Infrared drying, microwave drying, air drying, drying kinetics, kinetics of quality attributes.*

1. Introduction

Large amounts of lemon fruits (*Citrus limon*) are produced worldwide for industrial uses and results in the accumulation of high amounts of by-products such as peels, seeds, cells and membrane residues. These by-products can be used for the production of molasses, pectins, essential oils, limonene and cattle feed (Bocco et al., 1998; Jeong et al., 2004; Li et al., 2006). However, some research studies have highlighted the potentially healthy attributes of the lemon by-product. Gorinstein et al. (2001) found levels of dietary fiber to be 14 g/100 g DM in the peels of lemons, in comparison to 7.34 g/ 100 g DM in the peeled fruit itself. In addition, lemon by-products are a good source of phenolic compounds especially the characteristic flavanone glycosides which mainly include naringin, hesperidin, narirutin, and neohesperidin (Kamran Khan et al., 2010).

Lemon peels, because of their high moisture content (more than 75 %) are highly perishable and so require a good conservation if they aren't used immediately.

Dehydration of foods is one of the most common processes used to improve food stability, since it decreases considerably the microbiological activity and minimizes physical and chemical changes during it storage (Barrozo et al., 2001). Also, it extends the shelf-life but it produces sensorial characteristics generally lower than those of fresh products. Comparative investigation of the effects of different dehydration processes on the product quality indicators and their degradation kinetics is always essential to choose the optimal process and select operating conditions.

2. Material and methods

2.1. Material

Fresh *Citrus limon*. v. lunari were picked in Beni Khalled (Nabeul, Tunisia) in their commercial maturity (yellowish color) . Whole citrus fruit were washed, strained with blotting paper and stored at 4°C until processing. The average weight of lemon is about 160 ± 8.03g. The peel represents ~30 % of total weight for lemon fruit. Fresh lemon peels were cut as cubes of 1 cm^2 before processing. The drying process was applied until the weight of the samples reduced to a level corresponding to moisture content of about 0.10 kg water/kg DM. The choice of this criterion is joined to the microbiological stability of the product.

2.2. Microwave drying

A programmable domestic microwave oven (type Samsung, TDS, M1714), with a maximum output of 850 W at 2450 MHz was used for the drying experiments. The drying was performed according to a preset power (100, 180, 300, 450 and 600 W). Moisture loss was measured by taking out and weighting the dish on the digital balance (GIBERTINI, Germany) periodically.

2.3. Air drying

Drying experiments were performed by using a convective pilot air dryer. The drier works as an open-loop system and is controlled by a computer, with temperature, relative air velocity and relative air humidity PID controllers and computerized data acquisition (Courtois et al., 2001). Drying experiments were carried out at 40, 50 and 60°C, with the humidity of ambient air (4-16 %) and an air velocity of 1.5 m s^{-1}.

2.4. Infrared drying

Drying experiences of the lemon peels were conducted by the means of an infrared moisture analyzer (Sartorius MA 40). Several tests were achieved for different infrared drying temperatures (40, 50 and 60 °C).

2.5. Quality parameters

Experimental kinetics quality attributes of lemon peels were established by measuring the changes of total phenol contents, color and water and oil retention capacities at different drying times. All measurements were determined in triplicate.

2.5.1. Total phenols content

For the determination of total phenols content, extracts was performed according to George et al. (2005). A sample (3 g, weighed with precision) was homogenized with
15mL of acetone (70:30, v/v) for 10 min. Mixture supernatants were then recovered by filtration (Whatman n°1) and constituted the raw extracts.

A solid phase extraction using a 1 cc-Oasis-HLB™ cartridge (Waters) was carried out on the raw extract of lemon peels to eliminate the water-soluble reducing interferences. Colorimetric correction was performed by subtracting interfering substances contained in the water washing extract to the raw extract. The absorbance of the resulting blue solution was

measured spectrophotometrically at 750 nm and the concentration of total phenols was expressed as g caffeic acid equivalent/100 g DM (CAE/100 g DM).

2.5.2. Color determination

The color of fresh and dried lemon peels was determined using a CR-200 Minolta Colorimeter calibrated with a white standard tile. The results were expressed as Hunter color values of *L*, a** and *b*,* where *L** was used to denote lightness, *a** redness and greenness, and *b** yellowness and blueness. The total color difference (*ΔE*) was determined using the following equation:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad \text{Eq. (3)}$$

The subscript "0" in both equations refers to the color of fresh sample of lemon peels

2.5.3. Technological properties

For the determination of the water and oil retention capacities, the removed samples were freeze dried at 200 mbars and -50 °C (Modulyod, freez dryer, Thermo Electro Corporation) milled and sifted through 0.1 mm mesh screen.

The water retention capacity (*WRC*) was determined according to Garau et al. (2007). Samples (0.5 g) were hydrated in excess (24 h) in a 50 mL tube, prior to centrifugation at 2000g for 25 min. Supernatant was decanted. Water retention was recorded as g water/g dry sample.

Oil retention capacity (*ORC*) was measured as the oil retention capacity. Samples (0.5 g) were mixed with sunflower oil (10 mL), centrifuged at 2000g for 20 min and the supernatant decanted. *ORC* was expressed as g oil/g dry sample.

2.6. Statistical analysis

Experimental results were presented as mean values ± standard deviations. Mean values were analyzed by ANOVA test using SPSS software® version 17.0.

3. Results and discussion

3.1. Phenol content degradations

Fig 1a, 1b and 1c show the variation of the total phenols contents versus drying time during drying at 40, 50, and 60°C for air drying and infrared drying and at 100, 180, 300, 450 and 600 W for microwave drying.

The initial phenols content of fresh lemon (v. lunari) peels was 2.451± 0.022 g CAE/100 g DM. Overall drying at different temperatures and microwave powers resulted in a significant ($p<0.05$) reduction in the total phenol contents. A maximum reduction of 64% in comparison with fresh lemon peels in the total phenolic content (PC) was observed in infrared dried lemon peels at 40 °C and in microwave dried lemon peels at 180 W. A probable reason for this could be the long drying time required at 40 °C for infrared drying and at 180 W for microwave drying to achieve the final moisture content of 0.10 kg water/kg DM. Garau et al. (2007) reported that longer drying times resulted in a reduction in phenol contents for orange by-products. The maximal phenolic contents were preserved after 19.5 min of microwave drying at 450 W (1.855 ± 0.016 g CAE/100g DM).

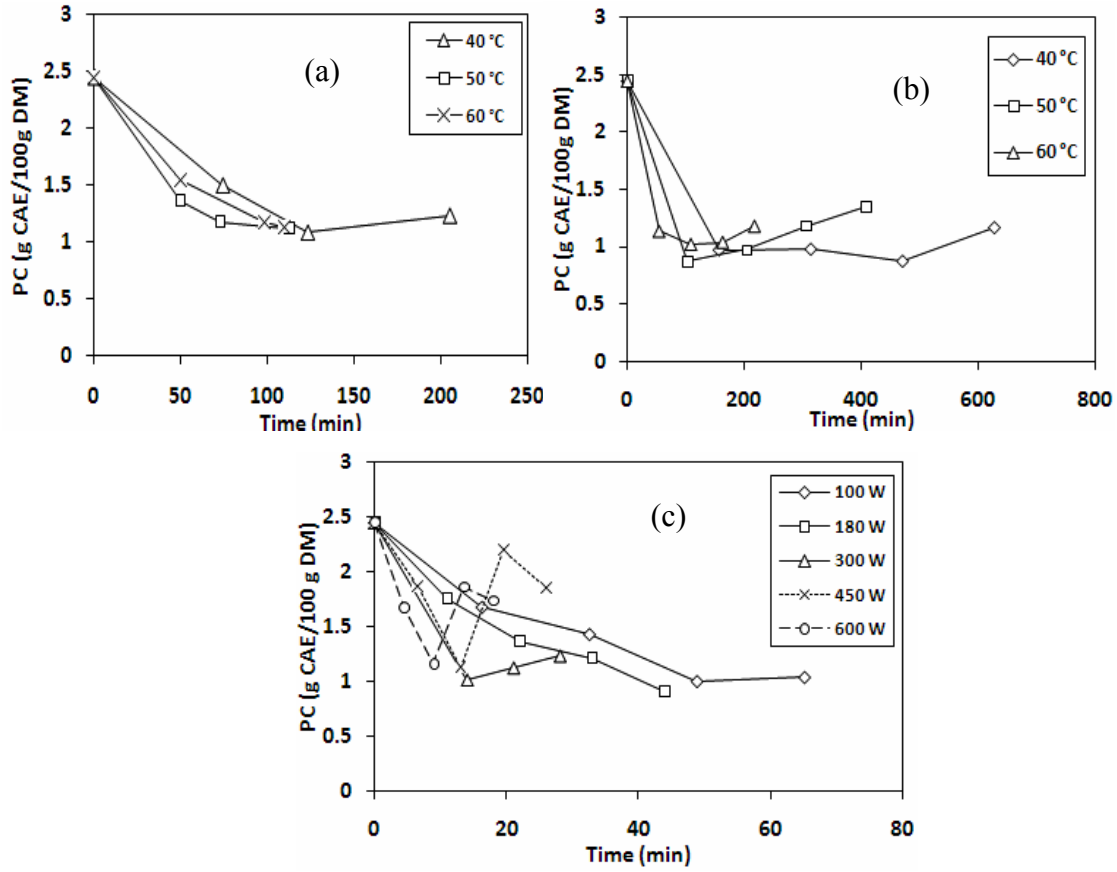

Fig. 1 – Variation of total phenol content during air drying (at 40, 50 and 60 °C) (a), infrared drying (at 40, 50 and 60 °C) (b) and microwave drying (at 100, 180, 300, 450 and 600 W) (c).

Fig 1a, 1b and 1c show that the total phenol content decreased linearly at the beginning of drying and then continue to decrease (air dying) or shows a significant increase at the end of

microwave drying (300, 450 and 600 W). Besides, total phenol content gradually increased as drying temperatures increased for lemon peels air dried at 40, 50 and 60 °C and microwave dried at 100 and 180 W.

During microwave drying at high power levels (450, 600W), phenol contents decreased after 9 and 13 min at 600 and 450 W than increased abruptly to attain 2.027±0.244 and 1.8±0.088 g CAE/100g DM at 450 and 600 W respectively. These results are in agreement with the findings of other researchers (Jeong et al., 2004; Xu et al., 2007), who found that microwave heat treatment can liberate the bound phenolic compounds in citrus peels and increase the amount of free phenolic compounds in the product. Que et al. (2008) indicated that the formation of phenolic substances occurred during oven drying at 70°C and mentioned that the formation of phenolic compounds might be due to the availability of precursors of phenolic molecules by non-enzymatic inter-conversion between phenolic molecules.

3.2. Color kinetics

Fig. 2 shows the effects of drying temperatures at 40, 50 and 60°C for air drying and at 40, 50 and 60°C for infrared drying and at 100, 180, 300, 450 and 600 W on the total color difference (*ΔE*) of dried lemon peels.

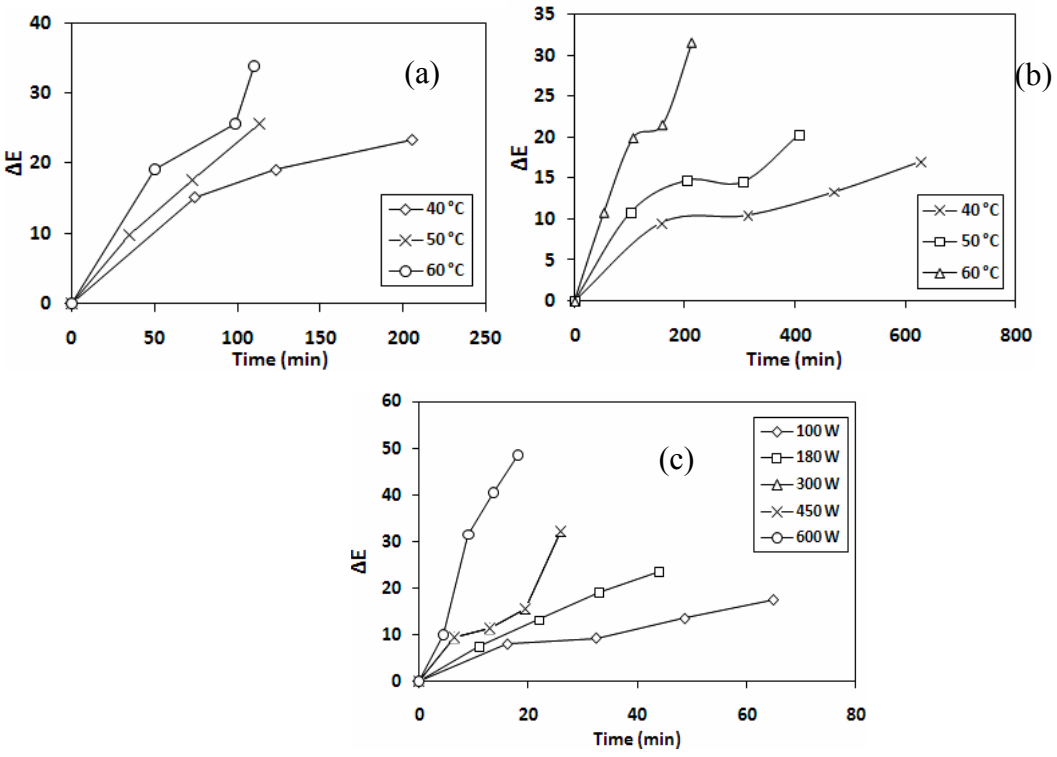

Fig. 2: Variation of total color difference (*ΔE*) of lemon peels during air drying (at 40, 50 and 60 °C) (a) infrared drying (at 40, 50 & 60 °C) (b) and microwave drying (at 100, 180, 300, 450 & 600 W) (c).

The total color difference increased with increasing drying time and air or infrared drying temperature and microwave power. Dried lemon peels had significant ($p<0.05$) color differences resulting from the browning reaction. In fact, samples at a given drying time do not have the same moisture content; hence the moisture decrease during drying and the corresponding reached water activities affects the non-enzymatic reactions (Troller and Christian, 1978).

3.3. Variation of water and oil retention capacities

Loss of water and volatiles, which occur during drying, results in major structural changes in materials that lead to textural and sensory characteristics different from the fresh product.

Fig.3 shows the variation of WRC and ORC of lemon peels during drying experiments; air drying, infrared drying (at 40- 60 °C) and microwave drying (at 100, 180, 300, 450 and 600 W). The highest values of water retention capacities were found in lemon peel samples (t=0min) (10.163 ± 0.627 g water/g DM). These values were near to those presented by Grigelmo-Miguel and Martin-Belloso (1999) for air dried orange peels (more than 11 g water/g DM).

Whatever the applied temperature and power, WRC of dried lemon peels decreased during drying. The infrared (40 -50 °C) and microwave (300, 450W) drying were in favour of the best water retention capacities. This result could be due to the fact that a higher temperature of drying creates a more rigid structure and, in consequence, produces the collapse of channels for water uptake. As a result, a smaller rehydration rate could happen (Ramallo and Mascheroni., 2012).

During drying, the product porosity increases as the water and volatiles are removed. However Krokida and Maroulis (1997) stated that the porosity of the final product could be controlled, if an appropriate drying method is chosen. Air-dried products have low porosity when compared to freeze, microwave and vacuum drying.

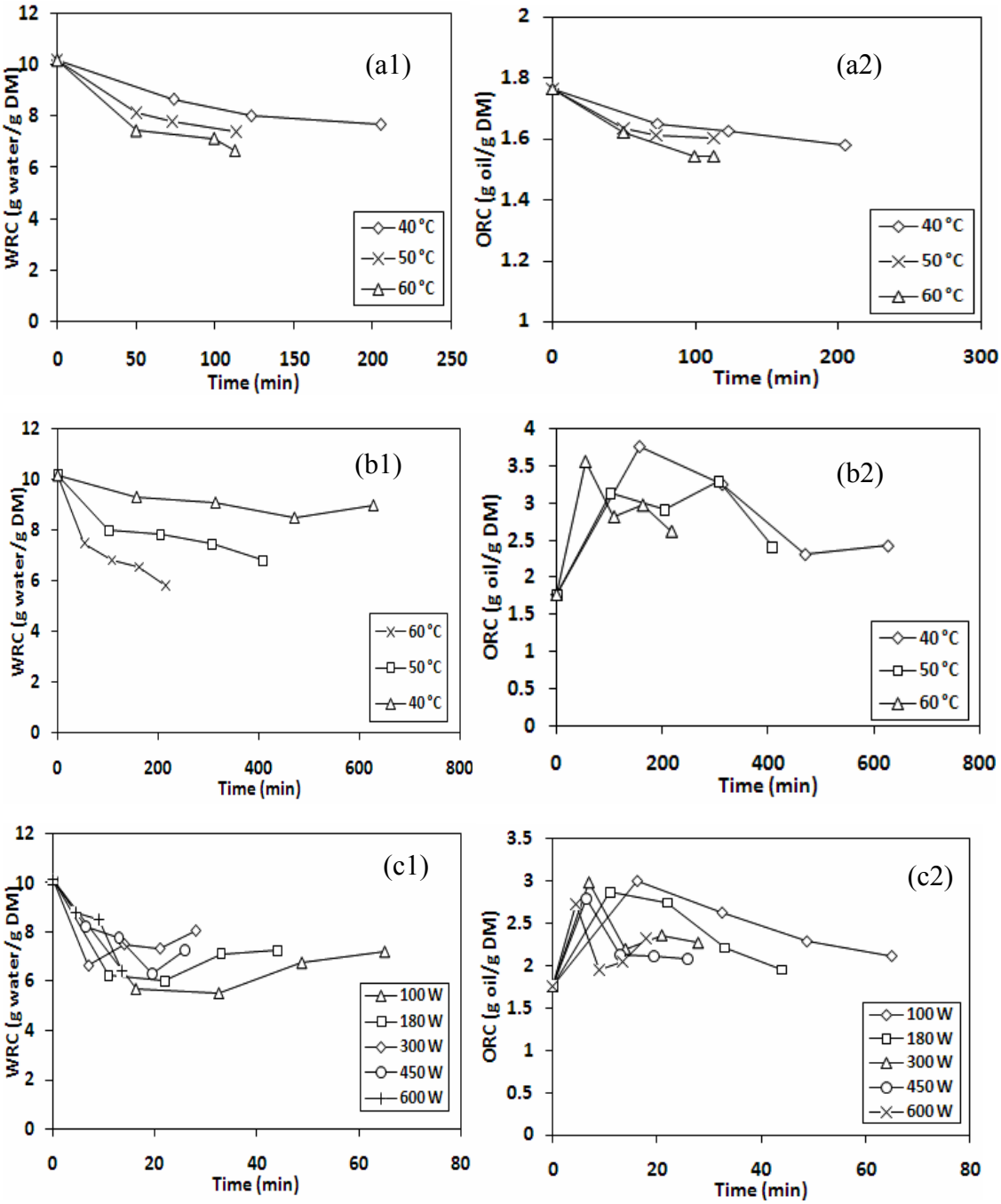

Fig. 3– Variation of water and oil retention capacities of lemon peels during air drying (at 40, 50 and 60 °C) (a1, a2), infrared drying (at 40, 50 and 60 °C) (b1, b2) and microwave drying (at 100, 180, 300, 450 and 600 W) (c1, c2).

For microwave drying, WRC decreases during drying but increased with the increase of applied microwave power. According to Aguilera and Stanley (1999) during fast drying processes, the product surface dries much faster than its core, a phenomenon that originates internal stresses that result in very cracked and porous product interior. On the other hand,

slow drying rates result on uniform and denser products (Brennan, 1994) with reduced re-hydration rate and capacity (Karathanos et al., 1996).

However, ORC decreased for air dried lemon peels and increased for microwave and infrared dried samples. Microwave and infrared drying processes could lead to changes of lemon peels at micro structural level which would be in favour of a best ORC.

4. Conclusions

The total phenol content decreased linearly at the beginning of drying and then values remain constant until the end of drying. However, microwave drying induces a gradual increased of total phenol content after 19.5 and 13.5 min of drying at 450 and at 600 W respectively. The maximal phenolic content was preserved for microwave drying at 450 W (1.855 ± 0.016 g CAE/100g DM). The maximal loss of total phenol content (63 %) was observed after 44 min of microwave drying at 180 W. WRC of dried lemon peels decreased during drying. The infrared (40 -50 °C) and microwave (300, 450W) drying were in favour of the best water retention capacities. The highest drying temperatures resulted in greater change in color. The microwave drying at 100 W and the infrared drying at 40 °C led to the lowest total color difference ΔE and preserved as well the yellowish initial color.

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ASSESSMENT OF SOLVENT EXTRACTABILITY EFFECT ON THE OLIVE KERNEL OIL AND BIOACTIVE COMPOUNDS RECOVERY

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ABSTRACT

Olive oil production represents an economic and social industrial activity that is highly relevant in the South European countries. One of the main by-products remaining after the olive oil manufacturing process is olive kernel. Olive kernel utilization is of significant economic interest, while in addition olive kernel oil presents very similar composition to that of olive oil. The Soxhlet extraction technique constitutes the common method for oil extraction from olive kernel that simulates the corresponding industrially used. The use of the extraction by solvent allows the olive kernel valorization by recovery of its residual oil. The oil extraction rate is influenced by a number of factors, including the size of the olive kernel particles and the intrinsic diffusion capacity of solvent facilitating oil extractability. Thus the solvent selection is considered the determinant factor for the extractability that can be investigated for the process optimization for both efficiency and economic reasons. In this study, various mixtures of solvents (n-hexane and petroleum ether) and different particle size of ground olive kernel (1,06 and 0,42 mm) were examined. Both of the examined factors proved to have a significant effect on the extracted olive kernel oil yield as well as on the recovery of the total unsaponifiable matter and its individual bioactive compounds such as stigmasterol and squalene. High particle size of olive kernel decreased the olive kernel oil yield but enhanced the total unsaponifiable matter. The highest oil yield was obtained using n-hexane: petroleum ether mixture in ratio of 3:1 and n-hexane: petroleum ether mixture in ratio of 1:3 for olive kernel's particle size 1,06 and 0,42 mm, respectively, accompanied by significant squalene and stigmasterol concentrations in extracted olive kernel oil.

Keywords: Olive Kernel, Solvent extraction, Unsaponifiable matter, Sterols, Squalene

1. INTRODUCTION

Olive (Olea europaea) is an evergreen tree that has been traditionally cultivated for olive oil and table fruit consumption. Despite olive trees' distribution over all continents, 97% of the world production of olive oil is concentrated in the Mediterranean countries, such as Spain, Italy, Greece, Portugal, Turkey, Tunisia and Morocco (Meziane and Kadi, 2008; Doymaz, Gorel and Akgun, 2004). Thus, olive oil production represents a valuable economic and social industrial activity for the South European countries (Rodriguez et. al., 2008).

The mechanical extraction process by pressing or centrifugation conventionally used for olive oil production has quite a large environmental impact due to the co-production of highly polluted olive-mill wastewaters and a semi-dry solid called 'olive cake' or 'olive kernel residue'. The amounts of these by-products vary mainly depending on the applied extraction technology (Hadj-Taïeb et. al., 2012; Rodriguez et. al., 2008; Saavedra et. al., 2006). Olive kernel residue consists of pulp pieces (21–33%), pit (42–54%), olive fruit skin (10–11%) and varied moisture content with higher in residues from centrifuge systems. Olive kernel usually contains oil that can exceed 8-12% depending on extraction system (Amarni and Kadi, 2010; Sánchez Morall and Ruiz Méndez, 2006; Doymaz, Gorel and Akgun, 2004). Therefore, olive kernel amounts delivered may be utilized for useful purpose as olive kernel oil presents very similar composition to that of olive oil regarding the triglycerides and essential fatty acids as well as the bioactive unsaponifiable materials content.

The use of the extraction by solvent allows the olive kernel valorization by recovery of its residual oil (Amarni and Kadi, 2010; Meziane and Kadi, 2008). The Soxhlet extraction technique constitutes the conventional method that simulates the corresponding used in oil industry for oil extraction from oilseeds or olive kernel (Virot et. al., 2007). According to the method the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium. Thus, it is considered generally a well-established technique that clearly surpasses in performance other extraction techniques (Wu et. al., 2011; Luque de Castro and Garcia-Ayuso, 1998).

Numerous theories have been proposed to explain the mechanism of oil recovery by solvent from ground and flaked oilseeds or oilseed cake residues (after mechanical process) (Baumler, Crapiste and Carelli, 2010). According to them, the performance of an extraction process is governed by both mass transfer and equilibrium phenomena. The oil extraction rate is influenced by a number of factors, including the thickness, size, shape and internal structure of the oilseed solid particles, and the intrinsic capacity for diffusion of solvent in the solid

flaked material (Baumler, Crapiste and Carelli, 2010; Tzia and Liadakis, 2003). Among all these factors, the effect of oilseed flakes' thickness is considered the most evident for the extraction process efficiency. The extraction apparent is argued that doesn't absolutely follow the diffusion's laws, but it is a complicated phenomenon which includes the oil dissolution and diffusion as well as the viscosity flux of the solvent into the solid's capillary pipes (Meziane, Kadi and Lamrous, 2006; Aguilera, 2003). Therefore, solid preparation is needed for an efficient extraction process.

The efficiency of extraction depends additionally on the solvent used, the extraction temperature, and the used extraction system (Tzia and Liadakis, 2003). Solvents such as hexane, ethanol, methanol, petroleum ether and acetone are the most commonly used in extraction of oil seeds. Hexane is often used for vegetable oil extraction mainly due to its dissolving ability and ease of recovery (Akaranta and Anusiem, 1996), while research has shown that petroleum ether could be used for oil extraction. Both solvents due to their chemically inert, high selective dissolving power and high volatility particularly suited for oil extraction. Other solvents such as acetone, ethanol and iso-propanol are also often used in oil extraction (Mani, Jaya and Vadivambal, 2007). So, the proper selection of solvent is considered the determinant for the extractability factor that should be investigated for the process optimization for both efficiency and economic reasons.

The minor components of oils and fats, which generally constitute the unsaponifiable matter, i.e. sterols, fatty alcohols, tocopherols, triterpene alcohols, and squalene provide important bioactive, nutritional and characteristic compositional properties that affect the quality of individual oils (Czaplicki et. al., 2011; Dhara, Bhattacharyya and Ghosh, 2010). Phytosterols possess the major part of the unsaponifiable fraction of vegetable oils, while the sterolic profile is characteristic for each oil indicating thus potential adulteration. Additionally, they are suggested for their functional properties i.e. anti-inflammatory antibacterial, antifungal, antiulcerative and antitumoral activities (Czaplicki et. al., 2011; Temime et. al., 2008). Squalene is also an unsaturated hydrocarbon with functional properties that is found in the unsaponifiable matter of olive and olive kernel oil.

The aim of this study was to examine the extraction of oil from olive kernel and to assess the extractability effect of most important factors affecting the extraction process, such as extracting medium solvent (various mixtures of n-hexane and petroleum ether) and the particle size of the olive kernel on the oil recovery as well as on recovery of the total unsaponifiable matter and its individual bioactive compounds such as sterols and squalene.

2. MATERIALS AND METHODS

2.1. Olive kernel

Freshly olive kernel obtained from a centrifugation system by a local olive oil mill. The initial moisture of the solid olive residue was 45,0 % (wet basis), that was reduced by air drying to 4,5 % (wet basis) before storage. This was done to avoid deterioration of the olive kernel oil and to facilitate the extraction. The air dried olive kernel was grounded by an electric mill (Retch ZM 1, Haan, Germany) so that the average diameters of particles of the solid were 1,06 and 0,42 mm.

2.2. Chemicals and solvents

Hexane, petroleum ether, ethanol, diethyl oxide of chemical purity and HPLC grade methanol and chloroform were purchased from Fisher Scientific (Fair Lawn, NJ). Reagent-grade potassium hydroxide was from Panreac Quimica S.A. (Barcelona, Spain). Standards of squalene and stigmasterol at least 98% pure were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3 Methods

2.3.1 Oil extraction procedure by Soxhlet

Soxhlet extractions were carried out using 40,00 g of dried ground olive kernel and 320 mL solvent (Liquid/Solid - L/S=8) at 50^{o}C for 8h. Two different particle size (1,06 and 0,42 mm) was tested. Hexane (n-H) and petroleum ether (PE) in different ratios were examined in extraction experiments. A mild temperature of 50^{o}C was chosen in extraction to avoid thermal degradation of bioactive compounds in the extracts. After the extraction, the miscella was distilled under reduced pressure by means of a rotary evaporator (BUCHI 461, Buchi Laboratotiums Tchnik AG, Flawil, Switzerland) and at temperature of 50^{o}C. The flask was then weighed and the operation repeated during 30 min until difference between two consecutive weights was less than 10% (m/m). Results obtained on the efficiency of extraction process were expressed as described hereinafter:

$$Yield\ (\%) = \frac{W_o}{W_s} \times 100$$

W_o and W_s are the weight of the olive kernel oil extracted from the sample (g) and the weight of the dried ground olive kernel (g), respectively. The extraction experiments were performed at least in duplicate.

2.3.2 Measurements

i. Unsaponifiable matter determination

The unsaponifiable fraction of the oil was determined using diethylether according to AOCS Official Method Ca 6b-53 and expressed in oil weight percent. The dried unsaponifiable matter was redissolved in 2 mL of HPLC eluant, filtered through a 0,45-μm membrane filter (Millipore, Bedford, MA), and the solution was used for chromatographic analysis of phytosterols and squalene.

ii. HPLC analysis of phytosterols and squalene

Analyses of phytosterols and squalene were carried out with an HP 1100 Series gradient HPLC system (Agilent Technologies, USA) combined with quaternary pump, diode array detector (Hewlett-Pachard, Waldbronn, Germany) and data analysis software (ChemStation for LC3D Software, Agilent Technologies, Waldbronn, Germany). A column (250 × 4,6 mm) packed with 5 μm particles Hypersil C18 (MZ Analysentechnik, Mainz, Germany) was used for the separation. The analyses were carried out with isocratic elution using methanol (100% v/v) at a flow rate of 1 mL/min. UV absorbance spectra were obtained in the range of 200–300 nm. The detection wavelength selected for sterol quantitation was 208 nm, which afforded the greatest response.

2.3.3 Statistical Analysis

Statistical analysis was performed using Statistica (statistica 7.0, StatSoft Inc.,Tulsa, OK, USA) statistical software. ANOVA was performed in order to evaluate the main effects of solvent mixtures and particle size on the oil yield and its compositional characteristics. Significant differences was defined at $P<0,05$.

3. RESULTS AND DISCUSSION

3.1 Extraction Yields

The results of olive kernel oil yield by various proportions of two solvents (hexane, petroleum ether) in the extracting mixtures and different particle size of ground olive kernel are shown in Table 1. As far as the effect of particle size is concerned, the oil yield increased significantly ($P<0,05$) as the particle size decreased; the oil yield increased from 8,41-9,69 to 10,26-12,21% as the particle size decreased from 1,06 to 0,42 mm with the highest oil yield

obtaining with an olive kernel particle size of 0,42 mm. Effect of intraparticle diffusion seems to gain importance for large particles causing appreciable decrease in the extraction yield; so part of the oil was not extracted due to the very long diffusion times of the solvent in the solid particles. This effect may be stronger with smaller particle sizes where intraparticle diffusion resistance becomes smaller because of the shorter diffusion path. By grinding of solid, more of the oil because is freed from the cells and therefore the oil yield was increased due to facilitated diffusion. Hence, extraction oil efficiency increased with decreasing of the particle size, as noted in the literature (Döker et al., 2010; Meziane, Kadi and Lamrous, 2006).

TABLE 1: Results of Oil Yield (%) from extraction experiments by various solvent mixtures and different particle size olive kernel.

Solvent mixtures	**Yield%**	
	Particle size	
	1,06 mm	**0,42 mm**
n-H	8,52	10,35
PE	8,41	10,57
n-H:PE 1:1	9,00	10,26
n-H:PE 2:1	9,16	11,16
n-H:PE 1:2	9,14	11,04
n-H:PE 3:1	9,69	11,87
n-H:PE 1:3	9,28	12,21

Furthermore, significant differences were found in the extraction yield of olive kernel oil depending on the different proportions of solvents used ($P<0,05$) with better mixture n-H:PE=3:1 or 1:3.

3.2 Unsaponifiable matter of extracted olive kernel oil

Total unsaponifiable matter content of extracted olive kernel oils are summarized in Table 2 for various proportions of two solvents (hexane, petroleum ether) in the extracting mixtures and for different particle size of ground olive kernel.

TABLE 2: Unsaponifiable matter (USM) of olive kernel oil extracted by various solvent mixtures and different particle size olive kernel.

Solvent mixtures	USM (%, w/w)	
	Particle size	
	1,06 mm	0,42 mm
n-H	2,47	1,68
PE	1,95	2,29
n-H:PE 1:1	2,12	2,08
n-H:PE 2:1	2,65	2,15
n-H:PE 1:2	2,36	2,26
n-H:PE 3:1	2,89	2,60
n-H:PE 1:3	2,82	2,69

The different proportions of solvents used resulted in olive kernel oils with significant different unsaponifiable matter ($P<0,05$) with the n-H:PE= 1:3 mixture providing higher USM% value. High proportion of n-hexane in solvent mixtures resulted in all cases in higher ($P<0,05$) percent of USM than petroleum ether.

3.3 Analysis of phytosterols and squalene

Based on phytosterol analysis, stigmasterol was used as main sterolic compound that was investigated in olive kernel extracted oils. Squalene and stigmasterol content (mg/100 g oil) of olive kernel oils are shown in Table 3 for various proportions of two solvents (hexane, petroleum ether) in the extracting mixtures and for different particle size of ground olive kernel. High squalene content was found in olive kernel oils extracted either by olive kernel particle size of 1,06 mm and/or by using high proportions of n-hexane in extracting solvent mixtures. As far as stigmasterol of olive kernel oils is concerned, n-H neat or in 3:1 mixture with PE enhanced its content.

TABLE 3: Phytosterols and squalene content of oils (mg/100 g oil) of olive kernel oil extracted by various solvent mixtures and different particle size olive kernel.

Component (mg/g oil)	**Solvent mixtures**						
	n-H	PE	n-H:PE 1:1	n-H:PE 2:1	n-H:PE 1:2	n-H:PE 3:1	n-H:PE 1:3
	Particle size: 1,06 mm						
Squalene	178,97	88,15	102,36	156,35	70,43	190,54	162,68
Stigmasterol	33,29	20,20	21,96	14,64	23,84	26,59	38,52
	Particle size: 0,42 mm						
Squalene	111,74	53,24	80,65	88,02	105,99	138,10	147,34
Stigmasterol	30,97	18,04	26,49	27,46	29,72	33,03	32,66

4. CONCLUSIONS

As was demonstrated, the various proportions of two solvents (hexane, petroleum ether) in the extracting mixtures and different particle size of ground olive kernel had a significant effect on the extracted olive kernel oil yield and on recovery of unsaponifiable matter. Among the particle sizes examined, the olive kernel granulated in 0,42 mm resulted in the highest oil yield. As for the various mixture solvent extractability effect, the highest oil yield was obtained using n-H: PE mixture in ratio of 3:1 or 1:3. Also, the results showed that the highest USM% was obtained by n-H:PE= 1:3 mixture. Finally, the extracted olive kernel oils resulted in significant content of squalene and stigmasterol making olive kernel a viable new source of bioactive compounds.

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EFFECT OF HOMOGENIZATION METHOD AND CONDITIONS ON OLIVE OIL W/O MICROEMULSION FORMULATION, STABILITY AND PROPERTIES

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ABSTRACT

Emulsion droplet size is crucial for many emulsion properties such as stability, color, appearance, texture, and rheology. W/o emulsions containing ultrafine droplets (d < 100 nm) named as microemulsions have a number of potential advantages over conventional emulsions for the encapsulation and delivery of hydrophilic substances (bioactive components) in foods and beverages. They are characterized by high optical clarity, physical stability and increased bioavailability. The aim of this study was to examine two methods (high speed or ultrasound homogenization) in microemulsion emulsification at 40°C emulsion performance. The methods/equipment used for emulsification were: (I) a high speed homogenizer (CAT Unidrive 1000, CAT Scientific, Ca., USA) at 8000, 10000 and 12000 rpm and (II) an ultrasonic generator VC 750 (Sonic & Materials) at 20 kHz at the amplitudes of 150, 225 and 300 W for 10 and 20 min. The w/o microemulsions obtained, consisted of refined olive oil, Tween 20 and water. The emulsification performance of each method was evaluated in terms of droplet diameter, polydispersity, ζ-potential, emulsion stability, turbidity and color. The prepared microemulsions properties were evaluated relatively to the preparation method. The results concluded that stable olive oil microemulsions were obtained with both methods. The influence of conditions (time, rpms, amplitude) regarding the emulsification used method was examined and moreover the most efficient method/conditions of emulsification were determined.

Keywords: Microemulsion, Olive oil, Emulsification methods, Ultrasound, Homogenization.

1.INTRODUCTION

Emulsions are mixtures of two or more immiscible liquids dispersed in each other as small spherical droplets with an interfacial layer consisting of some necessary surfactant material

and are normally thermodynamically unstable. However, emulsions containing ultrafine droplets (d < 100 nm), also known as microemulsions, are exceptionally stable approaching thermodynamic stability. Small droplet size enhances emulsion stability since the Brownian motion may be sufficient for overcoming gravity; meaning that creaming or sedimentation should not occur even on prolonged storage (McClements 2007). The small droplet size also prevents flocculation of the droplets as well as their coalescence. The long-term physical stability of microemulsions makes them unique providing a number of potential advantages over conventional emulsions. Microemulsions are thermodynamically stable, transparent, and isotropic dispersions. They are suitable as micro reactors for the synthesis of food flavors, as media for the solubilization of water or oil insoluble nutraceuticals and as the delivery system for controlled release of water-soluble nutrients, flavors and colorants in foods (Flanagan and Singh 2006).

For the formulation of microemulsions, either a large amount of energy or surfactant or the combination of both of them is required in order to lower the size of the inner droplets as much as possible (McClements 2007). However, many surfactants are not permitted in foods whereas others can only be added at low concentrations. As a result emphasis is given in the homogenization method in order to lower the amount of surfactant needed for the stabilization of the microemulsion. Various emulsification techniques have been used in this regard, among them stirring, high speed homogenizing (often referred as toothed disc dispersing), colloid milling, and ultrasound high-pressure homogenization (McClements et al, 2012).

The high speed homogenization assembly consists of a rotor housed concentrically inside the stator with two or more blades and a stator with either vertical or slant slots. As the rotor rotates, it generates a lower pressure to draw the liquid in and out of the assembly, thereby resulting in circulation, reduction of droplet size and emulsification.

In ultrasound emulsification, the energy input is provided through so-called sonotrodes (sonicator probe) containing a piezoelectric quartz crystal that can expand and contract in response to alternating electrical voltage. As the tip of the sonicator probe contacts the liquid, it generates mechanical vibrations and therefore cavitation occurs (Abismai 1999). Cavitation is the formation and the collapse of vapor cavities in a flowing liquid. The collapse of these cavities causes powerful shock waves to radiate throughout the solution in proximity to the radiating face of the tip, thereby breaking the dispersed droplets and homogenizing the liquid. Studies comparing ultrasonic emulsification with rotor–stator dispersing found ultrasound to be competitive or even superior in terms of droplet size and energetic efficiency (Freitas et al, 2006).

Olive oil was chosen as the emulsion's main ingredient because of its high nutritious value and its great stability to oxidation. It is superior compared to other vegetable oils as it is used in natural form and has unique flavor and other appealing sensorial characteristics. The olive oil with its natural antioxidants, in combination with its fatty acids and phenolic fraction serves many beneficial health effects (Ambrosone et al, 2009). Concerning olive oil, macroemulsion studies have mainly taken place whereas microemulsions have been examined only as enzymatic systems (Xenakis et al, 2011).

Tween 20 (polyoxyethylene sorbitan monolaurate) was chosen as emulsifier because of its stability and relative non-toxicity which allows it to be used as a food grade ingredient while according to references is capable to form a microemulsion without needing the addition of a co-surfactant (Bera et al, 2012). The addition of co-surfactant was avoided since they can cause toxicity and irritation, even destabilization and destruction of the microemulsion upon dilution due to the partitioning of the co-surfactant out of the interfacial region into the continuous phase (Cho et al, 2008).

The aim of the present work was to evaluate the effect of homogenization method (high speed or ultrasound homogenization) on the formulation, stability and properties of w/o microemulsions prepared with refined olive oil and Tween 20 without the addition of a co-surfactant.

2. EXPERIMENTAL PROCEDURE

2.1 Materials

Refined olive oil was generously donated by Minerva S.A., Greece and stored at 8ºC. Polyoxyethylene sorbitan monolaurate (Tween 20, HLB=16,7) was purchased from Across Organics (New Jersey, USA).

2.2 Methods

2.2.1 Preparation of the microemulsions

(I) High speed homogenization: In the first step, olive oil and surfactant were mixed at 40°C using a high speed homogenizer (CAT Unidrive 1000, CAT Scientific, Ca., USA) at either 8000, 10000 or 12000 rpm for 10 min. The Tween 20 ratio tested was 4% g surfactant/ g of the final mixture. In the second step, distilled water was added in drops (50 μL per 30 s) under continuous homogenization of the same speed as step 1 to reach 2% w/w of the final mixture. Then the mixture was left to homogenize for 10 or 20 min.

(II) Ultrasound homogenization: In the first step, olive oil and Tween 20 were mixed at 40°C using an ultrasonic generator VC 750 (Sonic & Materials) at 20 kHz at the amplitudes of 150, 225 and 300 W for 10 min. The surfactant ratio tested was 4% g surfactant/ g of the final mixture. In the second step, distilled water was added in drops (50 μL per 30 s) under continuous homogenization of the same amplitude as step 1 to reach 2% w/w of the final mixture. Then the mixture was left to homogenize for 10 or 20 min.

2.2.2 Emulsion stability Index (ESI%)

The emulsion stability was determined by measuring the extent of the gravitational phase separation. Creaming and sedimentation values were determined from the ratio of cream and sediment volumes over total volume of emulsion samples up on standing scale tubes. For this test, 20 mL of the prepared emulsion were transferred into a glass test tube and stored for 4 weeks at room temperature (25 ± 1 °C). Emulsion stability index (ESI) was estimated with the following equation (1) as the percentage of the initial emulsion height (HE), height of cream layer (HC) and height of the sedimentation phase (HS):

$$ESI(\%) = 100 \times (HE-(HS + HC))/HE \quad (1)$$

2.2.3 Particle size Distribution and ζ-potential

DLS (Zetasizer nano-zs 2000, Malvern Instruments Ltd., UK) analysis of the microemulsions was performed to determine the droplet size and the droplet size distribution as well as the ζ-potential of the droplets in the microemulsion. An air-cooled argon ion laser operating at 488 nm was used to illuminate samples. 1 mL of solution was injected into sample cell, and then the measurement was performed at 25 °C. ζ-Potential was obtained according to Helmholtz-Smoluckowski equation, and the data were corrected by determining the sample viscosity, refractive index and dielectric constant.

2.2.4 Turbidity

The turbidity of the emulsions was measured by a Turbimeter (Hach 2100N, Loveland, CO, USA). For the measurement, 10 mL of the emulsion were purred into a glass cell which was placed into the turbimeter. The temperature of the emulsion was set at 25ºC.

2.2.5 COLOR MEASUREMENT

The color of the prepared emulsions was measured using a colorimeter (Minolta CR 200, Minolta Corp, Osaka, Japan) and quantified in terms of tristimulus coordinates, such as the XYZ or L*a*b* systems specified by the Commission International de l'Eclairage (CIE). In the present work 5 mL of the sample while still being stable were measured at 25^{o}C in a glass plate. The variable E which stands for the total color of the microemulsions is derived by the equation (2):

$$E = \sqrt{a^2 + b^2 + L^2} \qquad \textbf{(2)}$$

2.2.6 STATISTICAL ANALYSIS

The experiments of microemulsion preparation were conducted three times, all measurements were replicated 3 times and their mean values are presented. Data measurements were subjected to analysis of variance (ANOVA) and statistical significance of mean values were determined using Duncan's multiple range test (at $P < 0,05$). A correlation matrix was applied using the STATISTICA release 7, statistical software (Statsoft 224 Inc., Tulsa, USA).

3. RESULTS AND DISCUSSION

In the present work, parallel studies on emulsification processes were conducted through mechanical high speed homogenization and power ultrasound. The conditions studied for each method were the time and the intensity (speed or amplitude in mechanical or ultrasound case respectively) of homogenization.

3.1 Emulsion Stability Index of the emulsions - ESI%

Commercial emulsions may be maintained for long time storage periods, and thus it is interesting to prolong their stability time (Whitehurst, 2004). For this reason in the studied systems, the influence of time on the Emulsion Stability Index (ESI) was investigated.

The Emulsion Stability Index (ESI) of all w/o microemulsions (Table 1) was significantly ($P < 0,05$) decreased during the 2 month-storage period at 25ºC. High speed homogenization proved to be extremely effective, stabilizing the samples throughout the studied storage period (2 months). The ESI was significantly affected by the method and the intensity of homogenization ($P<0,05$). The samples can be grouped to those prepared by low and medium intensity and those prepared with high intensity. Ultrasound homogenization was ineffective at the amplitudes of 150 and 225 W but effective at 300W. The reason for this was that at low

amplitudes large droplets remained in the emulsion causing destabilization of the microemulsion system.

TABLE 1. Emulsion Stability Index (ESI) indicating the extent of the gravitational phase separation of the microemulsions during storage at room temperature 2 months after the emulsification

Method	Homogenization Intensity (rpm/W)	Emulsification Time (min)	ESI
High speed	8000 rpm	10	99
		20	99
	10000 rpm	10	100
		20	100
	12000 rpm	10	100
		20	100
Ultrasound	150 W	10	96
		20	97
	225 W	10	98
		20	99
	300 W	10	100
		20	100

3.2 Dynamic Light Scattering Measurements

DLS measurements were carried out to evaluate the size, the polydispersity and the ζ-potential of aqueous domains in olive oil microemulsions. The particle size distribution is fundamentally considered as the most important characteristic for the evaluation of emulsion stability (Spernath and Aserin, 2006). The effect of the preparation method and conditions of the olive oil microemulsion system on the resultant droplet size was investigated. Figure 1 shows the Mean Droplet Diameter (MDD) of the microemulsions as a function of intensity and time for the two homogenization methods.

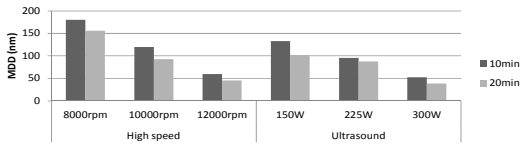

FIGURE 1. Influence of intensity and time for the two methods of homogenization on on the Mean Droplet Diameter (nm) of the studied microemulsions

The method, the intensity and the time of homogenization affected significantly the mean droplet diameter ($P<0,05$). The droplet size tended to be decreased with increasing intensity and time of homogenization. Ultrasound homogenization resulted generally to lower diameters; the minimum droplet size attained was 38, 3 nm by ultrasound homogenization at

300W amplitude for 20 min. The samples can be grouped according to the intensity and the method of homogenization. The samples that were prepared with high intensity high speed homogenization were found to have similar properties with samples prepared with high intensity ultrasound homogenization.

Polydispersity is an index (Polydispersity Index - PDI) indicating how the droplets are dispersed in the continuous phase. Low PDI values mean monodisperse emulsions with high stability while high PDI values indicate polydisperse emulsion with low stability respectively.

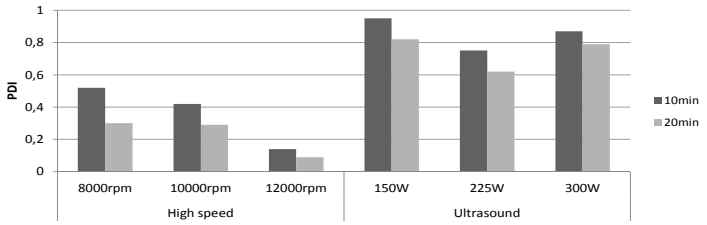

FIGURE 2. Influence of intensity and time for the two methods of homogenization on the PDI of the studied microemulsions apotelesmat toy PDI by different methods/conditions of homogenization

Significant differences among PDI values of the microemulsions (Fig.2) were resulted by the type of homogenization method (P<0,05). Ultrasound homogenization presented high PDI values in contrast with the high speed homogenization, indicating low stability, as it was confirmed by the ESI measurements. Ultrasound homogenization, although very effective is not homogenously efficient in the whole volume of the emulsion leading to the formation of polydispersed distribution of droplets. The ζ-Potential is a parameter commonly used for the evaluation of microemulsion stability, especially for microemulsions with non-ionic surfactants it can be used to analyze the charge of the system.

TABLE 2. Influence of intensity and time for the two methods of homogenization on the ζ-potential (mV) of the studied microemulsions

Method	Level (rpm/W)	Time (min)	ζ-potential
High speed	8000 rpm	10	-0,854
		20	-0,851
	10000 rpm	10	-0,841
		20	-0,803
	12000 rpm	10	-0,845
		20	-0,865
Ultrasound	150 W	10	-0,875
		20	-0,842
	225 W	10	-0,835
		20	-0,842
	300 W	10	-0,866
		20	-0,874

High absolute ζ-potential values correspond to stable dispersions, while low ζ-potential values are indicative of potential instable dispersions, since the dispersed droplet of the microemulsion tend to aggregate (Fanun 2009). Table 2 shows significant different values of ζ-potential derived from different values of homogenization intensity (P<0,05). Higher ζ-potential values showed the samples prepared with high homogenization intensity regardless of the method and the time of homogenization. Generally ζ-potential appeared low value deviation as it was expected since the composition of the system remained the same (emulsifier, water concentration) (McClements, 2012). Notwithstanding, small variations of ζ-potential values found among the samples can may be attributed to potential modification of the interface layer between the droplets and the dispersant by the homogenization intensity.

3.3 Turbidity

The stability of microemulsions was further studied by means of turbidity measurements. Transparent and homogenous emulsions are, also, considered to be monodisperse microemulsions. In the present study the turbidity values varied from 31 to 251 NTU.

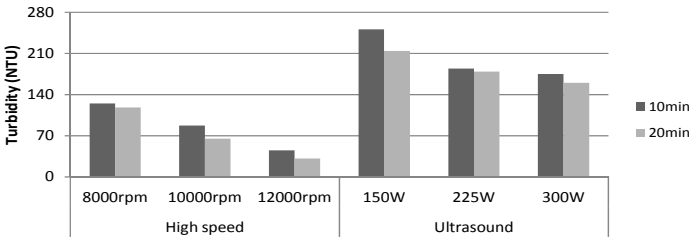

FIGURE 3. Influence of intensity and time for the two methods of homogenization on the turbidity of the studied microemulsions

Turbidity (Fig. 3) was significantly affected by the time, intensity homogenization method (P<0,05). The turbidity of the microemulsion decreased along with the increase of the homogenization time and intensity. As with the PDI, the use of ultrasound homogenization led to higher turbidity values indicating high droplet polydispersity.

3.4 Color measurements

An understanding of the structural basis of emulsion color depends on an understanding of the physical processes occurring when a light beam interacts with an emulsion and is subsequently detected by an eye or instrumental detector (León, 2006).

TABLE 3. Influence of intensity and time for the two methods of homogenization on the color (E) of the studied microemulsions

Method	Level	Time (min)	E
High speed	8000 rpm	10	46,26
		20	42,36
	10000 rpm	10	45,69
		20	41,98
	12000 rpm	10	40,65
		20	39,25
Ultrasound	150 W	10	46,28
		20	45,98
	225 W	10	46,75
		20	45,84
	300 W	10	45,98
		20	45,23

The color of the microemulsions E (Table 3) was significantly affected by the homogenization method (P<0,05) and not by the time or the intensity. High speed homogenization resulted to lower values of color (E) especially in high intensity.

4. CONCLUSIONS

The results of this study showed that it is possible to prepare stable olive oil w/o microemulsions both with high speed or ultrasound homogenization without the addition of a co-surfactant. In the studied system, the comparison of two types of emulsification processes, the first one using high speed homogenization and the second one involving power ultrasound, showed several interesting results in favor of the high speed method. Although ultrasound homogenization is more effective at lowering the mean droplet diameter, it led to more polydispersed and unstable distributions. On the other hand high speed homogenization led to more stable microemulsions, less turbid and with lower PDI. Thus, high speed homogenization can be suggested as more efficient for the preparation of stable w/o microemulsion systems whereas ultrasound homogenization should be used probably along with some other method of homogenization in order to lead to stable nanoemulsions.

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RELATIONSHIP BETWEEN WHEAT FLOUR EXTRACTION RATE AND DOUGH RHEOLOGICAL PROPERTIES

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ABSTRACT:

The effect of different extraction rates (64%, 82% and 90%) or bran content of wheat flour on the rheological properties of the wheat flour dough was investigated. A complete study of the rheological behavior of the wheat flour dough was performed by using different methods namely alveograph, farinograph, gluten index, zeleny and SDS sedimentation. The correlation coefficient among the various parameters of applied methods was also determined and the most suitable method in assessment of flour attributes recognized. The lower bran concentration (extraction rate 64%) in flours showed the better pronounced effect on dough properties yielding strengthened dough. Several expected associations were found among applied methods, in spite of the fact that no significant correlation among all parameters of the methods was observed, some parameters of the measuring methods had very strong correlation at significance 1% that included farinograph water absorption, tenacity (P), extensibility (L), P/L, swelling index (G) of alveograph, gluten index, zeleny and SDS. Among the applied methods, most of the alveograph parameters had significant correlation ($r=1$, $p<0.01$) together and it might be used as the recommended method to assess dough rheology.

Key words: Alveograph, Farinograph, Rheology, Extraction rate.

1. INTRODUCTION

Among the cereal flours, only wheat flour can form three-dimensional viscoelastic dough when mixed with water. The microstructure of wheat kernel has been well understood, consisting of an embryo or germ (2-3%), bran (13-17%), and an endosperm packed with starch granules in a protein matrix (about 75-80%) (Phan-Thien et al. 1997). After milling and sieving, wheat grains are separated into flour, bran and germ, with flour consisting mainly of

endosperm (Phan-Thien et al. 1997). Characterization of rheological properties of dough is effective in predicting the processing behavior and in controlling the quality of food products (Song and Zheng 2007). Although the production of baking products is considered the most accurate method in quality evaluation (Hoseney 1994), in order to assess flour-quality attributes, several predictive tests which are closely related to wheat flour quality are frequently used in wheat industry (Colombo et al. 2008). Gluten is the main base of the wheat dough and is the protein that only exists in wheat and rye and many baking properties in wheat flour are related with this protein. Decreased functional quality of final products such as bread has been ascribed to a dilution of functional gluten proteins (Pomeranz et al. 1977). Farinograph, and alveograph are the most common empirical instruments used for characterizing dough rheology. Tests based on these instruments are useful for providing practical information for the baking industries while they are not sufficient for interpreting the rheological testing, especially in the linear viscoelastic region, has been used to follow the structure and properties of dough and to study the functions of dough ingredients (Janssen et al. 1996; Miller and Hoseney 1999). Thus it is necessary to look for the suitable methods of evaluating gluten quality and dough rheology which we can select the proper flour for our aimed purpose (Hruskova and Smejda 2003). The choice of assessment method is influenced by several factors such as country, wheat class, intended end use, time and cost (Gains et al. 2006). Today, dynamic rheological tests has become a powerful and preferred approach for examining the structure and the fundamental properties of wheat flour dough and proteins because of its characteristic and sensitive response to the structure variation of wheat flour dough and proteins (Song and Zheng 2007).

In this study, we focus our attention on dynamic rheological characteristics of wheat flour dough with various extraction rates namely 64%, 82% and 90% by different assessment methods and influence of extraction rate on the rheological behaviors of flour dough is outlined. Moreover, the correlation among the parameters of applied methods through statistical analysis, were determined in order to judge about the quality of the flour more accurately.

2. MATERIALS AND METHODS

2.1. Materials

Commercially available soft white wheat flours were procured from the Tak flour factory (Karaj, Iran), and were stored in sealed containers, in a cold room (5-7 °C), until use. samples

were prepared on a roller mill (Buhler, Switzerlan) and flours with 64%, 82% and 90% extraction rate were coded as A, B and C respectively.

2.2. Methods

2.2.1. Flour analysis

In order to determine the main characteristics of flours, some important quality tests such as protein content (AACC, 46-12), moisture (AACC, 44-16), ash (AACC, 08-01) and zeleny sedimentation (AACC, 56-60) were performed (AACC, 2000). Also the content of damaged starch and falling number were determined according to approved method 76-30A, 56-81B respectively (AACC, 2000).

2.2.2. Dough rheological characteristics

Farinograph characteristics were determined according to the (AACC, 54-21) method (AACC.2000). The following parameters were determined in a Brabender farinograph: water absorption—percentage of water required to yield dough consistency of 500 BU (Brabender Units), dough development time (DDT, time to reach maximum consistency), stability (time during dough consistency is at 500 BU), mixing tolerance index (MTI, consistency difference between height at peak and to that 5 min later) and elasticity (band width of the curve at the maximum consistency).

alveograph test was performed using an alveograph (Chopin (NG), France) following the standard method (AACC, 54-30A) (AACC.2000). The following alveograph parameters were automatically recorded by a computer software program: tenacity or resistance to extension (P), dough extensibility (L), curve configuration ratio (P/L ratio), the deformation energy (W), swelling index (G) and elasticity (P200/P ratio). Gluten index and SDS sedimentation were determined according to (AACC, 38-12A) and (AACC, 56-70) respectively (AACC.2000).

2.2.3. Statistical analysis

The results were expressed as the mean of three replicates ± SD. The data were statistically analyzed using the statistical analysis system software package. Analyses of variance were performed by application of ANOVA procedure. Significant differences between the means were determined using Duncan multiple range test. Also the correlation coefficients were determined and tested for their significance.

3. RESULTS AND DISCUSSIONS

3.1. Chemical characteristic

Damaged starch in the flours was 6.57%. As expected, this result was the same as those found in soft wheat flours (Gaines, 2000); the falling number value on 14% moisture basis was 456 s. Other chemical characteristics of flours (Table 1) indicated a wide variation in the quality characteristics of flours by different extraction rates.

TABLE 1-The chemical composition of flour samples

Flour sample	Extraction rate(%)	Ash(%)	Moisture(%)	Protein(%)	Zeleny sedimentation(ml)	SDS sedimentation(ml)	Gluten index
A	64	0.46± 0.030^{c}	13.9±0.03^{a}	11.6±0.00^{c}	30.0±0.00^{a}	65.0±0.00	96.1±1.74
B	82	0.82±0.010^{b}	12.3±0.01^{b}	12.0±0.00^{b}	19.0 ±0.00^{b}	59.6±1.52	73.8±3.12
C	90	1.2±0.51^{a}	10.7±0.51^{c}	12.1±0.00^{a}	14.0 ±0.00^{c}	46.0±1.00	29.0±2.06

Values followed by different letters are significantly different ($P < 0.05$).

Values are means±SD of three replicates.

There was a significant difference ($P<0.05$) among the flours in moisture, ash, protein, gluten index, zeleny and SDS sedimentation. The values for ash varied from 0.46% to 1.40%, moisture 10.68% to 13.97% and protein 11.60% to 12.1%. As expected, protein content and ash content were higher in 90% extraction rate flour than the other flours (82% and 64% extraction rate). Sahlstrom et al. (1993) found similar results in whole meal flour (100% extraction rate) than in white flour (68-73% extraction rate). The sedimentation volume also varied significantly (Table 1) and decreased with an increase in the extraction rate flour. The gluten index value varied from 96.1 to 29.0 indicated stronger gluten network in flour with lower extraction rate.

3.2. Effects of extraction rate on farinograph parameters

The results of farinograph measurements summarized in table 2. There was a significant difference ($P<0.05$) among the flours in water absorption and DDT. Concerning water absorption, the higher bran concentration individually promoted the largest increase in water absorption; water absorption in sample C was the highest (66.30%) and in sample A was the lowest (53.20%). This means by increasing the flour extraction rate, the bran content of flour is higher and water absorption will increase (Sliwinski et al. 2004).

TABLE 2- The quantity of the farinograph analysis of flour samples

Flour sample	WA(%)	DDT (min)	S(min)	MTI(BU)	E (BU)
A	53.2±0.35^{c}	1.9±0.05^{c}	7.8±0.35^{a}	38.0±0.00^{c}	89.0±4.00^{a}
B	58.9±0.20^{b}	3.0±0.28^{b}	2.8±0.15^{b}	93.0±5.51^{a}	45.0±3.51^{c}
C	66.3±0.30^{a}	4.3±0.06^{a}	2.9±0.35^{b}	74.0±1.53^{b}	55.0±2.00^{b}

Values followed by different letters are significantly different ($P < 0.05$). Values are means± SD of three replicates. WA (water absorption); DDT (dough development time); S (stability); MTI (mixing tolerance index); E (elasticity).

The inclusion of a higher amount of bran in the dough formulation usually resulted in increased dough water absorption due to the higher levels of pentosans present in bran (Sanz Penella et al. 2008). Sudha et al. (2007) suggested that the differences in water absorption are mainly caused by the greater number of hydroxyl groups in the fiber structure that allow more water interaction through hydrogen bonding than in refined flour.

The time required for the dough development or time necessary to reach 500 BU of dough consistency (DDT) was lower in sample A (1.95 min) containing less bran than sample B (3.00 min) and sample C (4.27). Bran concentration had a positive significant linear effect in the time to reach maximum consistency, which is in agreement with previous results (Laurikainen et al. 1998). In addition, the increase in development time was attributed to the effect of the interaction between fibres and gluten that prevents the hydration of the proteins, affecting the aggregation and disaggregation of the high molecular weight proteins in wheat (Sanz Penella et al. 2008). The stability value is an indication of the flour strength, with higher values suggesting stronger dough (Rosell et al. 2001). Dough containing less bran (sample A) exhibited more stability than the other samples. Conversely, mixing tolerance index (MTI) values were significantly increased at higher bran concentration. Bran has the effect of softening in dough and by increasing bran in flour, the destruction of the rheological properties of the dough and the farinographic properties such as water absorption and MTI of the flour increases; in contrast, DDT and stability of the dough decrease (Goesaert et al. 2005). There was a significant difference ($P<0.05$) among the flours in elasticity and the elasticity of dough was reduced by increasing bran and the highest elasticity was observed in sample A.

3.4. Effect of extraction rate on alveograph parameters

The effect of flour samples with different extraction rate on the alveograph parameters is shown in table 3. There was a significant difference ($P<0.05$) in flour samples among the

most parameters of alveograph. Dough resistance to deformation or tenacity (P) is a predictor of the ability of the dough to retain gas (Indrani et al. 2007). This parameter increased with increasing the bran. In A, B and C samples that extraction rate increases respectively, consequently, P factor increases.

TABLE 3-The quantity of the alveograph analysis of flour samples

Flour sample	P (mmH2O)	L(mm)	E(%)	G(mm)	W($\times^{10-4j}$)	P/ L
A	53.6±1.53^{b}	84.0±2.64^{a}	46.2±0.87^{a}	20.4±0..32^{a}	136.6±2.52^{a}	0.64±0.040 ^{b}
B	54.3±0.05^{b}	63.0±7.00^{b}	28.6±1.34^{b}	17.6±1.02^{b}	89.6±7.64^{b}	0.87±0.120^{b}
C	66.6±3.05^{a}	43.0±1.00^{c}	22.8±0.62^{c}	14.6±0.20^{c}	88.6±4.72^{b}	1.55±0.11^{a}

Values followed by different letters are significantly different ($P < 0.05$). Values are means±SD of three replicates. P (tenacity); L(dough extensibility); E (elasticity); G (swelling index); W (deformation energy)

It is worthy to remark that the resistance results from the extensograph are not comparable to the resistance obtained with the alveograph because of the differences in principles involved in the measurements (Rosell et al. 2001).

Likewise, the extensibility of dough (L), an indicator of the handling characteristics of dough, was greatly reduced by increasing bran content, dropping to almost half of sample A extensibility with increasing bran in sample C (from 84 mm at sample A to 43 mm at sample C). As resulting of the bran increase on both dough resistance and dough extensibility, the P/L ratio (which gives information about the elastic resistance and extensibility balance of a flour dough) was augmented in dough containing more bran. Sample C yielded dough with the highest P/L ratio (1.55 vs. 0.64 in sample A and 0.87 in sample B).The deformation energy (W), swelling index (G) and elasticity from sample A to sample C reduced. The observed effect agrees with reduction of rheological properties of flour by increasing bran content of flour found by Collar and Scantos (2007).

3.5. Correlation coefficients among rheological parameters of applied methods

Statistical tests were performed to look for relationships among rheological parameters of farinograph ,alveogragh, gluten index, zeleny and SDS sedimentation methods in three kinds of flour (table 4).

TABLE 4-Correlation coefficients (r) [a] for farinograph, extensograph and alveograph with fundamental rheological parameters

parameter	1	2	3	4	5	6	7	8
1								
2	1**							
3	1**	1**						
4	0.5	0.5	0.5					
5	1**	1**	1**	0.5				
6	1**	1**	1**	0.5	1**			
7	1*	1*	1*	0.5	1*	1*		
8	1*	1*	1*	0.5	1*	1*	1*	
9	1*	1*	1*	0.5	1*	1*	1*	1*

Level of significance:[a] r=1, p=0.01 (**). 1- P (tenacity or resistance to extension); 2- L(dough extensibility); 3- G (swelling index); 4- W (deformation energy); 5- P /L; 6- water absorption; 7-Gluten index;8-Zeleny;9-SDS

Regarding the results of rheological tests, for farinograph and alveogragh method, some parameters were considered as the most important parameters to assess the dough rheology. Water absorption parameter in farinograph method, and also P, L, G, W and P/L parameters of alveograph were considered as the fundamental rheological parameters.

The results showed many significant correlations with the current rheological results. Alveograph parameters such as P, L, G and P/L had very strong correlation ($r = 1$, $P<0.01$), the comparison of methods revealed high correlation ($r = 1$, $P<0.01$) among farinograph water absorption, P, L, G, P/L of alveograph, gluten index, zeleny and also SDS sedimentation.

4. CONCLUSIONS

In summary, we reported some rheological data on different flours dough and correlations among the methods. The data point to the following conclusions:

- Rheological characteristics of wheat flour dough were affected by the flour extraction rate and increasing the extraction rate had negative effect on dough rheology;
- Combination and comparison of methods can be useful in the evaluation of wheat flour quality;
- Parameters such as farinograph water absorption, alveograph P, L, G, P/L ratio and also gluten index, zeleny and SDS sedimentation had significant correlation together. In conclusion these parameters were comparable to each other to assess the dough rhology;
- Alveograph among the applied methods can be considered as the most suitable method in order to predict the flour quality.

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KEEPABILITY OF SAFFRON AND BEETROOT PIGMENTS BY MEANS OF ENCAPSULATING EDIBLE POLYMER BLENDS

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ABSTRACT:

In recent years, the use of natural colorants has been steadily increasing due to trends in consumer preference towards natural components with functional properties. Basic natural sources of pigments are saffron and beetroot which are typically used as colorants in quite a wide range of food products. Beetroot pigments derived by extraction consist mainly of two major water-soluble fractions, betacyanins (red - violet colour) and betaxhantins (yellow - orange colour), while saffron include crocins, a carotenoid family with biologically active effect on human health. However, because of their poor stability and their heat and light liability, a protective encapsulation is needed for their feasible usage in food products. The encapsulation has found numerous applications in the food industry for coating colorants in an effort to increase their shelf life until they are added in a real food system as well as for the development of novel food products containing functional ingredients. The encapsulation process basically consists of the preparation of an oil-in-water emulsion, containing the encapsulating agent and the pigment-core, which is then dried, commonly using spray or freeze-drying techniques. The selection of the encapsulation agent is very crucial for an efficient encapsulation and it depends on the final application of the encapsulated pigment. The aim of this work was to extract pigments from saffron and beetroot, to attain an efficient encapsulation of them using gum Arabic as encapsulating agent as well as to study the keepability of the encapsulated pigments and their incorporation in a chewing gum model system.

Keywords: Natural pigments, Encapsulation, Saffron, Beetroot, Arabic gum, Chewing gum

1. INTRODUCTION

In recent years, the use of natural colorants has been steadily increasing due to trends in consumer preference towards natural components with functional properties. Basic natural

sources of pigments are beetroot and saffron which are typically used as colorants in quite a wide range of food products. Beetroot pigments derived by extraction consist mainly of two major water-soluble fractions, betacyanins that confer the red - violet color to beetroot and betaxhantins, a yellow - orange colorant also present in beetroot in lesser proportion to betacyanins (Pitalua, Jimenez, Vernon-Carter and Beristain, 2010).

Beetroot pigments are typically used as a colorant at levels of 4-25 $mgkg^{-1}$ in a wide range of dairy and confectionery products as well as in meat substitutes. However, the use of beetroot pigments in foods is limited due to their poor stability and their heat and light liability (Serris and Biliaderis, 2001). On the other hand, saffron pigments include crocins, a group of water-soluble carotenoids (Raina, Agarwal, Bhatia and Gaur, 1996), which are glycosyl esters of 8,8′-diapocarotene-8,8′-dioic acid (crocetin) (Tarantilis, Tsoupras and Polissiou, 1994) with biologically active effect on human health. Saffron pigments are typically used as a colorant at levels of 1±260 ppm in a wide range of culinary, bakery and confectionery preparations as well as in alcoholic and non alcoholic beverages. Being highly unsaturated, are prone to *trans* → *cis* isomerization and oxidation reactions, leading to losses of colorant power and nutritive value (Tsimidou and Biliaderis, 1997). Therefore, a protective encapsulation is needed for the feasible usage of the afore-mentioned natural pigments in food products.

The encapsulation has found numerous applications in the food industry for coating colorants in an effort to increase their shelf life until they are added in a real food system as well as for the development of novel food products containing functional ingredients. Encapsulation of carotenoids makes them easier to handle and improves their stability to oxidation (Dziezak, 1988). The encapsulation process basically consists of the preparation of an oil-in-water emulsion, containing the encapsulating agent and the pigment-core, which is then dried, commonly using spray or freeze-drying techniques.

The selection of the encapsulation agent is very crucial for an efficient encapsulation and it depends on the final application of the encapsulated pigment. In our previous works various edible polymers examined in fennel oleoresin (Chranioti and Tzia, 2012) and saffron volatile flavour substances (Chranioti, Papoutsakis, Nikoloudaki and Tzia, 2012) encapsulation. Among the materials used in the food industry as encapsulating agents, gum Arabic is one of the most important ones since it is nontoxic, odorless, tasteless, with excellent emulsification capacity, low viscosity in aqueous solution, and very good retention properties (Gabas, Telis, Sobral and Telis-Romero, 2007). Gum Arabic is described as a highly branched arabinogalactan protein, while both protein and polysaccharide moieties are fundamental to the functional properties of this polysaccharide (Dickinson, 2009). The quite unique behavior

of gum Arabic is attributed not only to its molecular structure, but also to its conformation-a globular like, random coil structure, that can be comparable to very few other polysaccharide–protein systems (Jafari, He and Bhandari, 2012). Gum Arabic has been also efficiently used in encapsulation food flavour processes (Chranioti and Tzia, 2013).

The aim of this work was to extract pigments from saffron and beetroot, to attain an efficient encapsulation of them using gum Arabic as encapsulating agent as well as to study the keepability of the encapsulated pigments and their incorporation in a chewing gum model system.

2. MATERIALS AND METHODS

2.1 Materials

Dried stigmas of saffron were provided directly from the "Cooperative of saffron, Krokos Kozanis" while the beetroot vegetable was purchased from a local market. Sorbitol and mannitol were from Cargill whereas gum base and lecithin were kindly donated from Kraft Foods and Biotrek-Greece, respectively. Gum Arabic was obtained from Chemicotechnica S.A. and used as encapsulating agent.

2.2 Methods

2.2.1 Preparation of aqueous saffron and beetroot extracts

Saffron was extracted with distilled water under continuous shaking in an ultrasound water bath (Elmasonic) at T=25 °C and at fixed-frequency of 30 kHz, while beetroots were washed, peeled and extracted with a commercial juice extractor. The water-soluble extracts were filtered, frozen overnight at -30 °C and lyophilized in a freeze dryer (Christ Alpha 1-4 LD Plus) at P = 0.017 mbar and T = −57 °C for 24 h. Both the water-soluble extracts and the corresponding freeze-dried pigments resulted were kept in the dark at −30 °C until used.

2.2.2 Encapsulation of water soluble extracts in gum Arabic by freeze-drying

Gum Arabic was selected as the encapsulating agent since based on our previous study (Chranioti and Tzia, 2013) it proved to be the most effective material in terms of encapsulating efficiency. An aliquot of 15 g of gum Arabic (GA) was dispersed individually in distilled water and final volume was made to 100 mL, while a fixed ratio of 0.33 of

pigment/GA was tested. The fixed ratio was selected as an average ratio within the best range presented in previous studies (Ahn et al. 2008; Hogan et al. 2001). The resulting solutions were frozen overnight at −30 °C (Shimada et al. 1991) and lyophilized in a freeze dryer (Christ Alpha 1-4 LD Plus) at P= 0.017 mbar and T=−57 °C for 48 h. The encapsulated pigments were converted into powder with help of a pestle and mortar.

2.2.3 Preparation of chewing gum model

The chewing gum ingredient formulation consisted of gum base (29.48 g / 100 g), sorbitol powder (37.24 g / 100g), glycerin (7.06 g / 100g), saturated sorbitol solution (25.82 g / 100 g) and Lecithin (0.40 g/ 100 g). For chewing gum preparation the gum base was initially melted (raised to 98-104 ^{o}C). Lecithin along with the pigment was then added to the molten gum base under mixing and after 2 min the mixture allows to cool. During cooling, the saturated sorbitol solution was added (under mixing for 2 min) followed by addition of about 50% of the sorbitol powder and further mixing for 2 min. At approximately 75 °C the remaining sorbitol powder was added and mixed for 2 min. Finally, the glycerin was added and further mixed for 1 min. Six different formulations (samples) of chewing gum were designed based on two different pigments (saffron and beetroot) either in extract (E), freeze-dried extract (FD-E) or encapsulated extract in Arabic gum form (AG-E) (see Table 1).

TABLE 1. Chewing gum formulations

Formulation.	*Abbreviation*	*Chewing gum samples*
1	S-E	Saffron extract
2	S-FD-E	Saffron freeze dried extract
3	S-AG-E	Saffron extract encapsulated in Arabic gum
4	B-E	Beetroot extract
5	B-FD-E	Beetroot freeze dried extract
6	B-AG-E	Beetroot extract encapsulated in Arabic gum

2.2.4 Color measurements

The color measurements of chewing gum samples were performed using a Minolta CR 200 colorimeter. The instrument was standardized each time with a white ceramic plate (L = 92.8, a = −0.8, b = 0.1). After standardization the L (lightness), a (red component) and b (yellow component) values were measured. The L parameter was taken for samples with both

pigments, while additionally *a* parameter was taken for samples with beetroot and *b* parameter for samples with saffron pigments respectively.

2.2.5 Texture measurements

The texture measurements of chewing gum samples were performed using a Texture Analyzer (Stable Microsystems Ltd., TA-XT2i, UK) and the evaluated parameters were hardness and gumminess.

2.2.6 Storage stability test

The chewing gum model samples were stored at 25 and 40 °C and during storage the changes in color and texture parameters of chewing gum samples were monitored.

2.2.7 Sensory evaluation

The sensory evaluation for color of chewing gum samples was carried out by a panel of expert assessors trained (ISO 5492:2008, ISO 5496:2006). The samples were evaluated using 1 10-point category scale with a scoring of color intensity: 0=degradation, 10=strong (fresh).

2.2.8 Statistical analysis

Statistical analysis of measurements results was conducted with the Statistica software (Statsoft 224 Inc., Tulsa, USA). Analysis of variance was performed by ANOVA procedure and significant differences ($P< 0.05$) between the means were determined by Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1 Color measurements

The color parameters of the fresh prepared chewing gum samples with both beetroot (*L*, *a*) and saffron (*L*, *b*) pigments depended significantly ($P< 0.05$) on the form of pigment used (extract, freeze-dried extract or encapsulated extract in gum Arabic). The evolution of color parameters of the chewing gum samples with beetroot and saffron pigments during storage at 25 and 40 °C is presented in Figures 1 and 2 respectively. Storage temperature affected significantly ($P< 0.05$) the *L* parameter; storage in increased storage temperature resulted in a decrease in *L* values in all the samples. During storage the *L* parameter of chewing gum products varied, however it must be noted that in beetroot pigments samples it remained

practically stable after six days storage. As far as the effect of pigment type for incorporation is concerned (Figures 1-I and 2-I), the *L* parameter presented the highest values ($P< 0.05$) in the case of extract form incorporation in the chewing gum samples for both pigments examined (average values 81.53 and 82.77 for beetroot and saffron extract, respectively).

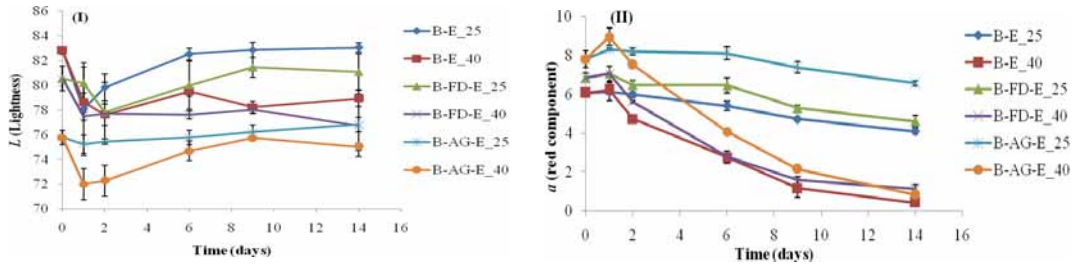

FIGURE 1. Changes of *L* (Lightness) (I) and *a* (red component) (II) color parameters of chewing gum samples with beetoot pigments during storage at 25 and 40 °C.

Concerning the *a* (red component) color parameter (Fig. 1-II), it decreased with storage time with lower degradation in samples stored at 25 °C. It was observed that the *a* color parameter almost disappeared after 2 weeks of storage at 40 °C (1.12, 0.83 and 0.41 for B-AG-E, B-FD-E and B-E, respectively). The *a* values were found in decreasing order as follows: B-AG-E > B-FD-E > B-E, showing that gum Arabic can offer protection against color degradation at storage temperature or even at accelerated temperature. The better protection provided to the beetroot extract by the use of an encapsulating agent polymer is in agreement with other findings as well (Pitalua, Jimenez, Vernon-Carter and Beristain, 2010).

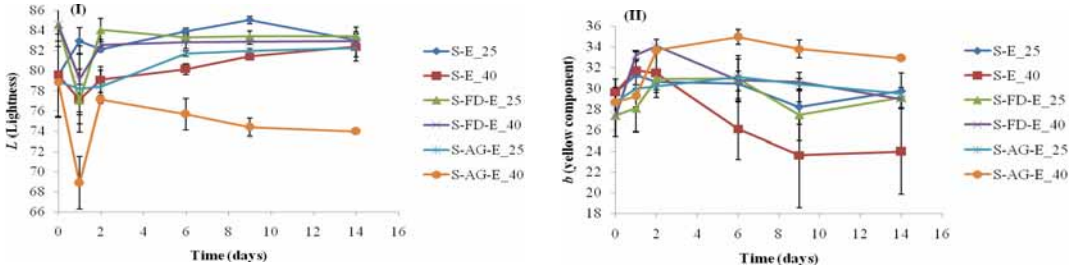

FIGURE 2. Changes of *L* (Lightness) (I) and *b* (red component) (II) color parameters of chewing gum samples with saffron pigments during storage at 25 and 40 °C.

Concerning the *b* (yellow component) color parameter (Fig. 2-II), it decreased with time independently of the storage temperature. A similar behavior of gum Arabic protection was observed also in saffron pigments as in beetroot. In particular, the chewing gum samples with the encapsulated in Arabic gum saffron extracts (S-AG-E) presented high *b* values (average values 29.97 and 32.21 for S-AG-E_25 and S-AG-E_40, respectively). The greater *b* values

observed in chewing gum samples with incorporation of freeze dried (S-FD-E_40) or encapsulated saffron extracts (S-AG-E_40) stored at 40 °C may be attributed only to the inherent color of the gum Arabic matrix and chewing gum base since color pigment was already diminished.

3.2 Texture measurements

The evaluated texture parameters (hardness and gumminess) of the fresh prepared chewing gum samples increased with time, however, without showing a certain trend based on the type of pigment and storage temperature (Figure 3 and 4).

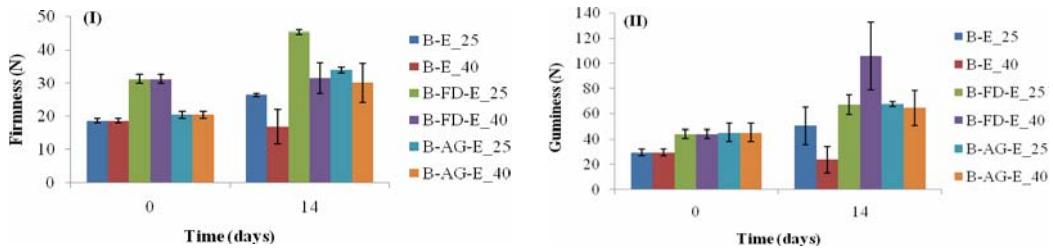

FIGURE 3. Changes of hardness (I) and guminess (II) texture parameters of chewing gum samples with beetroot pigments during storage at 25 and 40 °C.

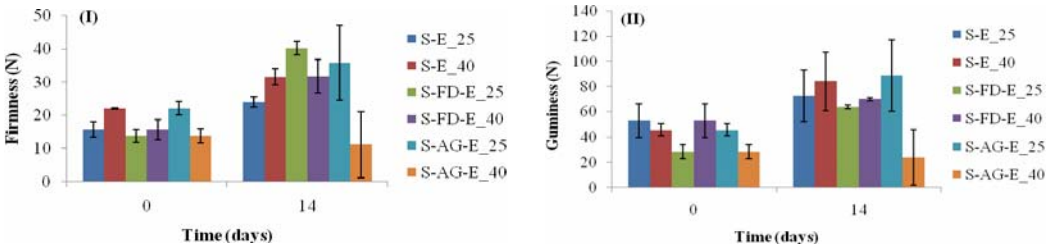

FIGURE 4. Changes of hardness (I) and guminess (II) texture parameters of chewing gum samples with saffron pigments during storage at 25 and 40 °C.

3.4 Sensory evaluation

From the results of color sensory evaluation, it was concluded that the use of encapsulated in gum Arabic pigments led to the highest acceptance scores, while an expected color degradation during storage was recorded for all products. All sensory color scores were in sufficient correlation with the respective objective color measurements (Figure 5).

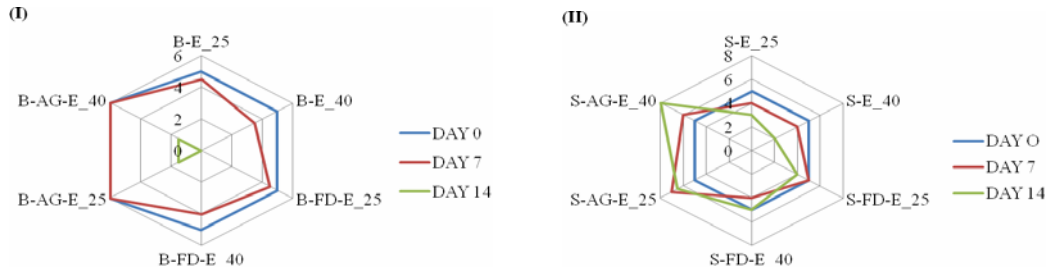

FIGURE 5. Sensory evaluation for color of chewing gum samples with beetroot (I) and saffron (II) pigments during storage at 25 and 40 °C.

4. CONCLUSIONS

Color parameters of the prepared chewing gum samples as well as their evolution during storage depended greatly on the used form for each pigment (beetroot and saffron) and storage conditions (time and temperature). The most sensitive color parameters to estimate the changes of chewing gum samples during storage were *a* for beetroot and *b* for saffron. The usage of encapsulated pigments is suggested since gum Arabic proved an encapsulating agent providing good keepability of pigment, while the effect on texture parameters should also be taken in account.

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The FRISBEE European project: Study and validation of tools for the assessment of the food cold chain

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ABSTRACT:

Effective control of storage and distribution is important for commercial viability of foods, since they often deviate from specifications. FRISBEE (**http://frisbee-project.eu)** is a Food Refrigeration Innovation for Cold Chain research IP EU project. Within FRISBEE a web-based database (**http://www.frisbee-project.eu/coldchaindb.html/**) was developed in which more than 9000 actual time-Temperature (t-T) profiles have been contributed for various food products (chilled and frozen) from all over Europe. Significant differences in t-T profiles of same products along the European cold chain were observed. The FRISBEE Cold Chain Predictor (FRISBEE_CCP), software also developed within FRISBEE objectives, allows the calculation of the remaining shelf-life (RSL) of a specific food product at different stages of the cold chain. This tool generates a representative t-T profile for any sequence of cold chain stages using Monte Carlo simulation. The RSL at each stage of the profile is calculated according to appropriate Equations, based on dominant deterioration factor. The developed FRISBEE_CCP and Cold Chain Database were applied for the evaluation of representative Cold chains (RSL estimation) for yoghurt, sliced cooked ham, ice cream and frozen spinach. The estimated RSLs from the tools were used to assess the cold chain for these specific products. For sliced cooked ham, the results are validated from field tests performed in 4 EU countries, in which a large sample of products is equipped with miniature data-loggers and followed in their real path from production through the different stages up to consumers homes. Overall, the developed FRISBEE tools offer the potential to effectively identify cold chain weak links and evaluate solutions for improving refrigeration and food product shelf life management along the food cold chain.

Keywords: FRISBEE, cold chain management, cold chain database, cold chain predictor

1. INTRODUCTION

The main shelf-life determining post-processing parameter in the cold chain of chilled and frozen food products is temperature (Evans, 1992; James and Evans, 1992 a,b ; Giannakourou and Taoukis, 2003). A modern quality and safety assurance system should rely on prevention through monitoring, recording and controlling of critical parameters during the entire product's life cycle that includes the post-processing phase and extends to the time of use by the final consumer (Evans et al., 1991; Giannakourou et al., 2001 ; Dubelaar et al., 2001 ;

Giannakourou and Taoukis 2002 ; Kennedy et al., 2005 ; Koutsoumanis et al., 2002 ;Koutsoumanis et al., 2005). Increasing attention should be focused on the role and the logistics of transport, storage and handling, and the benefits of taking a supply chain perspective are being appreciated and pursued. Temperature conditions in the chilled distribution chain (Broekmeulen, 2001; Browne and Allen, 1998) determine the risk potential, the shelf life and final quality of chilled products processed and packed under Good Manufacturing Practices and Good Hygiene Practices (Tijkens et al., 2001 ; Wells and Singh, 1989). Since in practice significant deviations from specified conditions often occur, temperature variability has to be taken into account for cold chain control and any logistics management system that aims on product quality optimisation at the consumer's end (Likar and Jevsnik. 2006 ; Giannakourou and Taoukis, 2002). The development of a Cold Chain Database web-based tool could significantly contribute to the determination of the weak links of the cold chain for a significant number of food products. Stage and product specific t-T information can be used for the quantitative description of cold chains within the FRISBEE project. The contributed data of the cold chain will allow simulation of realistic cold chain scenarios based on actual cold chain data and can lead to corrective actions aimed to optimizing efficiency and commercial shelf-life.

2. MATERIAL AND METHODS

A systematic data collection for identification and evaluation of the weak links of the cold chain for different types of chilled and frozen products was performed. Within European FRISBEE project (Food Refrigeration Innovations for Safety, consumers Benefit, Environmental impact and Energy optimisation along the cold chain in Europe), a web-based platform (hosted in the link **http://www.frisbee-project.eu/coldchaindb.html**) has been built for t-T data collection, maximizing information retrieval with user friendliness.

A menu driven web-based questionnaire accompanies or incorporates the basic information that in all cases has the form of t-T data. Additionally, some basic information apart from t-T profile should be specified to the extent it is available by the data provider such as the type of food product, the packaging, the recommended storage conditions, the stage (step) of the cold chain, geographical and seasonal information, information concerning the storage and distribution equipment, specifications of data collecting equipment, the position of the data collecting equipment and the format the contributed data should have. The processing of these data addresses the needs of the FRISBEE project and the needs of the users. Therefore, the web-based database provides information (output) useful and usable for the users (including data providers and ultimately European researchers, regulators, industry and even the consumer). The web-based platform

was developed in order to organize input and provide easy and systematic access the contributed information and it is hosted in **http://www.frisbee-project.eu/coldchaindb.html**. Application of an effective tool as a reliable predictive system of the shelf life of the product at any point of its marketing route would benefit food industries and retailers. The FRISBEE Cold Chain Predictor (FRISBEE_CCP) software was developed to give the users the ability to calculate the remaining shelf life of a specific food product at different stages of the cold chain corresponding to a representative t-T profile built from a sequence of cold chain stages.

3. RESULTS AND DISCUSSION

Cold Chain Data Collection Platform

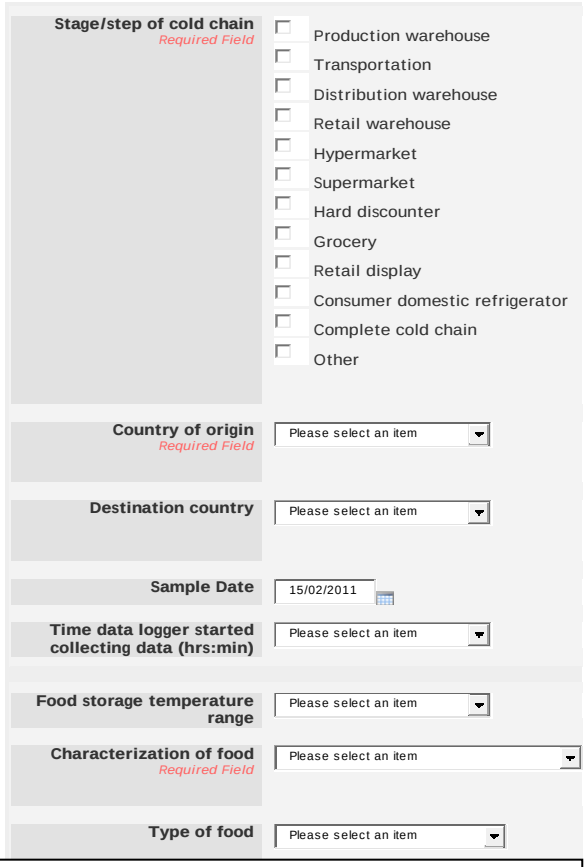
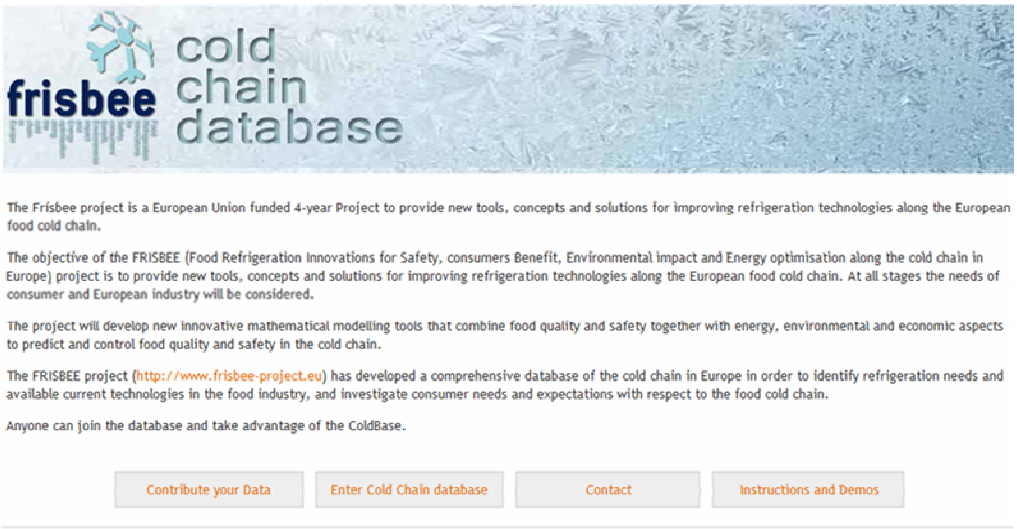


Figure 1. FRISBEE Cold Chain Data Collection

Within FRISBEE a web-based platform (hosted in the link **http://www.frisbee-project.eu/coldchaindb.html**/) has been built for data collection, maximizing information retrieval with user friendliness. This platform consists of a menu driven web-based software retrieving information to accompany the contributed food product time-temperature data. Some of the most important questions to be answered from the user include the stages of the cold chain (i.e. production warehouse, transportation etc.), the country of origin and the destination country of the products and some descriptive information of the product such as the food storage temperature range (chilled, frozen etc.), the characterization (fresh unprocessed, processed ready to eat etc.) and type of food (meat and meat product, vegetable etc.), the packaging (air packaged, vacuum packaged etc.) and the recommended food storage conditions (Picture 1). Significant differences in time-temperature profiles of same products along the European cold chain were observed.

Cold Chain Database

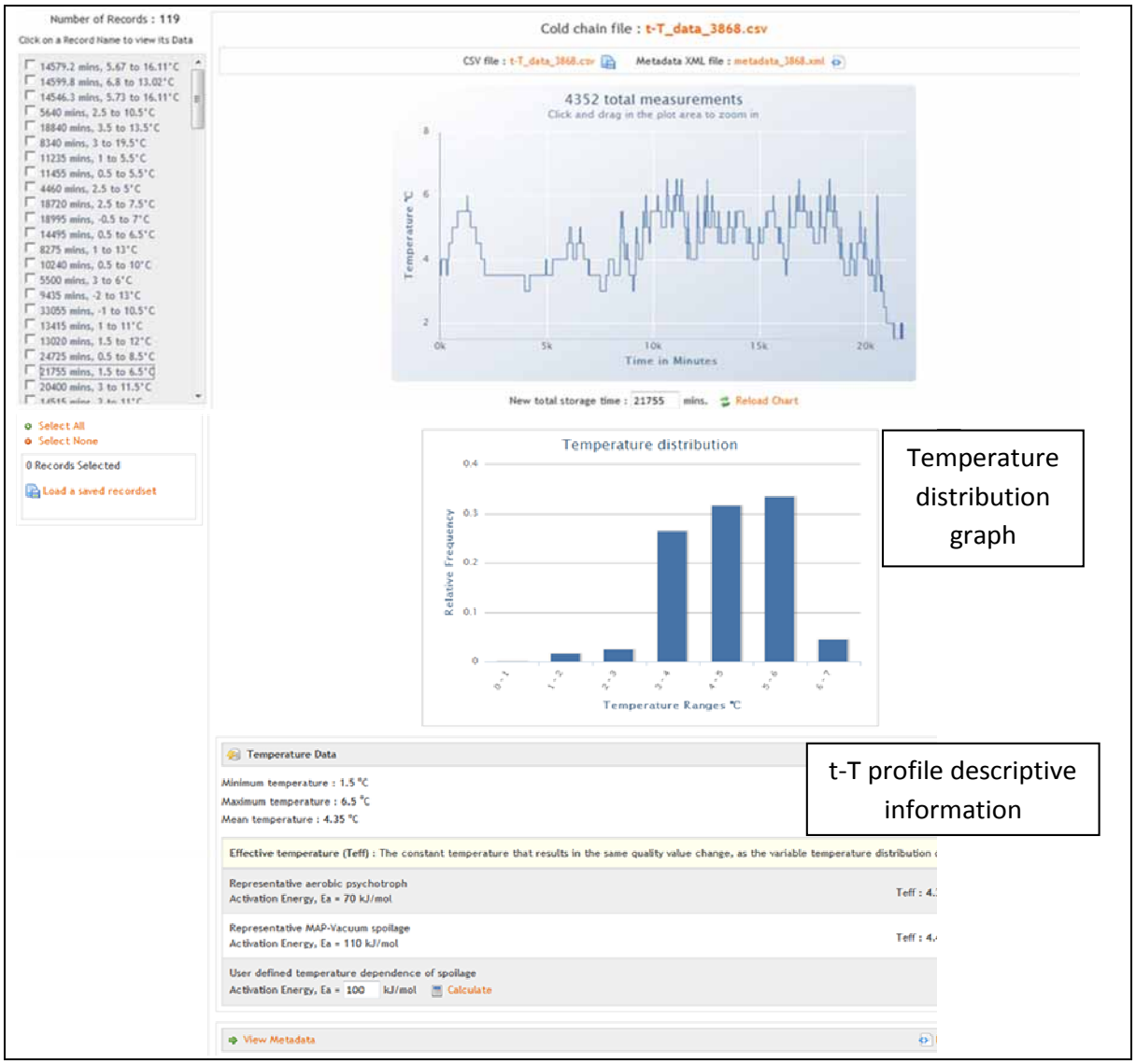
Cold Chain Database (hosted in the link **http://www.frisbee-project.eu/coldchaindb.html**) (Picture 2) has been constructed in order to develop a user friendly on line platform where collected data from all cold chain stages (Data collected in the Cold Chain Data Collection Platform) can be retrievable and available to be used from candidate users (consortium members, beneficiary members, industry and research institutes).



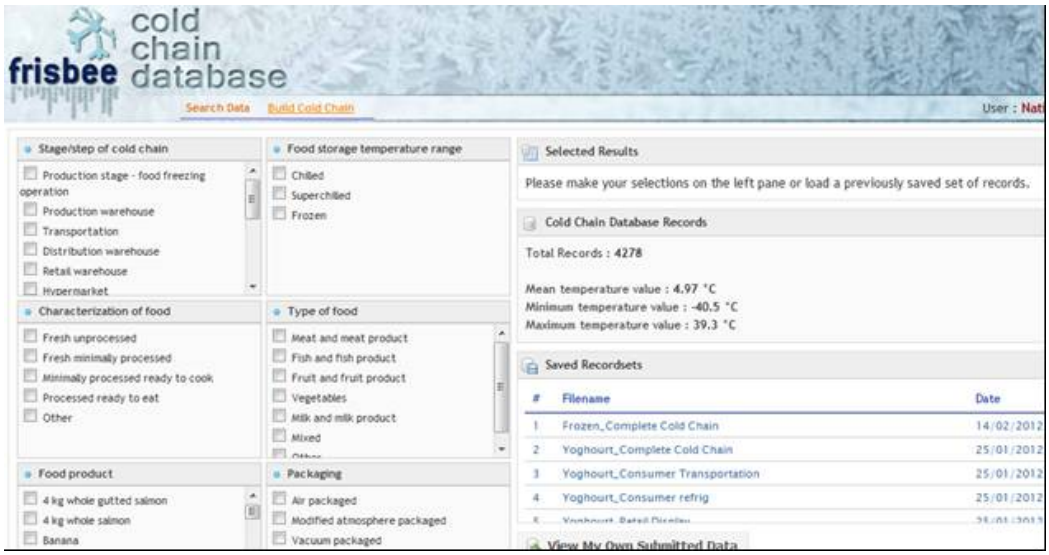
Picture 2. Cold Chain Database web site (**http://www.frisbee-project.eu/coldchaindb.html)**

One is able to retrieve t-T profiles of specific products along the cold chain using search criteria such as Stage/step of the cold chain, Food storage temperature range, Characterization of food, Food product etc. All received data were further processed in such a way that the output of the web-based database includes (Picture 3): actual t-T profiles, Mean, min and max value of temperature for the whole t-T profile, Effective temperature of the t-T profile. To demonstrate the integrated effect of the temperature variability on product quality, the term of the effective temperature T_{eff} is introduced. T_{eff}, which is defined as the constant temperature that results in the same quality value as the variable temperature distribution over the same time period, is based on the Arrhenius model and integrates, in a single value, the effect of the variable temperature profile.

Up to now the Cold Chain Database consists of more than 9000 t-T profiles and is being continuously updated with new data uploaded from an expanding network of contributors. In this database, the user can build a specific sequence of cold chain stages for specific food product based on user defined search criteria (Picture 4).



Picture 3. Description of the Cold Chain Data Collection Platform output



Picture 4. Cold Chain Database web site where the user can select the "Build Cold Chain" application.

Cold Chain Predictor Software

Cold Chain Predictor (v1.1) is a software tool designed in the framework of FRISBEE project. The purpose of this tool is to simulate a cold chain by building a t-T history from the contributed profiles. This tool is based on nearly 9000 t-T profiles obtained for different food products along the European cold chain. The FRISBEE Cold Chain Predictor (FRISBEE_CCP), software developed within FRISBEE objectives, allows the user to estimate the distribution graph of (effective) temperature for a specific stage of a selected food product (Picture 5) and to calculate the remaining shelf life of the food product at different stages of the cold chain (Picture 6), if quality decay data are known (Figure 1).

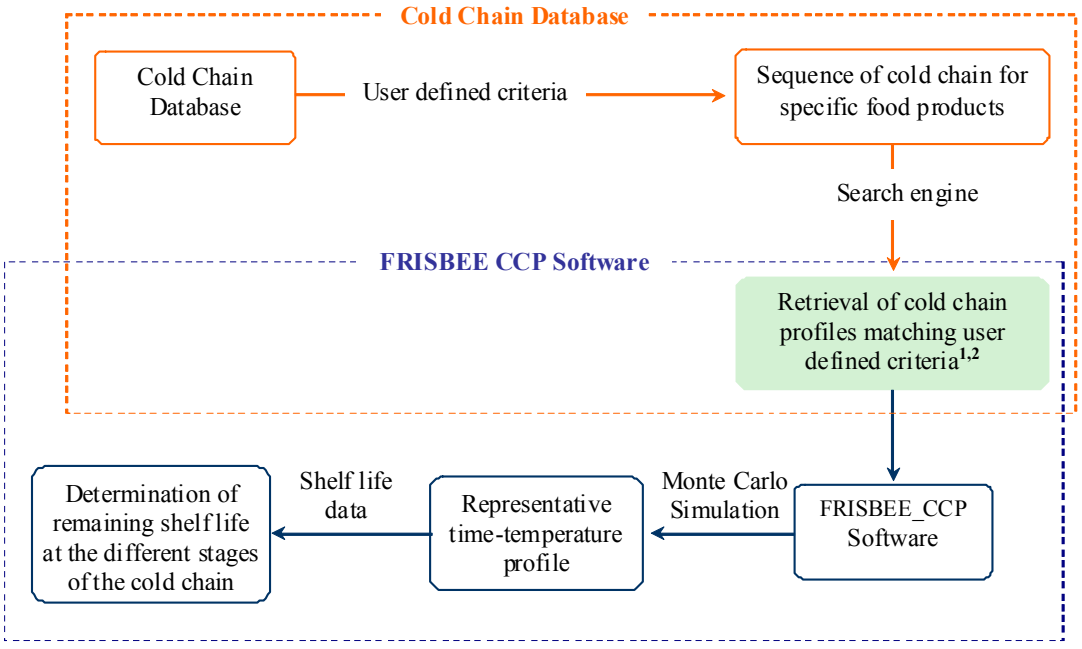
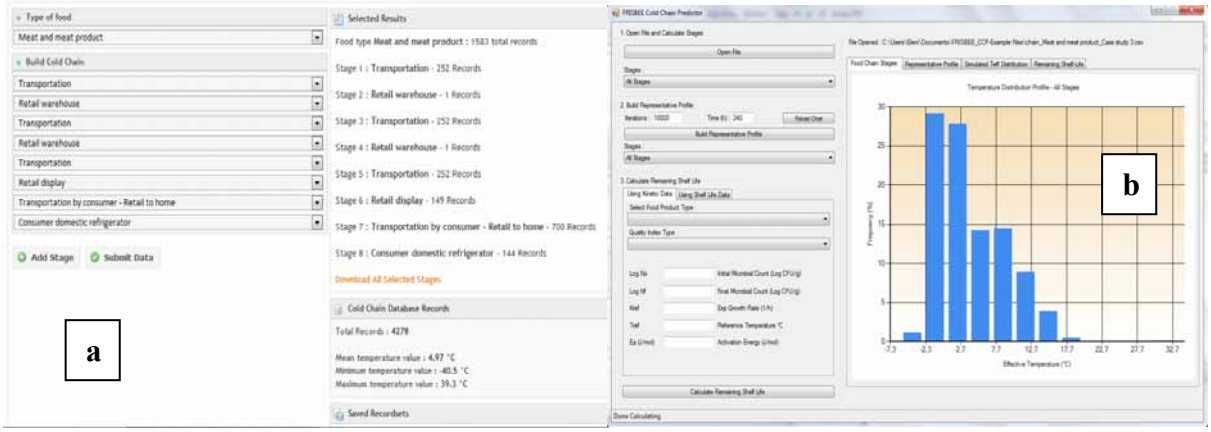
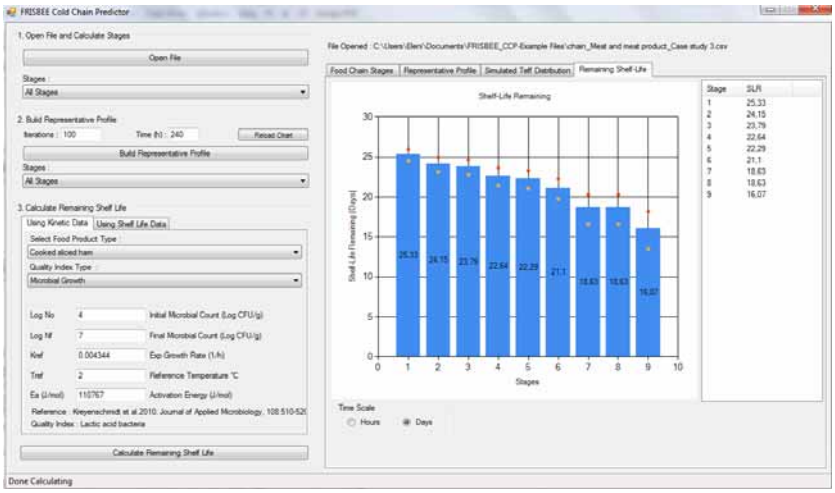


Figure 1. Illustration of FRISBEE_CCP Software-Option 1: Build Representative Profile



Picture 5. (a) Build of a sequence of cold chain stages for meat products and **(b)** determination of the representative profile and temperature distribution for all stages (example).



Picture 6. Calculated remaining shelf life of meat product at each stage of the cold chain based on a built representative profile using kinetic data (example).

Assessment of the Food Cold Chain using FRISBEE Tools

The main objectives the FRISBEE project may be summarized in Figure 2. Using multiple real data from the database realistic representative sequences of user defined cold chain stages may be built. The FRISBE Cold Chain Predictor (FRISBEE CCP) software was designed to reproduce by Monte Carlo simulation the most likely time/temperature distribution for each defined stage of the cold chain and to estimate for a selected food product, going through the cold chain, the remaining shelf-life after each individual stage.

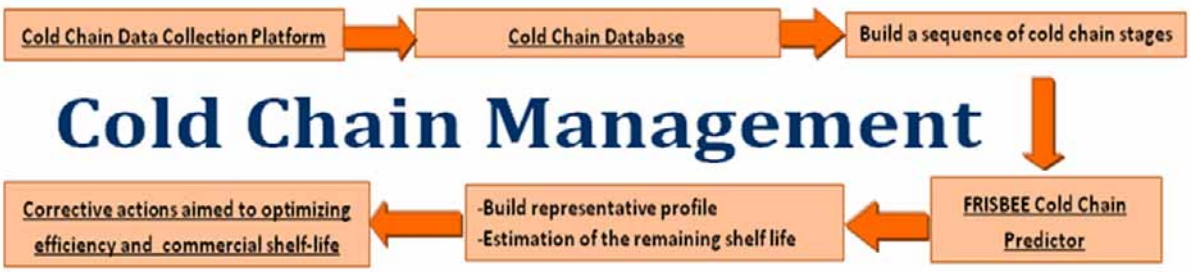
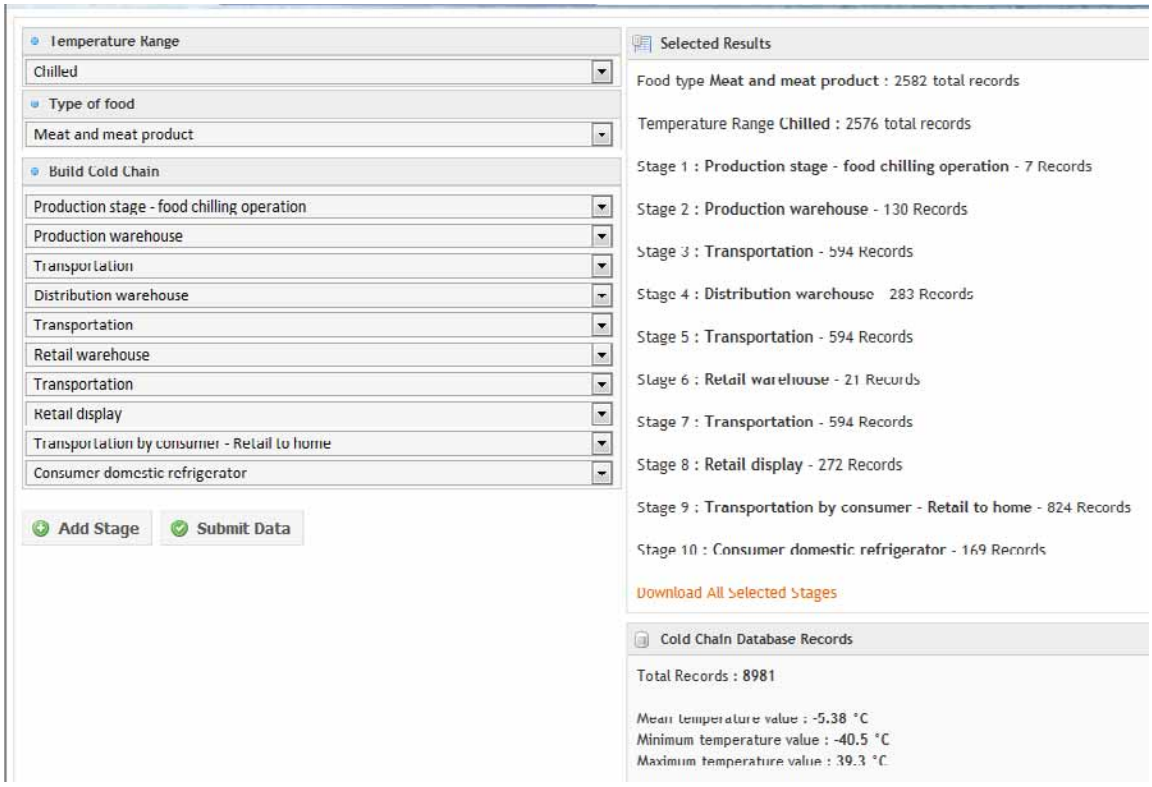


Figure 2. Summarized FRISBEE objectives for the Cold Chain Management.

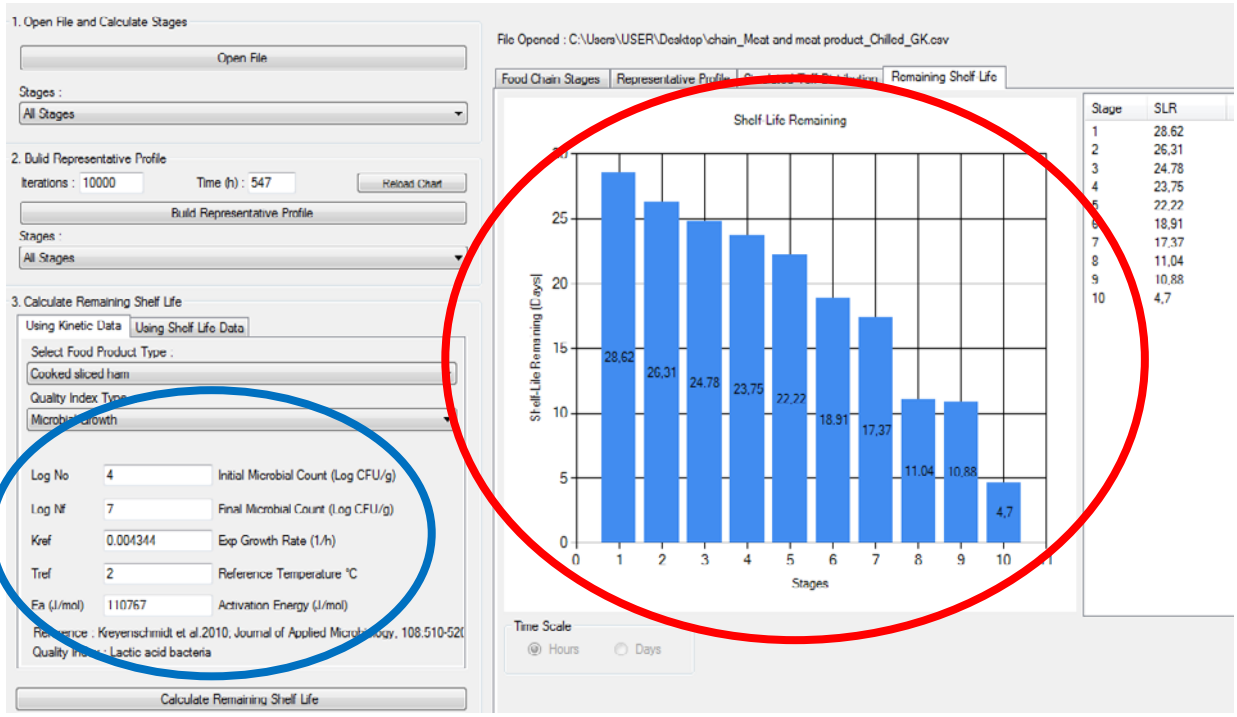
For meat products, there are over 2580 records of contributed profiles with real time-Temperature data measured at different cold chain stages from production to domestic storage. Building a sequence of real cold chain stages (Picture 7), a representative t-T profile may be built for meat products. This sequence consists of data production stage-food chilling operation, production warehouse, transportation, distribution warehouse, retail warehouse, retail display, transportation by consumer to home and consumer domestic refrigerator. Combining the representative profile with the kinetic deterioration data from published data (Kreyenschmidt et al., 2009), the remaining shelf-life of sliced cooked ham may be estimated

at any stage of the built cold chain, under actual temperature conditions, considering Lactic acid bacteria growth as the dominant quality deterioration parameter (Picture 8). The respective work may be conducted for other products as well, such as yoghurts, ice cream, frozen spinach etc. The obtained results for the sliced cooked ham were validated by conducting field test in 4 European countries (Greece, France, UK, Belgium). The received data from the field tests (estimated remaining shelf-life before consumer consumption) confirm the effectiveness of the FRISBEE tools for the cold chain management.



Picture 7. Sequence of cold chain stages for the building of a representative profile for meat products. Output of the Cold Chain Database (number of records for each cold chain stage are presented).

Overall, useful information from the Cold Chain Database may be retrieved and used to run realistic scenaria for the behaviour of food products along the cold chain avoiding costly and time-consuming field tests. The FRISBEE CCP offers thus the potential to effectively manage and improve cold chain weak links using appropriate shelf-life decision systems leading to an optimized handling of products in terms of both safety and quality. Using this tool, one can efficiently manage the food cold chain and undertake corrective actions at predetermined important stages of the cold chain. This could result in significant increase of the remaining shelf-life at the end of the cold chain.



Picture 8. Estimation of the Remaining Shelf-life for sliced cooked ham at any stage (within red circle) of the representative profile built using the FRISBEE Cold Chain Database and FRISBEE CCP tools combined with kinetic data of the deterioration factor (within blue circle).

CONCLUSIONS

Development and application of the European cold chain database as a tool for food products cold chain management has been described. The aim is the estimation of the remaining shelf-life of food products after each stage of the cold chain, based on a large number of t-T profiles available in the database and retrievable based on user defined criteria. Simulations of alternative distribution scenarios based on real cold chain data can be executed based on which corrective actions could be applied on the important stages for maximizing remaining shelf-life.

ACKNOWLEDGEMENTS

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EDIBLE COATINGS APPLICATION IN IMPROVING STORABILITY AND ENHANCING QUALITY CHARACTERISTICS OF TABLE OLIVES

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ABSTRACT:

Olives (Olea europaea) are of the oldest cultivated fruits, largely found in the Mediterranean countries. After fermentation they are usually stored in brine in plastic or glass containers and cans. The aim of this study is to introduce a new type of preservation and packaging of table olives that can maintain their quality characteristics for longer time by using edible coatings combined with modified atmosphere packaging (MAP). Green and black table olives either with or without kernel were examined while the edible coatings of hydroxy-propyl-methyl-cellulose (HPMC) and chitosan were applied. The table olives preserved in brine were treated with an aqueous solutions of 1% HPMC or chitosan, then packed under normal or modified atmosphere and stored under ambient (25˚C) temperature conditions. Modified atmosphere conditions applied in coated olives' packaging were selected based on a previous experimental study and were: 80% CO_2 - 20% Air. During storage, the olives were examined for their weight loss and for physicochemical characteristics including color, texture (firmness), pH and titratable acidity (expressed as lactic acid/100 mg of olive pulp). Sensory evaluation was also conducted by a trained panel throughout the storage period. Based on the obtained results, the coating application led to reduction of weight loss, maintenance of color and firmness and extended olives' shelf-life. Comparing the two coatings applied, chitosan in most cases proved more efficient than HPMC in olives' preservation maintaining their quality characteristics.

Keywords: Edible coatings, modified atmosphere packaging, table olives, chitosan, HPMC.

1. INTRODUCTION

Interest for table olives (olea europaea) has increased considerably in recent years, either for their high nutritional value or for their high concentration in antioxidants, phenolic compounds and unsaturated lipids. Table olives are a key element of the Mediterranean diet and are frequently utilized in the modern diet. The world production of table olives reaches 1.500.000 tn/year. The most common varieties of table olives commercially available in the international market are: Spanish style green olives, naturally olives (Greek-style) and black ripe olives (Californian style), for which elaboration processes are well-established in the literature. In general, the processing that table olives undergo before becoming available for consumption includes decrease of glucoside oleuropein (that causes the bitter tastes of the fruit) and fermentation by microorganisms (that cause the generation of natural compounds which enhance aroma and flavor as well as prolong the olives sustainability) (Sabatini, Perri and Marsilio, 2009; Garrido Fernandez, Fernandez Diez and Adams, 1997).

Edible coatings have long been utilized for preservation and shelf life extension of food products. The basic principle of edible coating is to control moisture loss and prevent deterioration of the product either by physicochemical or microbial agents with the application of a semipermeable membrane/film on the surface of the product. The implementation of the coating on fresh agricultural products is commonly carried out by dipping in or spraying (Li and Zhao, 2007). Common edible films and coatings consist of proteins, polysaccharides, and lipids, which include natural waxes and resins. Additionally, emulsifiers and plasticizers are added to improve coating performance. Apart from preservation reasons edible films can also be applied as carriers of food additives like colourants, flavoring agents, antioxidants, or antimicrobial compounds (Valencia-Chamorro, Perez-Gago, del Rio and Palou, 2009). The most important property of an edible coating is the ability to shelter the food product by moisture, oxygen and carbon dioxide thus maintaining the products freshness. Edible coating is ideal for minimally processed food products like vegetables and fruits (Vargas, Pastor, Chiralt, McClements and Gonzalez Martinez, 2008).

Modified atmosphere packaging (MAP) is considered to be one of the most important food preservation techniques that maintain the natural quality and extend the storage life of fruits and vegetables without compromising the quality and sensory characteristics of the latter. The basic principle of MAP is the packaging of the product in carbon dioxide and oxygen concentrations different from normal atmosphere and therefore preventing oxidization phenomena, ethylene production and microbial growth. Thus MAP contributes to maintaining

freshness of the product as well as quality and safety during storage (Xing, Li, Xu, Jiang, Yun and Li, 2010).

Chitosan is a high molecular weight copolymer consisting of β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units with the latter usually exceeding 60%. Chitosan exhibits antibacterial and antifungal activity as well as film-forming properties therefore makes an ideal material for edible food coatings (Elsabee and Abdou, 2013). It has been proven that chitosan acts as a fat trap, reducing the absorption of cholesterol in the human body by 20-30% and as a fiber interfering with the modulation of the duration of the intestinal transit. Additionally chitosan has a natural ability to form active edible or biodegradable films, therefore chitosan coating can be expected to limit contamination on the food surface (Vargas, Pastor, Chiralt, McClements and Gonzalez Martinez, 2008).

Hydroxy-propyl-methyl-cellulose (HPMC) is also suitable for edible coatings as it appears to regulate moisture transfer in food systems. Cellulose ether films -deriving from aqueous or aqueous-ethanol solutions of methyl-cellulose (MC), hydroxyl-propyl-methyl-cellulose (HPMC), hydroxyl-propyl-cellulose (HPC) and carboxy-methyl-cellulose (CMC) tend to have moderate strength, are resistant to oils and fats, and are flexible, transparent, odourless, tasteless, water-soluble and moderate barriers to oxygen and moisture (Villalobosa, Hernandez-Munoz and Chiralt, 2006).

2. MATERIALS & METHODS

Green and black olives in brine and two edible films based on HPMC (1% w/v) and chitosan (0,8% w/v) were examined. HPMC was easily dissolved in hot water (~80°C) and chitosan was dissolved in an aqueous solution of 1% v/v glacial acetic acid. The coating was applied by dipping the olives in the coating solution. The coated olives were packed in modified atmosphere of low oxygen (80% CO_2 - 20% Air) and stored at ambient (25°C) temperature conditions for 7 months (183 days). During storage the olives were examined for their color (MINOLTA CR-200) with the CIE L*a*b* Color Scale, texture (Texture Analyser XT2 - Stable Microsystems), weight loss, titratable acidity (expressed as lactic acid/100 mg of olive pulp) and their sensorial characteristics by a panel of 10 trained assessors. The shelf-life of the coated packaged olives was also evaluated based on the overall acceptance.

3. RESULTS AND DISCUSSION

3.1 Color

Figures 1-4 present the total color value E for the table olive samples during their storage.

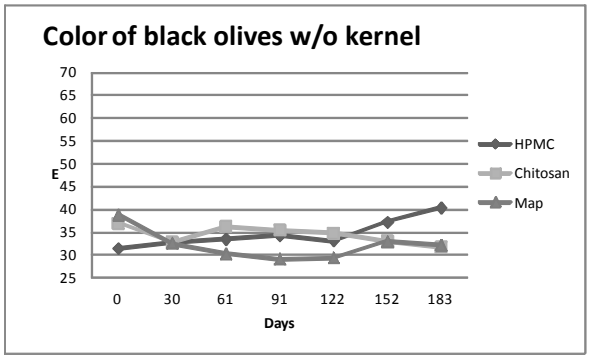

Figure 1: E color value of black table olives without kernel

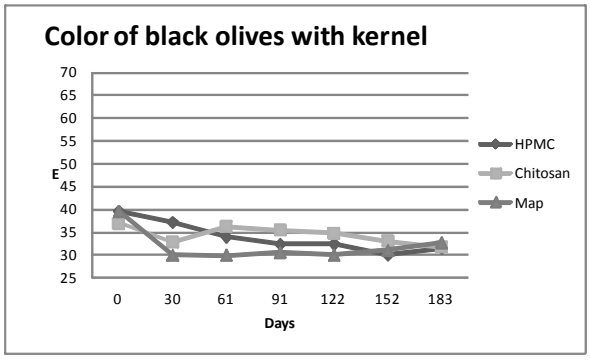

Figure 2: E color value of black table olives with kernel

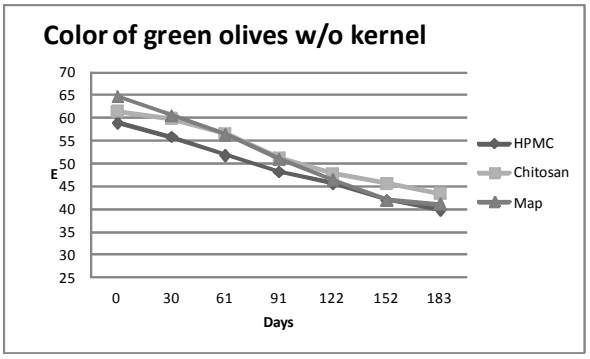

Figure 3: E color value of green table olives without kernel

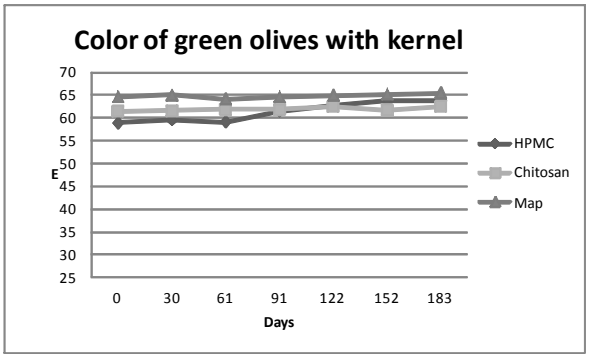

Figure 4: E color value of green table olives with kernel

In general the change of the color of the olives, either green or black, was very low. The olives retained their color during the 7 months of storage. The coating did not affect the color of the olives significantly ($P<0,05$). Therefore the coated olives with HPMC and chitosan showed similar alteration of color during storage with the non coated olives.

3.3 Texture Analysis

Texture analysis was performed on samples from different varieties and with different coating (black or green olives with or without kernel not coated and coated with chitosan or HPMC). The characteristic measured was the firmness of the olive exterior (skin) and interior (flesh). Figures 5-12 show the firmness of each sample during the 7 months of storage.

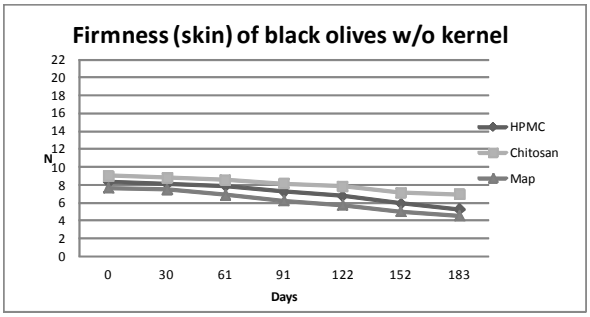

Figure 5: Firmness of the skin of black table olives without kernel

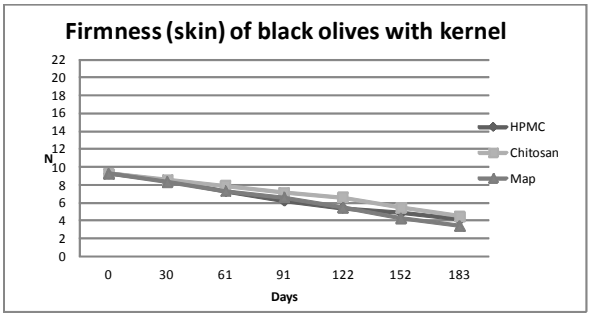

Figure 6: Firmness of the skin of black table olives with kernel

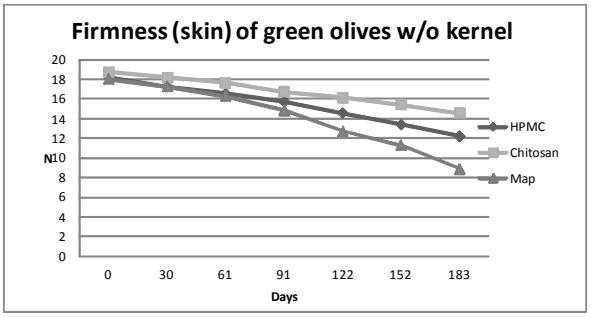

Figure 7: Firmness of the skin of green table olives without kernel

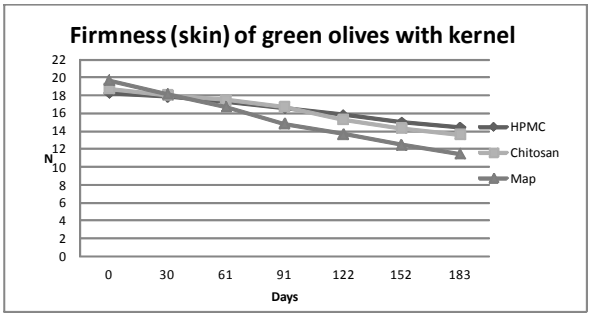

Figure 8: Firmness of the skin value of green table olives with kernel

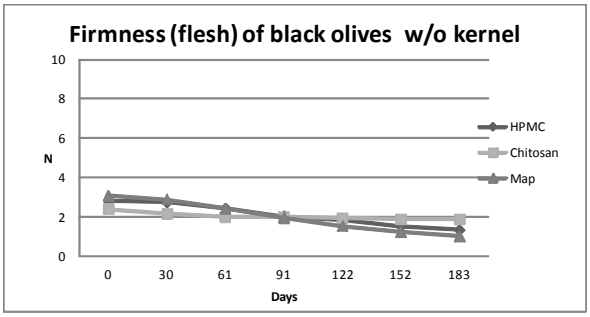

Figure 9: Firmness of the flesh of black table olives without kernel

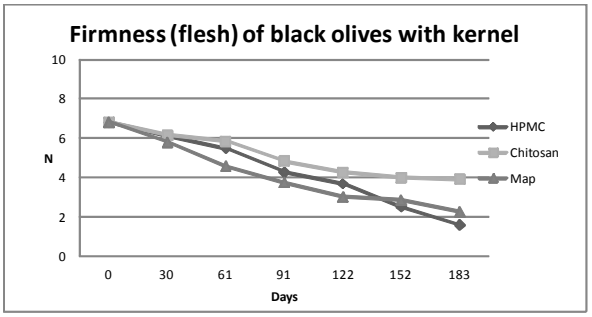

Figure 10: Firmness of the flesh of black table olives with kernel

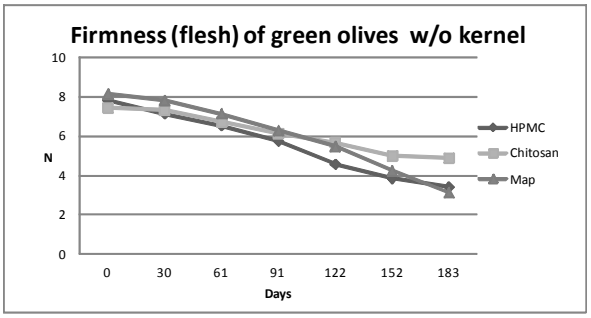

Figure 11: Firmness of the flesh of green table olives without kernel

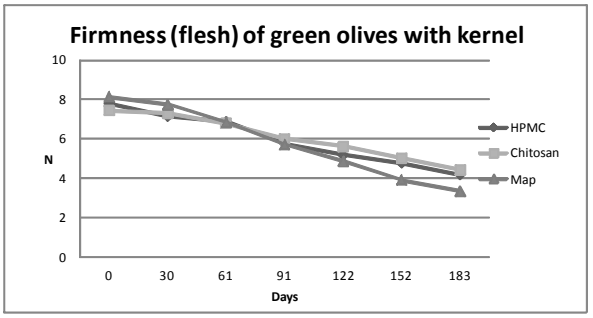

Figure 12: Firmness of the flesh of green table olives with kernel

In general black olives were less firm than the corresponding green. All samples showed that the olives' firmness was reduced with time, however the firmness of coated olives decreased

with slower rate compared with the non coated. Therefore coating olives with HPMC or chitosan hinders the softening of the olives' skin and flesh. Of the two coatings studied, chitosan presented lower firmness reduction rate than HPMC, thus preserving the texture of olives more effective than HPMC.

3.3 Weight loss

During storage all samples, either black or green, and both coatings presented a significant loss of weight compared with the fresh ones (values at time zero). This was attributed to the water migration from the body of the olives to the environment. Figures 13-16 present the weight loss of the olives during storage.

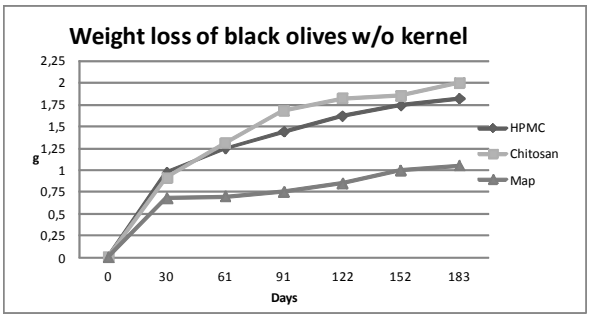

Figure 13: Weight loss of black table olives without kernel

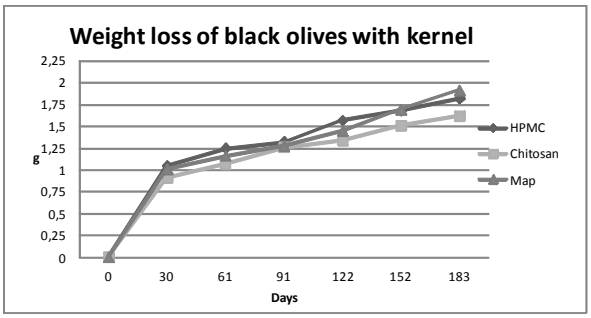

Figure 14: Weight loss of black table olives with kernel

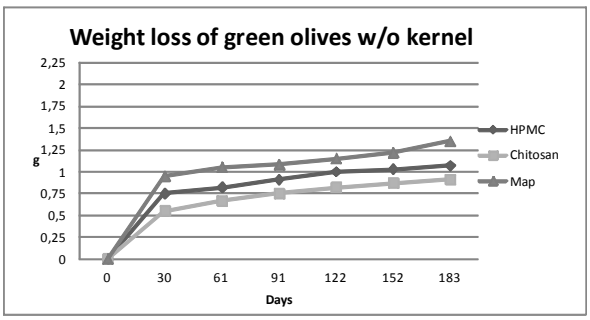

Figure 15: Weight loss of green table olives without kernel

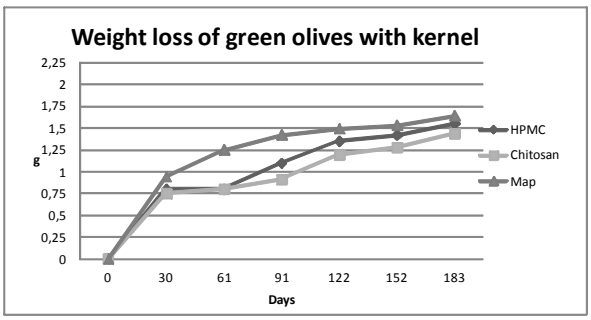

Figure 16: Weight loss value of green table olives with kernel

According to figures 13-16, the non coated olives presented the highest loss of weight, while those coated with HPMC presented the least. Therefore the coating of olives either by chitosan or HPMC hinders the loss of moisture. However, the ability of chitosan to reduce the moisture loss is higher than that of HPMC .

3.4 Tirtatable acidity

The increase in the tirtatable acidity of olives is a result of the lactic bacteria presence in their package. Table olives are fermented by lactic bacteria and the latter produce lactic acid thus increasing the tirtatable acidity of the olives. According to Codex Alimentarius, table olives must present acidity values higher than 0,4 m/m in terms of lactic acid (CODEX STANDARD FOR TABLE OLIVES CODEX STAN 66-1981 (1981, revised 1987); however high acidity is not acceptable because it is a sign of spoilage.

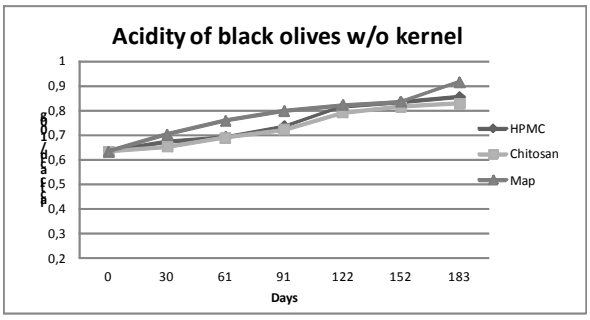

Figure 17: Acidity of black table olives without kernel

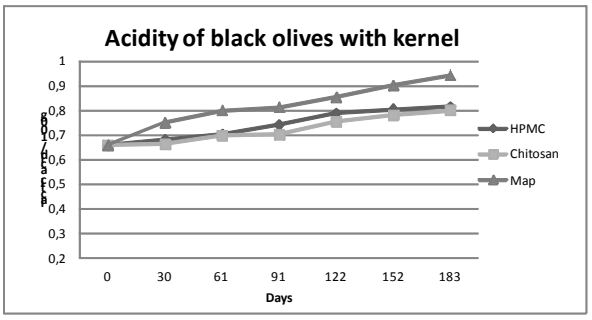

Figure 18: Acidity of black table olives with kernel

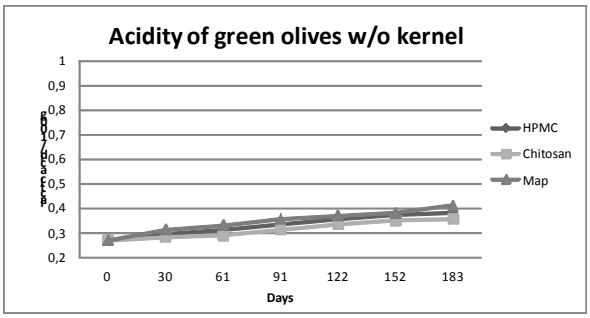

Figure 19: Acidity of green table olives without kernel

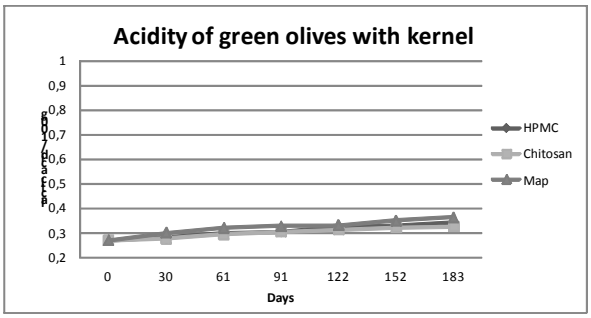

Figure 20: Acidity of green table olives with kernel

The acidity of the olives during the 7 months of storage presented low increase in all cases of olives and coatings (black or green with or without kernel not coated and coated with chitosan or HPMC), signifying that MAP packaging and coating with HPMC or chitosan results in the preservation of the initial value of acidity for at least 7 months. Especially chitosan and HPMC coated olives presented even lower increase in acidity that the non coated. Therefore the combination of coating with chitosan or HPMC with MAP packaging enchases the sustainability of table olives in terms of lactic acid generation.

3.5 Sensory Evaluation

Sensory evaluation of the olives' samples was conducted by a team of 10 trained panelists according to ISO 13300-1:2006 for their total acceptance on a 1 to 10 scale (1 represents a product totally inappropriate for consumption and 10 represents an excellent sample). Figures 21-24 present the scores each sample received versus storage time.

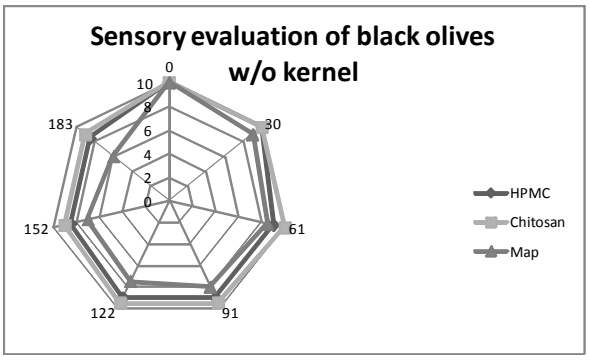

Figure 21: Total acceptance of black table olives without kernel

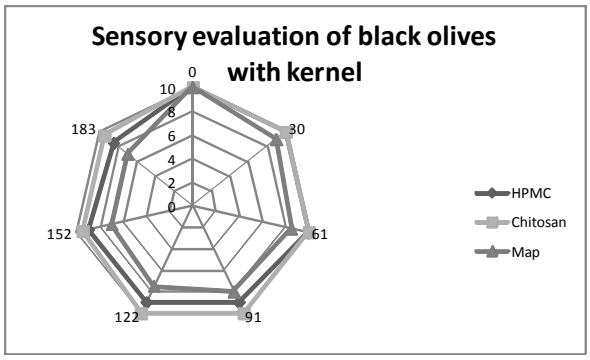

Figure 22: Total acceptance of black table olives with kernel

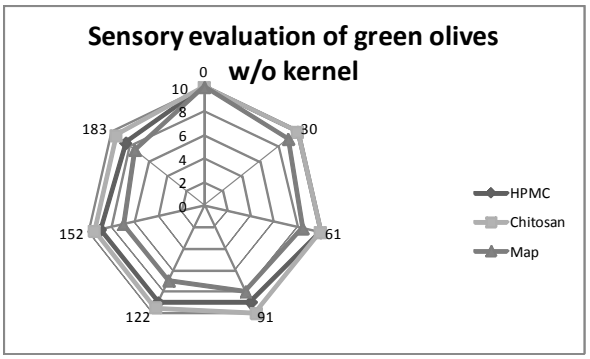

Figure 23: Total acceptance of green table olives without kernel

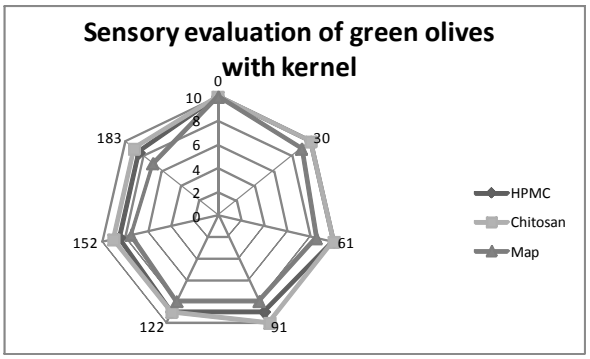

Figure 24: Total acceptance of green table olives with kernel

All samples were considered acceptable by the panel throughout the 7 months storage period. The scores in total acceptance of samples packed in MAP with no coating started to decrease soon after the 4th month of storage while the coated olives, either with HPMC or chitosan scored over 8 even after 7 months of storage. Especially olives packed in MAP and coated by chitosan scored 9, signifying that even after 7 months of storage the olives, either black or green, were only slightly degraded as compared to the initial samples and the liking of the panelists. In general MAP packaging is suitable for the storage of table olives prolonging the shelf life of the latter for more than 7 months. Especially when combined with edible coating,

either HPMC or chitosan, it prolongs the shelf life and the sensory characteristics of table olives even more effectively.

4. CONCLUSIONS

Concluding MAP packaging combined with HPMC or chitosan coating of green and black table olives with or without kernel results in the extension of the shelf life of the latter for more than 7 months. The combined effect of packaging and edible coating resulted in the preservation of the color of the olives, reduction in weight loss, slow reduction of firmness, low rate of acidity increase as well as maintenance of the sensory characteristics to similar values as the initial ones (samples at time zero). Of the two coatings compared, chitosan showed slightly better results than HPMC in the properties studied in the current work.

ACKNOWLEDGMENTS

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COMPARATIVE INACTIVATION KINETICS OF CRUDE AND PURIFIED CYSTEINE PROTEASES SUBJECTED TO HIGH PRESSURE PROCESSING

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ABSTRACT:

Plant sulfydryl proteases such as actinidin and ficin may be used as meat tenderizers, milk clotting agents for the development of novel dairy products or as proteolytic agents in biotechnological procedures. The main issue with regards their application is the control of their enzymatic activity after the desired extent of proteolysis. High Pressure processing (HP) could be used for enzyme activity regulation.

The objective of the present work was to systematically study high pressure (0.1-900 MPa) combined with temperature (30-80^{o}C) inactivation of the proteolytic activity of purified and crude actinidin (obtained from kiwi fruit Hayward var., *A. chinensis*) and ficin (obtained from fig latex, *Ficus carica caprificus*). Purified enzymes appeared to be significantly resistant to pressure treatment (yielded lower enzyme inactivation rate in similar treatments) when compared to crude enzymes. The results imply that the enzymes environment (composition and pH) play an important role in their stability.

The results of this research allow determination of the type of enzyme (purified form or enzyme extract) and processing conditions to be used efficiently in food industry applications.

Keywords: cysteine proteases, High Pressure Processing, kinetic modelling

1. INTRODUCTION

Ficin and actinidin are commercially important plant cysteine proteases found in latex of *Ficus carica* species and kiwi fruit flesh (*Actinidia chinensis*), respectively. These enzymes have many similarities to papain that is the most studied plant cysteine protease, with respect to structure, catalytic mechanism and activation by reducing agents. On the other hand, they

can differ in the amino acids lining, the substrate-binding pocket and in the substrate specificity.

Plant cysteine proteases can be used in food and beverage industry like meat tenderization (Sullivan and Calkins, 2010), removal of chill haze in beer, milk coagulation (Katsaros et al., 2010 ; Lo Piero et al., 2011), improvement in the processing quality of cereals (Feldberg and Baker, 1971) and gelatin hydrolysis. In recent years, many researchers have reported that actinidin and ficin can aid processing, improve the product sensory charecteristics and increase the efficiency of operation in dairy industry. Recent studies conducted by Katsaros et al. (2010) suggested the application of either kiwi fruit powder rich in actinidin or kiwi juice in the production of dairy products as well as the application of high pressure as proteolytic activity regulator. Another study reported the use of actinidin as a potential alternative of natural calf rennet for the coagulation of milk leading to novel dairy products (Lo Piero et al., 2011).

One of the major disadvantages for the industrial application of these enzymes in food industries is that they are extremely stable enzymes in terms of resistance to thermal, solvent and chaotropic denaturation. This provides difficulties in terms of developing an effective protocol to assure inactivation in the final product prior to consumption. Technologies such as High Pressure (HP) processing and Pulsed Electric Fields (PEF) that have potential to inactivate microorganisms and enzymes, while providing advantages with regards sensory and nutritional quality, could be employed. However, the data on the inactivation kinetics using HP on proteases are scarce in literature. In previous works, the pressure and thermal stability of commercially available papain and ficin proteases (Katsaros et al, 2009a) and of actinidin extracted from kiwi fruit Hayward var.,(*A. chinensis*) (Katsaros et al, 2009b) was investigated. Purified ficin and papain showed significant thermal and pressure stability, requiring intense process conditions for adequate inactivation.

The objective of the present work was to systematically study thermal and high pressure inactivation of the proteolytic activity of purified and extracted forms of actinidin (obtained from kiwi fruit Hayward var., *A. chinensis*) and ficin (obtained from fig latex, *Ficus carica caprificus*) during combined HHP (from 200 to 900 MPa) and temperature (from 30 to 80^{o}C) treatments. Mathematical models have been used to describe pressure–temperature dependence of inactivation rate constants of enzymes. Such models can be a useful tool in designing and optimizing high pressure processes.

2. MATERIALS AND METHODS

2.1 Enzymes

Actinidin extract from kiwi pulp

Fresh ripe kiwi fruits (*A. chinensis*), Hayward var. grown in Pieria, Northern Greece were obtained directly by the Association Cooperatives of kiwi fruit producers. The kiwi fruits were peeled and pulped. The pulp was centrifuged at 10,000 rpm for 15 min. The supernatant was filtered and the clear juice (pH 3.9), that had a significant proteolytic activity due to the endogenous actinidin, was used for the experiments.

Purified actinidin

Actinidin was extracted from kiwi juice (Hayward var., *A. chinensi*.) using the purification procedure described by Brocklehurst et al. (1981). After a number of steps such as centrifugation, ammonium sulphate fractionation (30-75%) and ultrafiltration, actinidin was purified by anion exchange (UNOsphereQ, Biorad) and gel filtration (Biogel P30, Biorad) chromatography. All received fractions were analyzed for proteolytic activity and protein concentration. The purified actinidin in 100mM phosphate buffer (pH 6.0) was used for the experiments.

Ficin extract from fig tree latex

Latex was collected directly from fig trees (*Ficus carica caprificus*) and stored at -30°C until used for the experiments. The latex was centrifuged at 10,000 rpm at 10°C. The centrifuged (crude) enzyme was dissolved in 100mM phosphate buffer (pH 7.0) and was kept for the conduction of the experiments.

Purified ficin

Purified Ficin powder (EC 3.4.22.3) used for the experiments were obtained from Sigma Chemical Co. (MI, USA). The ficin powder was dissolved in 100mM phosphate buffer (pH 7.0) and was used for the experiments.

2.2 Substrates

The hydrolytic activity of ficin and actinidin were determined using substrate pGlu-Phe-Leu p-nitroanilide (GPLNA) and nitroanilidine-Z-N-BOC-L-lysine-4-nitrophenyl ester,

respectively. These substrates were obtained from Sigma Chemical Co. (MI, USA). All chemicals used were analytical grade.

2.3 Enzymatic activity assays

The hydrolytic activity of ficin was determined using GPLNA (pGlu-Phe-Leu p-nitroanilide) as a low molecular weight substrate. The enzymatic activity was calculated from the initial rate of GPLNA hydrolysis by determining pNA liberation within the given period of time. The hydrolytic activity of actinidin was determined using nitroanilidine-Z-N-BOC-L-lysine-4-nitrophenyl ester as a low molecular weight substrate. The enzymatic activity was calculated from the initial rate of ester hydrolysis by determining nitroanilidine (NA) liberation within the given period of time.

2.4 Thermal treatment

The effect of temperature treatment on the ficin and actinidin stability was examined. 0.5 ml aliquots in thin-walled glass test-tubes were placed in water-baths (WB/OB 7–45, MEMMERT GmbH + Co., KG, Schwabach, Germany) at temperatures in the range 40–80^{o}C for various periods of time. During thermal treatment temperature was monitored and recorded at 2 s intervals in a multichannel datalogger (CR10X, Campbell Scientific, Leicestershire, UK) by a type T thermocouple placed inside a capillary used as a temperature indicator.

2.5 High pressure treatment (HP)

High pressure treatments were conducted using a laboratory scale HP equipment with a maximum operating pressure and temperature of 1000 MPa and 90˚C, respectively (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland) consisting of a high pressure unit with a pressure intensifier and a multivessel system of six vessels of 45 mL capacity each. The initial adiabatic temperature increase during pressure build up was taken into consideration in order to achieve the desired operating temperature during pressurization. Pressure and temperature were constantly monitored and recorded (in 1 s intervals) during the process.

3. RESULTS AND DISCUSSION

3.1 Thermal inactivation of cysteine proteases

Data for isothermal inactivation of ficins and actinidins could be accurately modeled applying a first-order kinetic model. For ficin from fig latex, the inactivation rate constants (k, min^{-1}) obtained at each process temperature were estimated as 0.0046, 0.0249, 0.0588, 0.1876 and 0.2259 min^{-1} for processing at 50, 60, 65,70 and 75°C, respectively. The corresponding k-values for the purified ficin in phosphate buffer (pH 7.0) were calculated 0.0011, 0.0158, 0.0592 and 1.0951 at 50, 60, 70 and 80 °C, respectively. Comparing these data and as evidenced by the plotting of remaining enzyme activity vs time for temperatures 50, 60 and 70°C (Fig. 1), the purified ficin appeared to be more resistant to heat treatment. The temperature dependence of inactivation rate constants of ficin in the temperature range studied could be described adequately by the Arrhenius equation with an activation energy value of 154±15 kJ/mol (R^2=0.985) obtained. The corresponding value for the purified enzyme was slightly higher (210±22 kJ/mol, R^2=0.989).

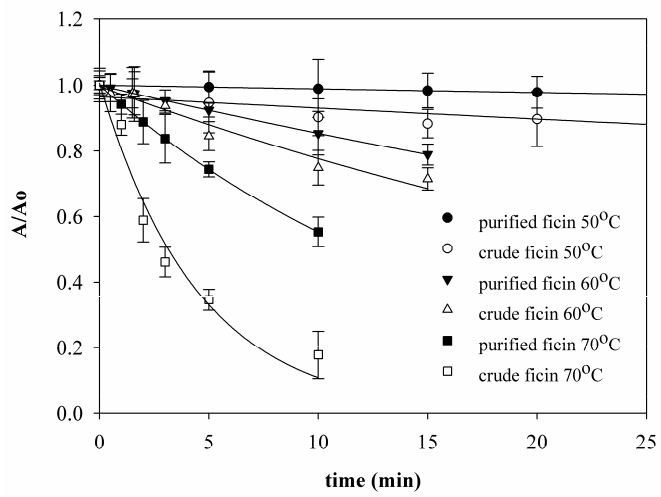

Figure 1. Thermal inactivation of purified and crude ficins at temperatures from 50°C to 70°C at ambient pressure.

Isothermal inactivation of purified and crude actinidin was investigated as well. As in the case of ficin, the purified form of actinidin is more stable to heat treatment. For purified actinidin, the inactivation rate constants (k, min^{-1}) obtained at each process temperature were estimated as 0.0183, 0.0367, 0.1567 and 0.4295 min^{-1} for processing at 65, 67.5,70 and 72.5°C, respectively, while the values of k for this enzyme in crude form was achieved at lower temperatures (k_{40^oC} = 0.0611 min^{-1}, k_{45^oC} = 0.3776 min^{-1}, k_{50^oC} = 1.6700 min^{-1}, k_{55^oC} = 5.8951 min^{-1}). The temperature dependence of inactivation rate constants of purified and

crude actinidin in the temperature range studied was also investigated and expressed by the E_a value (426±23kJ/mol and 267±42 kJ/mol for purified and crude actinidin, respectively).

3.2 High pressure inactivation of cysteine proteases

Investigation of the effect of both pressure and temperature on the inactivation of proteolytic activity of ficins and actinidins (purified and crude form) was carried out. HP inactivation of studied enzymes was described by first-order kinetics, as in the case of thermal inactivation. Inactivation rate constants of the enzymes studied increased with increasing pressure and temperature levels applied.

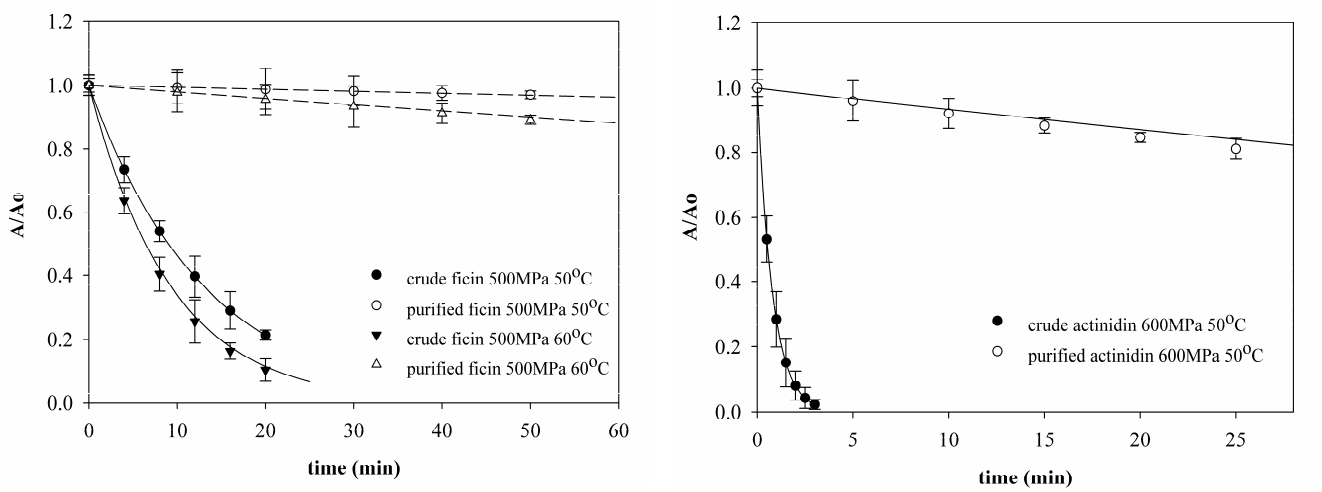

Figure 2.a. Purified and crude ficin inactivation during processing at 500 MPa and various isothermal conditions (50 and 60°C). **b.** Purified and crude actinidin inactivation during processing at 600 MPa and 50 °C.

In Figure 2 a and b the high pressure inactivation curves of ficin and actinidin (crude and purified forms) are represented. Analyzing the results, it can be observed that high pressure inactivation of purified enzymes was slower compared to the crude enzymes indicating that the purified enzymes were more pressure stable. This conclusion could be attributed to the pH value or to the level of enzymes purification. The pH of buffer solution for both enzymes was close to 7 indicating that the enzyme pressure stability is increased at neutral pH values. Severe conditions based on combining high temperatures and high pressures were necessary to completely inactivate the purified enzymes.

Having as variables pressure and temperature and taking also into consideration the effect of pressure on E_a and the effect of temperature on V_a, a multi-parameter equation (Polydera et.

al, 2004) was used to predict the inactivation rate constant at any combination of pressure and temperature conditions (Eq. 1).

$$k = k_{ref_{P,T}} \cdot \exp\left\{ \begin{array}{l} -\frac{E_{a_P}}{R} \cdot \exp\left[-B \cdot (P - P_{ref})\right] \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \\ -\frac{A \cdot (T - T_{ref}) + V_{a_T}}{R} \cdot \frac{(P - P_{ref})}{T} \end{array} \right\} \quad \textbf{(Eq.1)}$$

The parameters of the equation for all the enzymes studied (crude and purified forms) were estimated using non-linear regression routine, using SYSTAT 8.0 (Statistics 1998, SPCC Inc., Chicago, III, USA)(Table 1)**.**

Table 1. Parameters of the multi-parameter equation for ficin and actinidin (crude and purified forms) inactivation at various pressure-temperature conditions.

PARAMETER	**Estimated values (crude ficin)**	**Estimated values (purified ficin)**	**Estimated values (crude actinidin)**	**Estimated values (Purified actinidin*)***
P_{ref} (MPa)	600	750	600	600
T_{ref} (K)	323	343	313	323
$k_{ref\ P.T}$ (min^{-1})	0.069±0.009	0.019±0.005	0.169±0.011	0.007±0.002
E_{aP} (kJ/mol)	68.9±15.5	117±26	104±14	59.2±10.3
V_{aT} (ml/mol)	-6.2±2.1	-11.6±3.2	-12.8±1.4	-9±1.9
B (MPa^{-1})	-0.0020±0.0004	0.6278±0.0014	0.2529±0.0012	-0.0020±0.0005
A (ml/molK)	-0.742±0.232	0.0028±0.0005	0.0007±0.0003	-0.265±0.0064
R^2	0.99	0.99	0.99	0.99

4. CONCLUSION

Thermal and high pressure-assisted thermal inactivation data of ficin and actinidin (purified and crude form) could be fitted to the first-order reaction model. In order to achieve the necessary inactivation, the adequate process could be described by a mathematical model. Purified enzymes appeared to be significantly resistant to pressure treatment. Similar

treatments performed in crude forms yielded higher enzyme inactivation rate, which implies that the matrix (composition and pH) play an important role in enzyme stability.

ACKNOWLEDGEMENTS

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EFFECTS OF DEXTROSE AND MODIFIED STARCH OF WAXY CORN CONTENT ON BREAD MECHANICAL PROPERTIES.

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ABSTRACT:

The aim of the present paper is the study of the effects of dextrose and modified starch of waxy corn content on bread mechanical properties.

In order to examine the effects, six dough recipes (S1,…S6) were tested. Additions of flour of two auxiliary materials/additives used for varying the softness and better maintainability of toast type breads. At the same time, bread samples without additives were taken from the standard output of the bakery plant. The additives used were dextrose and modified starch of waxy corn with amylopectin content and no amylose.

The overall samples of the six dough recipes (six treatments) were produced based in the classic production and methodology which is used by the bakery industry "Ortoulidi" without alterations in the basic recipe. Then all the different bread samples were cut into slices of toast form in Larissa, within the next day of their production in the bakery, using the special type machine toaster and they all have the same dimensions and packaged in a special film per 10 slices for a total of 20 bread samples for each recipe. Twenty samples were used in order to open one every day and each sample was considered using the force- deformation Analyzer device ZWICK available in the Manufacturing Engineering Laboratory of the Department of Biosystems Engineering in TEI of Larissa in Greece. Firmness is accepted as a measure of freshness and quality. The firmness method is useful for measuring freshness and quality in bakery product development and quality control. The test method principle is quantitatively measure the force required to compress the bread sample. One slice of bread 25mm thick or two slices, each 12.5mm thick can be used. The slices were cut mechanically, the end three slices were discarded and the crusts were not removed. A 38.1mm Ø probe (TA4/1000) at a

test speed of 2mm/s was used. The location of slice testing is the centre of the bread slice(s) avoiding non-representative areas of crumb.

The study of the resulting curves of compresion force vs elastic deformation revealed that the sample with the smallest change in work strain with time is the S2 containing the lesser amount of starch. Also as starch increases the slope of the work elastic deformation in relation to the time grows thereby hardens abruptly bread. This means that adding thermostable modified waxy starch in the formulation is a disadvantage while dextrose addition is increasing the softness of the bread and the period it remains soft.

Results showed that the addition of modified waxy maize starch does not improve and rather worse texture and no longer gives the softness of the bread toast and brioche produced by a near recommendation dough. In the contrary, the addition of dextrose to the recipe improves texture/softness of bread and preserve it for a long time and therefore must be used as an additive.

The results of the the six (6) dough recipes with different additive compositions revealed that the tested samples of recipes S2 (1.5% dextrose on flour and 5% modified starch of waxy corn content) and S1 (1% dextrose on flour and 0% modified starch of waxy corn content) were extremely successful, possibly due to modulation/reduction of water activity which sustains better the bread and provides sustained softness/ firmness.

Keywords: Dextrose, modified starch of waxy corn, firmness method, bread mechanical properties.

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