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IMPACT OF BIOACTIVE COMPOUNDS ON IMPROVING THE OXIDATIVE STABILITY OF GROUNDNUT AND MUSTARD OILS

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ABSTRACT
Physical and chemical properties of groundnut oil and mustard oil are studied at different concentrations of two antioxidants Tocopherol and Tert- Butyl Hydro Quinone (TBHQ). Classification of vegetable oils in terms of their usage safety, stability in transport, easy reuse characteristics and overall health benefits is vital to categorize them for quality. Hence, to increase the shelf life of oil in cooking and pipelining, vegetable oils are added with different concentration of antioxidants to improve the permanence. Properties like viscosity, relative density, electrical quality factor and acid value are analyzed with the addition of antioxidants. Variation of viscosity with temperature for different concentration of oil was observed. Empirical equations like Wright's ASTM Equation and Akerlof - Oshry's relating viscosity with temperature are computed and compared and Wright model (R^2= 0.999-0.995) is highly correlated. The experiential value of relative density and acid value show good variation with the addition of tocopherol even below 10 mg/100 mL and random variations with TBHQ. The present study also states that addition of TBHQ to groundnut oil do not produce any synergistic effect with the antioxidant in the oil and also do not impede the oxidation reaction effectively.

1. Introduction

Vegetable oils are also much used in the food industry and are found to have an exponential rise with developing civilizations over the past few decades. Extraction and production of vegetable oil are more in South America and south East Asian countries. They are the suppliers of edible oil to every part of the world (Fasina, 2006). Hence the physical and chemical property of the oil should be maintained for economic transfer. The individualistic properties of oil are pre-determined by their constituent molecules and their behavior at various cooking conditions. With continued exposure to atmosphere at higher heating conditions, the oils achieve a state called rancidity where the double bonds of the unsaturated fatty acids undergo cleavage to release oxidative products (Augustin, 1987). The subsequent effect observed in the oils is highly harmful for consumption. The super-oxides and other free radicals released as the aftermath products of oxidation are capable of eliciting chain reactions within the cell machinery, leading to permanent DNA damage (Augustin, 1987; Williams, 1999).

Lipids, the predominant substrates of oils usually exist in a non-radical singlet state and at times of high exposure to heat; the rate of

radical formation is greatly accelerated. When these lipid radicals are formed in the oil, they tend to react with the free oxygen ions in sequential chain reactions, catalyzing auto-oxidation (Soriguer, 2003; Rubalya and Neelamegam, 2015). Thermal oxidation occurs faster than auto-oxidation while the primary oxidation product peroxide gives rise to secondary oxidation products like aldehydes and ketones. These secondary products are the major reason for causing rancidity in oil (Kochhar, 2000).

As an inherent measure of reducing the risk factors, oils contain components called antioxidants that inhibit the process of oxidation, operating at a certain limit (Becker, 2007; Jan Pokorny, 2001). Antioxidants are often extracted from both their natural sources and chemical sources. Tocopherols, Ascorbic acid, Carotene-compounds, phyto-sterol, sterol, oryzanol, etc. are the most commonly occurring antioxidants in oil by nature. There are some synthetic antioxidants like Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), Propyl Gallate (PG) and Tert- Butyl Hydro Quinone (TBHQ) added by the distributers to improve the shelf life of the oil (Rubalya and Neelamegam, 2012; Kousuke Hiromori, 2016). Some antioxidants, for example, Tocopherol exists in various forms like α-, β-, γ-, δ-tocopherols (Jan Pokorny, 2001). These antioxidants also play a pivotal role in extending oil's shelf life and keeping it away from rancidity, thereby enabling a manageable extent of oil reuse. Tocopherol and its derivatives are lipophilic antioxidants that are capable of active alkoxyl and peroxyl scavenging. The most important role of tocopherol lies in protecting lipid containing food substances from oxidative stress and damage during prolonged usage and storage (Rubalya and Neelamegam, 2012). TBHQ is a hydro quinone derivative that is extensively used in industries as stabilizers that restrict or stop auto-polymerization of organic peroxides. As a preservative for saturated vegetable oils, TBHQ is found to be fairly inert without causing many changes to the oil it was added. The flavor and odor of the oil remains the same even after TBHQ addition (Kousuke Hiromori, 2016).

The addition of antioxidants is done in significantly low concentrations that are capable of delaying or inhibiting the oxidation of lipid substrates in the oil. It is important that the antioxidants are stable, non-reactive with undesirable substrates, non-toxic and capable of trans-membrane passage. It is also a notable quality of a good antioxidant when the color, flavor, texture and the skeletal physical properties of oil remain the same before and after its addition. Addition of high concentration of antioxidant will alter the behavior of oil leading faster in producing toxic effect in human health (Jun Li, 2014). In addition, antioxidants plays a significant role in donating H+ ions to $RCOO^-$ radical and hence in preserving the quantity of fatty acids that alters the physical and chemical properties of oil. This can be used to enhance the shelf life of oil under storage and also reduce the transportation costs by lowering the viscosity of oil which leads to efficient pumping (Jan Pokorny, 2001). Viscosity of oil plays a major role when oil is transported in bulk across different compartments or through pipes.

Polar molecules (peroxides, ketones, free fatty acids, triglycerides etc.) in the oil can follow the changes in the direction of the electric field, when it is kept in an external field. As the frequency continuously increases, the dipole motion can no longer keep up with the changing field due to its relaxation time (Jun Li, 2014). Quality factor of oil is the ratio of permittivity with imaginary loss in the medium at external electric field. The permittivity of edible oils at room temperature ranges from 3.0 to 1.9 with respect to frequency (Rhet de Guzman, 2009). In the present work, physical and chemical parameters; viscosity, relative density, electrical quality factor and acid

value are experimented at different concentration of antioxidants - tocopherol and TBHQ. This study will help understanding the inherent antioxidant property of the oils as well as their behavior in the presence of added natural and synthetic antioxidants. The variation of viscosity with respect to temperature and the concentrations of antioxidant give an understanding of oxidative stability in the oil.

2. Materials and methods

To compare the oxidative stability of Groundnut (*Apios americana*) oil and Mustard oil (*Brasicca compestriss*), the oils fresh samples were purchased from a local supermarket in Trichy, Tamil Nadu. The basic composition of oil was noted down for reference. The various reagents used in physical parameters were purchased from SLR (Standard Laboratory Reagent) grade. TBHQ (tert- butyl hydroquinone) and α-tocopherol antioxidants were procured from Sigma Aldrich Chemicals India Private Ltd. The antioxidants used for this study were both natural and synthetic of origin. Tocopherol was purchased as Vitamin E EVION capsules from a local pharmacy. 16 samples with groundnut and mustard oils along with different concentration of antioxidants were prepared and 2 samples of fresh oil were taken for the study. The maximum concentration used in the studies, as per FDA (Food and Drug Administration) rules, is 0.02%. Thus a gradient of antioxidant concentrations was dissolved in the oil for preparing the samples. Increasing concentration of antioxidants (0mg, 5mg, 10mg, 15mg and 20mg) was added in five 100 mL samples of groundnut and mustard oil. These 20 samples were kept in dark bottles with a paper being wrapped around them to offer protection from external atmospheric conditions and thereby ambient temperature was maintained.

Heating was provided by keeping the oil sample in a copper beaker and placed in a water bath. The whole set up is aided by an electric device. The range of temperatures maintained for the oil samples is 303K, 313K, 323K, 333K, 343K, 353K, 363K and 373K.

A. Physical parameters

*a) Measurement of Viscosity***:** Kinematic viscosity of oil using laminar flow was experimented using redwood viscometer (ASTM D445-American Society for Testing and Materials) manufactured by New Delhi-Associated Instrument Manufacturers, India Private Limited.

*b) Measurement of Relative Density***:** Relative Density of the oil samples was measured using a Pycnometer (ASTM D891-09) calibrated with an accuracy range of ± 0.2 kg m^{-3}.

*c) Measurement of Dielectric Constant***:** Dielectric constant of the oil was measured by the capacitive method (DCK-001,Mittal enterprises, New Delhi, India) at 2MHz. Dielectric constant from 1Hz to 10MHz was measured in accordance with the standard of IEC 61620. All dielectric measurements follow ISO 9001:2000.

B. Chemical parameters

a) Measurement of Total Acid Value (TAN): Acid Value is measured by a simple titrimetric analysis method. Oil samples with varying concentrations of antioxidants are dissolved in solvent system containing ethanol and diethyl ether. Using 0.1N KOH as burette solution and phenolphthalein as indicator, titration is carried out till an end point of pale pink is observed for about 10-15 seconds. The set up follows ASTM D664.

C. Statistical analysis

The independent parameter temperature and dependent parameter viscosity listed in Table 1 were fitted with Akerlof and Oshry's Equation and Wright ASTM using SPSS (version 12). Akerlof and Oshry's Equation relating viscosity to temperature was

correlated for 18 oil samples and determination coefficient R^2 was observed between 0.871 and 0.994. Whereas Wright ASTM equation using least square fitting exemplify determination coefficient R^2 ranges between 0.992 – 0.999. Table 2 and 3 explicate the computed values of constant A, B, C, D and R^2 for the oil sample with different concentration of antioxidants with the temperature.

3. Results and discussions

A. Physical Property

a)Variation of viscosity with temperature

Viscosity variation with respect to temperature was determined for pure groundnut and mustard oil also other 16 samples added with natural and synthetic antioxidants. Table 1 illustrates the variation of kinematic viscosity with the change in the concentration and temperature 303 – 373K. It was observed that as temperature increases there's a consequential decrease in the viscosity of the oil. Groundnut oil has larger 34% of polyunsaturated fatty acids (PUFA) and 47% of monounsaturated fatty acids (MUFA) (El-Shami, 1992). With the addition of natural antioxidants the % of variation of viscosity decreases in steps of 52.6%, 46.7%, 42.6%, 42.4% and 41.9%. Viscosity has direct relationship with the chemical composition of oils such as chain length of fatty acids and unsaturated compounds. Viscosity is negatively correlated with unsaturation; hence it decreases with increase in unsaturated fatty acids (Kim, 2010). There is more rapid decrease in viscosity with increase as the % of PUFA is high compared to MUFA. The polyunsaturated fatty acid exists in *cis* form and there is no interaction between the molecules. Hence with increase in temperature due to thermal acceleration the molecule gets agitated in the direction of flow and the viscosity decreases. Addition of TBHQ with groundnut oil was pragmatic that the % of variation of viscosity with increase in concentration and temperature differ in steps of 75%, 78.8%, 82.3% and 73%.

Mustard oil has 12% of oleic acid (C18:1), *Erucic* acid 42% (C22:1), PUFA 21% (Wanasundara and Shahidi, 2005), hence it has more percentage of monounsaturated fatty acids. The % of variation of viscosity with concentration of tocopherol and temperature found to be 63.2%, 56%, 53.8%, 52%, 51.4%. Whereas with the addition of TBHQ, 68%, 71.7%, 74.4% and 73%. Early studies have stated that viscosity is highly correlated with polyunsaturated fatty acids than monounsaturated fatty acids (Choe and Min, 2007; Kim, 2010). Mustard oil has high percent of long chain erucic acid. Hence the % of variation of viscosity in mustard oil is high compared to groundnut oil. The studies also exemplify the synergistic effect of added antioxidant with antioxidants in oil. The maintenance of viscosity would be much useful in the transportation of oil from one part of the world to another which is at different temperature reducing the cost of transportation and pipelining. The behavior of viscosity with the addition of natural antioxidant is more cooperative compared to TBHQ. Hence addition of tocopherol to the oil in retaining the property would be more beneficial.

Variation of viscosity with temperature can be investigated to analyze the correctness of the computed value with the experimental, predict the intermediate value of viscosity at any temperature and quality analysis of the food samples (Hosahalli Ramaswamy, 2015). Empirical modelling equations were employed to understand the viscosity-temperature correlations. The equation was fitted with the experimental values using least square fitting and the constants with the correlation coefficient (r^2) was analyzed using SPSS software and tabulated in Tables 2 and 3.

Table 1. Viscosity of Groundnut and Mustard Oil Varying with Temperature added with antioxidants.

Type Of Oil	Anti – Oxidants	Concentration mg/100 mL	Viscosity x 10^{-6} (m^2/s)							
			303 K	313 K	323 K	333 K	343 K	353 K	363 K	373 K
Groundnut Oil	α Tocopherol	0	56.45	44.9	36.32	22.02	20.21	17.36	14.31	11.26
		5	46.93	36.9	29.7	22.6	19.04	16.32	13.63	10.99
		10	43.92	34.76	27.28	21.5	18.24	15.72	13.17	10.68
		15	42.64	33.26	26.14	20.77	17.39	14.78	12.42	10.13
		20	42.2	32.74	25.27	19.99	16.16	13.26	11.07	8.95
	TBHQ	0	56.45	44.9	36.32	22.02	20.21	17.36	14.31	11.26
		5	75.81	42.56	28.38	21.41	18.62	12.39	14.74	7.94
		10	78.92	43.07	28.59	21.69	18.5	14.53	12.56	8.05
		15	82.46	48.74	32.28	23.61	21.59	17.27	13.3	8.36
		20	73.32	43.64	29.36	22.66	19.83	15.95	13.38	8.69
Mustard Oil	α Tocopherol	0	63.38	46.91	34.49	25.72	20.69	17.13	13.84	10.47
		5	56.39	42.88	32.56	25.11	20.43	16.96	13.97	11
		10	54.1	41.06	31.29	24.42	19.61	16.02	12.86	10.57
		15	52.21	39.76	30.33	23.62	19.11	15.74	12.57	10.36
		20	52.61	39.50	28.77	21.55	17.33	14.32	10.9	8.81
	TBHQ	0	63.38	46.91	34.49	25.72	20.69	17.13	13.84	10.47
		5	68.32	51.81	39.95	31.3	24.88	20.02	16.33	13.46
		10	72.04	59.74	49.93	42.03	35.63	30.4	22.54	20.1
		15	74.53	49.48	34.06	24.22	17.72	13.29	10.2	7.99
		20	73.24	49.22	34.23	24.54	18.08	13.64	10.53	8.28

Table 2. Akerlof and Oshry's Modelling Equation relating viscosity and Temperature.

Type Of Oil	Values	$\eta = A/T + B + C*T + D*T^2$								
		Pure	0.05 g/L Toco	0.10 g/L Toco	0.15 g/L Toco	0.20 g/L Toco	0.05 g/L tbhq	0.10 g/L tbhq	0.15 g/L tbhq	0.20 g/L tbhq
Groundnut Oil	r^2	0.989	0.986	0.994	0.994	0.985	0.873	0.871	0.893	0.893
	A $x10^4$	3.2	2.8	2.59	2.52	2.60	4.60	4.80	5.13	4.46
	B	486.4	395.1	369.1	365.1	377.7	1111	1155	1121	982.5
	C	-3.02	-2.45	-2.29	-2.26	-2.35	-6.86	-7.14	-6.95	-6.08
	D x 10^{-3}	4.06	3.30	3.05	3.02	3.14	9.56	9.95	9.62	8.40
Mustard Oil	r^2	0.972	0.963	0.971	0.971	0.965	0.972	0.983	0.982	0.985
	A $x10^4$	4.06	3.51	3.39	3.26	3.40	4.29	4.17	5.03	4.96
	B	673.3	541.8	514.3	493.3	553.4	640.6	372.8	993.0	953.1
	C	-4.19	-3.37	-3.20	-3.07	-3.45	-3.99	-2.34	-6.18	-5.94
	D x 10^{-3}	5.68	4.50	4.30	4.12	4.66	5.35	2.92	8.50	8.10

Table 3. Wright's ASTM Modelling Equation relating viscosity and Temperature.

Type Of Oil	Values	Ln Ln(η) = A + B*Ln T								
		Pure	0.05 g/L Toco	0.10 g/L Toco	0.15 g/L Toco	0.20 g/L Toco	0.05 g/L TBHQ	0.10 g/L TBHQ	0.15 g/L TBHQ	0.20 g/L TBHQ
Groundnut Oil	r^2	0.996	0.997	0.997	0.998	0.998	0.998	0.990	0.996	0.999
	A	6.625	6.079	5.993	6.174	6.805	8.391	8.544	8.266	7.909
	B	-2.283	-2.213	-2.182	-2.256	-2.510	-3.130	-3.190	-3.074	-2.936
Mustard Oil	r^2	0.997	0.998	0.998	0.998	0.996	0.990	0.995	0.992	0.992
	A	7.124	6.611	6.679	6.755	7.568	6.355	4.809	9.160	8.921
	B	-2.621	-2.419	-2.484	-2.480	-2.807	-2.308	-1.681	-3.434	-3.338

Table 4. Dependence of Relative Density, Acid Value and Dielectric Constant of Groundnut and Mustard oil with the concentration of antioxidants.

Oil	Antioxidant	Concentration mg/100 mL	Acid Value mg (KOH / g)	Dielectric Constant	Relative Density (g/L) at 25 °C
Groundnut Oil	α – Tocopherol	0	3.402±0.001	3.59±0.06	0.926±0.002
		5	3.235±0.07	3.25±0.03	0.914±0.003
		10	3.107±0.01	3.14±0.05	0.918±0.009
		15	2.224±0.04	3.25±0.03	0.919±0.007
		20	1.910±0.001	3.31±0.08	0.916±0.006
	TBHQ	0	3.446±0.01	3.59±0.06	0.926±0.002
		5	3.328±0.02	3.59±0.04	0.919±0.004
		10	3.305±0.08	2.98±0.05	0.923±0.006
		15	2.562±0.05	2.89±0.01	0.920±0.004
		20	2.210±0.001	2.99±0.02	0.924±0.001
Mustard Oil	α – Tocopherol	0	5.213±0.003	2.98±0.02	0.904±0.006
		5	4.202±0.002	3.09±0.0	0.921±0.005
		10	4.190±0.01	3.31±0.07	0.913±0.008
		15	3.202±0.001	3.33±0.05	0.920±0.002
		20	2.524±0.04	3.23±0.03	0.924±0.005
	TBHQ	0	5.516±0.02	2.98±0.02	0.904±0.006
		5	4.473±0.04	3.23±0.07	0.926±0.005
		10	4.370±0.003	3.21±0.05	0.929±0.007
		15	3.617±0.002	3.26±0.01	0.915±0.009
		20	2.724±0.04	3.14±0.04	0.908±0.006

Values are expressed by mean ± SD of three independent observations, with significance p-value<0.001

i. Akerlof and Oshry's Equation

Temperature and Viscosity dependent models are a key analytic tool to determine oil quality and antioxidant capacity. Using appropriate viscosity measurements at set temperatures, one can qualitatively assess the activity of the antioxidant added and also the nature of the oil at that state. Antioxidants increase the stability of oil by preventing rapid proliferation of free radicals which lead to primary oxidized products like peroxides, ketones, aldehyde, esters etc. This leads to a decrease in overall viscosity of sample. Thus, compared to pure oil the viscosity of oils with antioxidants must have a lesser viscosity at any temperature.

$$\eta = A/T + B + C \cdot T + D \cdot T^2 \quad (1)$$

Behavior of oxidative stability and flow characteristics of ground nut oil and mustard oil with temperature. The r^2 value lies in the range of 0.871 to 0.994 for the groundnut oil added with tocopherol and TBHQ. It is observed from the table the variation of viscosity with additional natural antioxidant is much correlated with temperature and has accuracy of 2-3%. The r^2 value for TBHQ is less and has deviation of viscosity from experimental to computed value of 7-9%. Which show TBHQ is not synergistic with the antioxidants in the oil. The constant A and B values are computed and tabulated in Table 2.

In mustard oil, the r^2 value lies in the range of 0.963 to 0.985 for the oil added with tocopherol and TBHQ. It is observed from the table the variation of viscosity with additional natural antioxidant and TBHQ is much correlated with temperature and has variation in accuracy of 3-5%. The data show that both the antioxidants are synergistic with the antioxidants in mustard oil. The constant A and B values are computed and tabulated in Table 2.

ii.Wright's ASTM Equation

$$\text{Ln Ln } \eta = A + B \cdot \text{Ln } T \quad (2)$$

This equation relates the behavior of unsaturation nature and flow characteristics of ground nut oil and mustard oil with temperature ([a]Rubalya, 2015). The r^2 value lies in the range of 0.996 to 0.999 for the groundnut oil added with tocopherol and TBHQ. It is observed from the table the variation of viscosity with additional natural antioxidant and TBHQ is much correlated with temperature and has deviation of viscosity from calculation less than 1%. The % of accuracy is less for TBHQ compared to tocopherol hence it is experiential to have less synergistic with the antioxidants in the oil. The constant A and B values are computed and tabulated in Table 3. These constants could be used in predicting the viscosity at any desired temperature within the range. In mustard oil, the r^2 value lies in the range of 0.992 to 0.998 for the oil added with tocopherol and TBHQ. It is observed from the table the variation of viscosity with additional natural antioxidant and TBHQ is much correlated with temperature and has deviation less than 1%. The % of accuracy is less for TBHQ compared to tocopherol hence it is comparatively less synergistic with the antioxidants in the oil. The constant A and B values are computed and tabulated in Table 3.

b) Variation of viscosity with concentration

Table 1 illustrates that addition of antioxidant decreases the viscosity of oil by inhibiting the formation of peroxide which initiate rancidity in oil. Concentrations of antioxidant play an important role in increasing the shelf life of the oil ([b]Rubalya, 2015). From Table 1 it is observed viscosity decreases with the increase in the concentration of tocopherol but random variation is observed with TBHQ. The behavior is neither linear nor non- linear hence attempt in correlating with empirical equations could not be done using the concentration of antioxidants. For tocopherol, the behavior of viscosity with increase in concentration up to 20 mg/100 mL has good correlation coefficients but with TBHQ there was poor correlation coefficient. The study indicates that the addition of natural antioxidants more synergistic compared to TBHQ.

c) Relative density

The ratio of the density of groundnut and mustard oil to the density of water was practical to be 0.926 for groundnut oil and 0.904 for mustard oil. The specific gravity value for mustard oil is low due to the high content of erucic acid. It was supported by the early research that oil which contains more % of monounsaturated fatty acids has less specific gravity value compared to the oil that has higher % of polyunsaturated fatty acids. Throughout the experiment, the overall stability of the oil is maintained with the addition of antioxidants. Table 4 show that relative density of groundnut oil decreases with increase in the concentration of antioxidants. With the addition of Tocopherol the specific gravity values get reduced by 1.29% and maintain the same with increase in concentration. Addition of TBHQ reduces the relative density value by 0.53% and retains the change. Mustard oil added with tocopherol increases the relative density value by 2.1% whereas the specific gravity value with the addition of TBHQ increases by 2.7%. The relative density of groundnut oil decreases with the addition of antioxidants whereas mustard oil the parameter increased. The increase is observed to be more with TBHQ may be due to the synergistic effect between the natural antioxidant present in the oil with the synthetic TBHQ. But it was observed with the presence of antioxidants, the relative density of the oil is fairly maintained as a constant, thus extending the original property of the oil for a longer shelf life.

B. Chemical Properties

a)Total Acid Value (TAN)

Acid value corresponds to the degree of acidity existing in the oil sample. Oils are known to contain inherent quantities of fats, which when heated beyond a range are converted into acid products. For examples, triglycerides are converted into glycerol and fatty acids. This increase in acid products is an accurate measure of oil quality and helps determining the state where oil reaches rancidity. To study the TAN value the oil sample was added with antioxidants, stir thoroughly until it becomes homogeneous. The sample was left for a couple of days and the variations of TAN with the concentration of antioxidants are illustrated in Table 4. It was experimental that acid values ranges from 3.402 to 1.91 mg KOH/g with the addition of tocopherol with groundnut and 5.213 to 2.524 for mustard oil. The addition of TBHQ to the groundnut oil, the TAN values varies from 3.446 to 2.213 mg KOH/g. Mustard oil added with TBHQ show slight increase in the acid value 5.516 to 2.724 mg KOH /g. Any how the antioxidants maintain fairly a constant rate of TAN value index to maintain the quality of oil.

b) Electrical property

Electrical quality factor was measured at frequency ranges from $1\text{-}10^7$ Hz as shown in Figure 1. The ratio of the dielectric constant and the loss from the capacitance and the resistance is termed as Quality factor (Q) represented below:

$$Q = 1/\tan ø = ε‘/ ε’’= Rp \cdot Cp \quad (3)$$

where the dielectric constant is represented as ε', capacitance of the parallel plate capacitive cell represented as $C_{p,}$ resistance of the sample is denoted as Rp and ø is the impedance phase angle. Dielectric constant depends on the polarization effect in the oil (Dilip Kumar, 2013). The variation of dielectric constant with respect to frequency ranges from $1\text{-}10^7$Hz is highly due to study the polarization of orientation, ionic and electronic variation of dipoles. The increase in passage of current induces furthermore dissipation of electromagnetic energy and thus results in a higher dielectric loss.

Quality factor Q is the important property in the excellence analysis of the oil which reflects the characteristics of polar compound with the change in the dielectric constant. The ratio of loss factor and dielectric constant (ε’’/ ε’) decides the quality factor of the oils (Fritsch, 1981). Figure 1 illustrates the observed Q-factor for pure groundnut oil in the presence of alternating electric field with the addition of tocopherol and TBHQ at different concentrations.

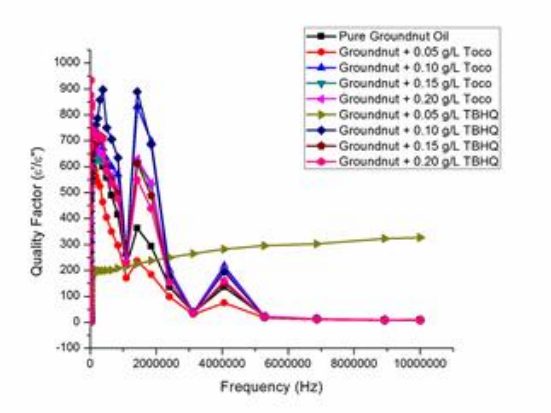

Figure 1. Dependence of quality factor of groundnut oil added with antioxidants at different concentration to the change in frequency

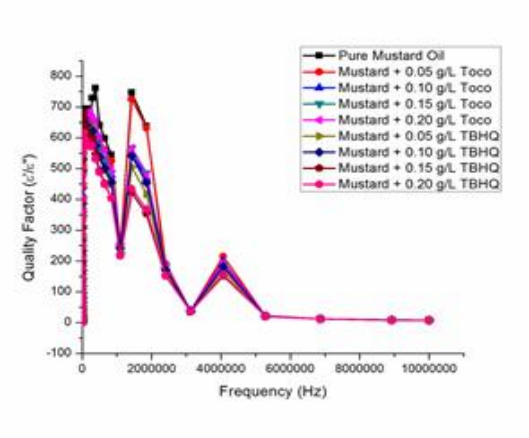

Figure 2. Dependence of quality factor of mustard oil added with antioxidants at different concentration to the change in frequency

The quality factor is less for groundnut oil added with TBHQ. Similar characteristic behavior is observed in Figure 2 for mustard oil where the quality factor of TBHQ is less. The quality peak is observed from low frequency region up to 5 MHz.

Dielectric constant is the measure of the ability of a substance to concentrate electric flux. With the release of oxidized compounds, the number of double bonds decreases in the oil (Pace, 1968; Dilip Kumar, 2013, Tjaša Prevc, 2013).

Table 4 shows the variation of dielectric constant with change in concentration at 2 MHz frequency. The experiments emphasize that addition of antioxidants at low concentration (10 mg/100 mL) shows better effect than higher concentration. One of the noninvasive methods used in the estimation of quality of the fats on frying is the dielectric constant, which is reliant on the sum of total polar compounds (Hamparsun Hampikyan, 2011). Decrease in dielectric constant with the addition of antioxidant inhibits the oxidation and increases the shelf life of the oil. The study also exemplifies the synergistic effect of added antioxidant with the antioxidant in oils. Best interaction is observed in the addition of tocopherol to the oil, increasing the stability of both oils. But addition of TBHQ does not maintain the stability in oils. It was exemplified by Becker, (2007) that tocopherol regenerates myricetin during auto-oxidation.

4. Conclusions

Groundnut and Mustard oils were mixed with antioxidants like tocopherol and TBHQ to test their ability to resist secondary product formation by oxidation. Various physico-chemical properties like viscosity, density and acid value along with electrical properties. Significant deviations in the parameters were noticed on addition of antioxidants indicating the synergistic effect. Akerlof and Oshry's model and Wright's ASTM model were used to relate the variability of viscosity with temperature. It was observed that Wright's model has high determination coefficient (R^2= 0.999). Oil added with TBHQ exhibited less co-relation compared to Tocopherol. Acid value of the oil is controlled with the addition of antioxidants; support the retarding degradation effect in the oil. The quality analysis elucidates the quantity of polar compound in oil. Assessing the impact of antioxidants on the physical and chemical properties of oil gives us an idea about designing the storage conditions of oil with respect to the environment. Antioxidant addition can be used to retain the quality level of the oil during transportation by conserving the viscosity across a wide range of temperatures.

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EFFECTS OF MODIFIED ATMOSPHERE PACKAGING SYSTEMS, LOW TEMPERATURE AND STORAGE TIME ON THE QUALITY OF FRESH MINIMALLY PROCESSED POMEGRANATE ARILS

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ABSTRACT

The effect of packaging method (air packaging; and modified atmospheres packaging including, MAP1: 5% O_2+85% N_2+10 % CO_2 and MAP2: 70% O_2+20% N_2+10% CO_2), storage temperature (4 and 8˚C), and storage time (0 to 30 days) were studied on chemical, physical and sensory characteristics as well as extending the shelf life of pomegranate arils**.** Mold was detected in arils stored under air packaging after 9 days. The samples packaged with low oxygen atmosphere show no mold attack during 30 days storage. Arils kept in MAP1 and stored at 4˚C maintained their texture and appearance better than those packaged under air and MAP2 packaging. The samples stored in MAP1 were firmer than those stored in air and MAP2 at the end of storage time.

1. Introduction

Pomegranate (*Punica granatum*, L.) is one of the most popular fruits worldwide, which is widely grown in many subtropical and tropical countries, including almost all Mediterranean countries (Gil et al., 1996a; López-Rubira et al., 2005). The total pomegranate production in Iran was 665,000 tons in 2003 (Anonymous, 2003). Pomegranate is consumed as fresh fruit and the seeds are the edible portion of the fruit which surrounded by a sweet and juicy pulp called arils **(**Gil et al., 1995; Melgarejo et al., 2000; Roy and Wasker, 1997). It is a very rich source of vitamins, fatty acids, mineral elements, polyphenolic compounds and high levels of anthocyanin which reduction of liver injury and prevents some types of cancer **(**Gil et al., 1996a; Caleb et al., 2012; Du et al., 1975; Fadavi et al., 2006; Lansky et al., 1998**).**

Pomegranate fruits contain a considerable amount of arils ranging between 518 to 740 g kg^{-1} of fruit weight depending on cultivar (Safa and Khazaei, 2003). Some studies have been reported on the physical and mechanical properties of fruits pomegranate fruits (Ekrami-Rad et al., 2011; Mansouri et al., 2010). The minimal processing of pomegranate arils mainly consists of washing and/or preprocessing (chlorine and antioxidant solutions), pH modifications, modified atmosphere packaging **(**MAP), and storage under temperature control. (Gil et al., 1995; Ayhan and Eştűrk, 2009; Babic et al., 1992; Sepulveda et al., 2000; Schlimme, 1995).

As a useful conservation method, MAP can be introduced for extending the shelf-life of different perishable food products such as fruits (Oğuzhan et al., 2013; Goktepe, and Moody, 1998; Silva, and White, 1994; Jouki, and Khazaei, 2013;

Jouki, and Dadashpour, 2012). In this method, increasing CO_2 and/or decreasing O_2 levels with diminish the rate of respiration and ethylene production leads to inhibition and of the enzymatic reactions. Moreover, MAP with a reduction in physiological activity of the object causes a better maintaining of the final quality (Soliva-Fortuny and Martin-Belloso, 2003; Dadashpour et al., 2014). In micro-controlled atmosphere, the use of polymeric films has also significant effect on controlling the quality losses. Sepulveda et al. (2000) investigated the influence of different types of semi permeable films and antioxidant solutions on the quality of pomegranate arils in passive modification. They reported that minimally processed pomegranate Var. Wonderful were able to be stored for 14d at 4˚C with the use of semi permeable film. Ayhan and Esturk (2009) studied the shelf life and overall quality of minimally processed and modified atmosphere packaged "ready-to-eat" pomegranate arils Hicaznar packed in PP trays sealed with BOPP film under 4 atmospheres including low and super atmospheric oxygen stored at 5˚C for 18d. They reported the selected packaging material combined with modified atmosphere at low storage temperature provided commercially acceptable shelf life of 18d, quality, and convenience for pomegranate arils sanitized with chlorine. In a similar study by Gil et al. (1996a), the influence of different washing treatments, storage temperatures (8, 4 and 1˚C) and actively or passively modified atmosphere packaging on the quality of the minimally processed pomegranate seeds was investigated. they reported the best out comes in quality and appearance without fungal attacks on off flavor development were obtained for pomegranate seeds washed with chlorine plus antioxidants sealed in OPP film, using an initial atmosphere actively modified stored for 7 d at 1˚C. Accordingly, the objective of this research was to study the effect of packaging method (air packaging and MAP technique in low and super atmospheric oxygen), storage temperature (4 and 8˚C), and storage time (0 to 30 days) on chemical, physical and sensory characteristics as well as extending the shelf life of pomegranate arils.

2. Materials and methods

2.1. Sample preparation

Pomegranate fruits (*Punica granatum* L.) cv. 'Malas-e-Saveh' were obtained from Agricultural Research Center of Saveh, Iran and harvested at the commercial maturity stage. The fruits were collected manually and transported on the same day to the Chemical Engineering Laboratory, University of Tehran, Iran. The damaged fruits were removed and the healthy fruits of uniform mass (with average weight of 280 g), size and appearance were used for tests. The fruits were stored in refrigerator at 4 and 8˚C and 75±5% RH and were kept there for a maximum of 30 days while the experiments were completed. The fruits arils were removed manually. To this, each fruit was washed in chlorine using a brush), drained, and then cut into pieces to separate the arils manually. The chemical, physical and sensory characteristics of fresh arils were measured and assigned as control treatment (without any treatment). Chemicals were supplied from Merck (Darmstadt, Germany).

2.2. Storage conditions

In this study, the effect of packaging method (air packaging with 21% O_2+79% N_2; and modified atmospheres packaging, including MAP1: 5% O_2+85% N_2+10% CO_2 and MAP2: 70% O_2+20% N_2+10% CO_2), storage temperature (4 and 8˚C), and storage time (0, 9, 12, 18 and 30 days) were studied on quality parameters of pomegranate arils, including fruit firmness, total Titratable Acidity (TTA), total soluble solids (TSS), pH, fungal decay and sensory evaluation (color, taste, freshness and overall acceptability). Factorial experiment was conducted as a randomized complete design with three replicates.

For each test, the arils samples of 120±5 g were packed in heat-sealed pouches (20×30 cm) made of oriented polypropylene (OPP)

film of 52μm thickness, 230 mL/(m^2 24 h bar) O_2 permeability, 889 mL/m^2 24 h bar CO_2 permeability and a water vapour transmission rate of 0.22 g/m^2 24 h (all values at 75% RH and 5˚C). Measuring and evaluation of the physical, chemical and sensory characteristics of control and treated pomegranate arils were conducted as follow.

2.3. Texture

Textural properties of the arils was carried out using a Testometric Machine M350-10CT (Testometric Co. Ltd., Rochdale, Lancashire, England) according to the method reported by Ayhan and Estuck (2009). For each test, twelve grams of arils were placed into a 28 cm^2 metal container and were compressed using a 5-cm diameter cylindrical probe. For all the tests, the maximum compression force (N) at probe displacement of 7 mm was measured and expressed as arils firmness. A total of 10 replications were made for each packaged aril sample and the average was reported. In this study, all the compression tests were conducted at a speed of 5 mm/s.

2.4. Chemical attributes

A total of 75g of arils were squeezed with hand pressed for extracting the juice and filtered using cheesecloth. The juice obtained was directly analyzed for Total titratable acidity (TTA), Total soluble solid (TSS) and pH. Total titratable acidity (TTA) was determined by titrating with 0.1mol/l NaOH to pH 8.1 and using 10ml of juice diluted with 50 ml of distilled water and expressed as citric acid% (AOAC, 1984). Total soluble solid (°Brix) values were analyzed by Bausch & Lomb Abbe-3L type refractometer at 20˚C. The pH of the arils was analyzed by a pH meter (Metrohm, Herisau, Switzerland) in duplicate measurements on day 0, 9, 12, 18 and 30 (Dadashpour et al., 2014; AOAC, 1984; Khazaei et al., 2011). All analyses were done as triplicate.

2.5. Fungal decay

The presence of fungi in each package was visually evaluated. The arils samples showing surface mycelia development were considered as decayed. Finally, all the samples were classified as presence (+) or absence (-) of fungi.

2.6. Sensory evaluation

Sensory evaluation of the packaged arils was included the assessments of color, freshness, taste and product acceptability. Tests were performed by a sensory panel of 12 trained judges (six females and six males) on a 5-point hedonic scales, where 5 corresponded to extremely liked and 1 corresponded to extremely disliked (López-Rubira et al., 2005; Ayhan and Esturk, 2009; Gil et al., 1996b). Scores of 3 and above were considered as acceptable for commercial purposes.

2.7. Statistical analyses

The experimental data for each independent parameter were individually subjected to analysis of variance by using SAS software (ver. 9.2). The data were analyzed to study the effects of packaging method, storage temperature, and storage time on different chemical, physical and sensory characteristic of pomegranate arils. The difference between the means of main and interaction effects was determined using the Duncan Multiple Range Test in 0.05 and 0.01 levels (López-Rubira et al., 2005; Ayhan and Esturk, 2009; Jouki and Khazaei, 2012; Jouki and Khazaei, 2014).

3. Results and discussions

3.1. pH

The results showed that (Table 1) the mean value of pH for fresh arils (without any treatment at 0 day storage) was in the range of 3.16±0.04, which was in agreement with that reported by Akbarpour et al. (2009). During the storage period, increasing on fruit's pH was observed between the first and the 12th day. These results are in agreement with obtained by Artes et al. (2000), who reported that at the end

of the shelf life, all treatments maintained or increased pH values, except pomegranate fruits in perforated PP at 5˚C, which had slightly decreased pH values.

Table 1. The effect of packaging methods, storage temperature and storage time on pH of pomegranate arils

Treatments	Temperature (°C)	Day 0	Day 9	Day 12	Day 18	Day 30
AP	4	3.163±0.049 ^{c}	3.440±0.157 ^{b}	3.536±0.070 ^{b,A}	3.440±0.030 ^{b,C}	3.710±0.140 ^{a,B}
MAP1	4	3.163±0.049 ^{b}	3.400±.0.098 ^{a}	3.356±0.810 ^{a,B}	3.323±0.068 ^{a,D}	3.380±0.017 ^{a,D}
MAP2	4	3.163±0.049 ^{c}	3.390±0.117 ^{b}	3.393±0.470 ^{b,B}	3.360±0.020 ^{b,D}	3.576±0.056 ^{a,C}
AP	8	3.163±0.049 ^{d}	3.393±0.060 ^{c}	3.580±0.060 ^{b,A}	3.760±0.036 $^{a, A}$	3.713±0.030 $^{a, A}$
MAP1	8	3.163±0.049 ^{c}	3.360±0.065 ^{d}	3.513±0.032 $^{c, A}$	3.630±0.020 ^{b,B}	3.720±0.026 $^{a, B}$
MAP2	8	3.163±0.049 ^{c}	3.370±0.111 ^{d}	3.590±0.020 ^{c,A}	3.716±0.030 ^{b,A}	3.796±0.015 ^{a,A}

[1]For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among packaging treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ between storage temperatures.
[2]AP: 21% O_2, 79% N_2; MAP1: 10 % CO_2 + 5 % O_2+85 % N_2; MAP2: 10 % CO_2+ 70 % O_2 + 20 % N_2.
[3] Values in the same column (A-D) and same row (a-d) with different superscripts are significantly different ($p < 0.05$).

Table 2. The effect of modified atmosphere packaging, storage time and temperature on TTA (%) of pomegranate arils

Treatments	Temperature (°C)	Day 0	Day 9	Day 12	Day 18	Day 30
AP	4	1.411±0.182^{a}	1.076±0.255^{ab,B}	0.845±0.205^{b,B}	0.818±0.024^{b,B}	0.687±0.035^{c,B}
MAP1	4	1.411±0.182^{a}	. 1.330±0.170 ^{a,AB}	1.236±0.075^{a,A}	0.290±0.314^{a,A}	0.883±0.075^{b,A}
MAP2	4	1.411±0.182^{a}	1.610±0.200^{a,A}	1.243±0.240^{,A}	1.096±0.114^{b,AB}	0.758±0.097^{c,AB}
AP	8	1.411±0.182^{a}	1.140±0.172^{b,B}	0.854±0.085^{c,B}	0.783±0.029^{d,B}	0.689±0.032^{c,B}
MAP1	8	1.411±0.182^{a}	1.360±0.210^{a,AB}	1.111±0.035^{b,A}	1.024±0.010^{c,AB}	0.845±0.052^{d,A}
MAP2	8	1.411±0.182^{a}	1.326±0.176^{a,AB}	1.145±0.012^{b,A}	1.061±0.029^{c,AB}	0.714±0.054^{d,AB}

[1]For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among packaging treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ between storage temperatures.
[2]AP: 21% O_2, 79% N_2; MAP1: 10 % CO_2 + 5 % O_2+85 % N_2; MAP2: 10 % CO_2+ 70 % O_2 + 20 % N_2.
[3] Values in the same column (A-D) and same row (a-d) with different superscripts are significantly different ($p < 0.05$).

Table 1 shows that storage temperatures had a significant increasing effect (p=0.08***) on the pH of stored packaged arils. The pH of arils stored at 4°C with the mean value of 3.39 was 3.5% less than that for the samples stored at 8˚C. It was also found that storage time had a significant increasing effect (p=0.08***) on the pH of stored packaged arils. At storage temperatures of 4˚C, increasing the storage time from 0 to 30 days, significantly increased

the mean value of pH from 3.16 to 3.56, a 13% increase (Table 1). The corresponding values for storage temperature of 8°C were 3.16 to 3.78, respectively with a 20% increase (Table 1). For both storage temperatures of 4 and 8˚C, the minimum variation in pH was belonged to arils packed under MAP1 (i.e. 5% O_2+85% N_2+10% CO_2).

Among the most often used gases in MAP (O_2, CO_2, and N_2), only CO_2 has significant and direct antimicrobial activity due to alteration of cell membrane function including effects on nutrient uptake and absorption, direct inhibition of enzymes, or decreases in the rate of enzyme reactions, penetration of bacterial membranes leading to intracellular pH changes and changes to the physicochemical properties of proteins (Farber, 1991). During the refrigerated storage at 4 or 8˚C, the pH increased significantly in AP or MAP-stored fruits, mainly due to a decrease in TA (Table 1). The pH of pomegranate arils increased from 3.16 to 3.71 and 3.81 at the end of storage time for air-packaged samples stored at 4˚C and 8˚C respectively, and was lower in samples under MAP1 than samples under MAP2. At the end of shelf life, all treatments significantly increased pH values, except MAP1 at 4˚C fruits**,** which had slightly increased values**.** However, no significant differences were found between MAP2 and control at 8˚C ($P \leq 0.05$). The difference became significant between low oxygen (MAP1) and the other applications were found at 8 and 4˚C. There was no significant difference between MAP1 at 8˚C and control at 4˚C, although. The pH of pomegranate arils packaged under low oxygen (MAP1) was significantly lower than the other applications of storage for all temperatures. The pH increased when stored at 8 and 4˚C but increasing this value at 4˚C was lower than at 8˚C.

3.2. Total Titratable Acidity

In general, no significant difference was observed between atmospheres in terms of total titratable acidity (TTA; $P>0.05$). However, storage time had significant effect on TTA at all applications ($P \leq 0.05$). TTA significantly decreased in all applications especially at the 12th day ($P \leq 0.05$) and stayed almost unchanged for the rest of the storage (Table 2). Decrease in acidity during storage is in agreement with the results of Artes et al. (2000) and Maghoumi et al. (2013). This could be related to metabolic activities of pomegranate during storage (Babic et al., 1992). Caleb et al. (2012) reported that the variability of pH, TSS, and TTA values could be explained by several factors such as cultivar differences and the relative solubility effect of CO_2 in water molecules surrounding the freshly packed pomegranate arils. TTA of arils decreased when stored at 8 and 4˚C but increasing this value at 4˚C was lower than at 8˚C. Thus, storage temperature at 4˚C was selected as the best for keeping quality non-significant differences were observed for control at 8˚C but there was a significant effect between control and other applications for all temperatures in terms of TTA ($P \leq 0.05$).

3.3. TSS

The TSS value of arils increased when stored at 8 and 4˚C. Hence, only slight differences were observed for TSS at 4˚C. At the 30 day of storage, TSS of pomegranate arils under enriched oxygen (MAP2) and stored at 8˚C was slightly higher than the other applications (Table 3). This result is in agreement with the results of Ayhan and Esturk (2009) for 18d. They reported that there was no significant but slight changes observed in TSS of pomegranate during cold storage. They also reported that changes in total acidity and TSS content influenced the diversity of the amounts of total phenolic content during storage, which in return affected the total anthocyanin content and total antioxidant activity and Anthocyanins are responsible for the color of the pomegranate seeds. In general, total anthocyanin content decreased as the storage time increased for all treatments.

3.4. Physical quality

The firmness characteristics of pomegranate arils are presented by Table 4. While the firmness value was 90.87 N day 0, it was significantly increased at 9 d of storage to 128.91, 116.96 and 112.23 for normal air (AP), MAP1 (Low oxygen) and MAP2 (enriched oxygen); respectively (for samples were stored under 4˚C). Increase in firmness during storage is in agreement with the results of Ayhan and Esturk (2009). There was no significant difference between MAP applications until 9 d of storage in terms of texture. However, at the end of storage time the most firmness was related to MAP1 (104.067 N) (Table 4). Ayhan and Esturk (2009) reported that Changes in firmness could be due to changes in water content during storage.

After 18 d of storage time firmness decreased when stored at 8 and 4˚C but decreasing this value at 8˚C was higher than at 4˚C. Shamsudin et al. (2009) surveyed physico-mechanical properties of the Josephine pineapple fruit. They reported the firmness of the fruits was decreased with the stage of maturity.

3.5. Sensory quality

Figure 1 shows the effects of MAP, storage time and temperature on the sensory attributes and acceptance of pomegranate arils during cold storage. The minimally processed pomegranate arils were acceptable in terms of product attributes such as aril color, freshness and taste under normal air, low and enriched oxygen atmosphere until the end of the storage time (30 days). Overall, the pomegranate arils packed with enriched oxygen and low oxygen were acceptable by the sensory panelists on 12th day; however, it was limited to 9 d for the normal air. The overall acceptance score of pomegranate arils packaged under low oxygen atmosphere was 3.82, which was higher than the acceptable level (score 3) on the storage day of 30. The pomegranate arils packed with normal air, enriched and low oxygen weren't acceptable by the sensory panelists on 30 day.

Jacxsens et al. (2003) reported that LAB showed low counts (about 5 log cfu g^{-1}) after 10 days of storage, without any trace of fermentative metabolism which could lead to the production of lactic and acetic acids responsible for off-flavour. It should be taken into account that high amounts of yeasts (>5 log cfu g^{-1}) can provoke an off-flavour of fresh-cut produce due to the production of CO_2, ethanol, organic acids and volatile esters (Babic et al. 1992; Fleet, 1992). In terms of colour, taste, freshness, overall acceptability, MAP1-treated arils were still acceptable for consumption up to 30 days at 4˚C. However, MAP2-treated arils were below the limit of usability according to the sensory evaluation, even when stored at 4˚C.

3.6. Fungal decay

The effects of modified atmosphere packaging on pomegranate arils were restricted in the counts of microbial flora with the concomitant benefit of prolonging refrigerated shelf-life on the samples. No mold growth was detected after 9 days of storage, but after day 9 mold growth was observed on the arils stored at AP (air packaging). These results were similar to those achieved by Lopez-Rubira et al. (2005), who reported that microbial counts of minimally fresh processed arils increased throughout shelf life at 5˚C. Even though they treated the arils with MAP2, the shelf life was limited to 12 d due to the mold growth. Farber (1992) stated that CO_2 has significant and direct antimicrobial activity due to alteration of cell membrane function including effects on nutrient uptake and absorption, direct inhibition of enzymes, or decreases in the rate of enzyme reactions, penetration of bacterial membranes leading to intracellular pH changes and changes to the physicochemical properties of proteins. No mold growth was detected during 30 days of storage on the arils in MAP1 (not shown). In our study, the arils were treated with chlorine. MAP1 (low levels of O_2) has been found to be effective in inhibiting mold growth.

Table 3. The effect of modified atmosphere packaging, storage time and temperature on TSS (°Brix) of pomegranate arils.

Treatments	Temperature (°C)	Day 0	Day 9	Day 12	Day 18	Day 30
AP	4	14.533 ± 0.642^{bc}	14.250 ± 1.298^{c}	$15.250\pm0.250^{b,A}$	$15.333\pm0.288^{ab,AB}$	$15.750\pm0.050^{a,AB}$
MAP1	4	14.533 ± 0.642^{b}	14.416 ± 1.025^{b}	$13.83\pm0.400^{b,B}$	$14.000\pm0.019^{b,B}$	$15.333\pm0.288^{a,C}$
MAP2	4	14.533 ± 0.642^{b}	14.083 ± 0.629^{b}	$14.166\pm0.288^{b,B}$	$14.166\pm0.288^{b,B}$	$15.706\pm0.090^{a,B}$
AP	8	14.533 ± 0.642^{b}	15.000 ± 1.000^{ab}	$15.933\pm0.115^{a,A}$	$16.033\pm0.057^{a,A}$	$16.100\pm0.100^{a,A}$
MAP1	8	14.533 ± 0.642^{bc}	14.333 ± 0.763^{bc}	$13.833\pm0.040^{c,B}$	$15.383\pm0.332^{b,AB}$	$15.866\pm0.125^{a,AB}$
MAP2	8	14.533 ± 0.642^{b}	14.750 ± 0.443^{a}	$15.666\pm0.377^{a,A}$	$15.706\pm0.476^{a,A}$	$15.933\pm10.115^{a,A}$

[1]For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among packaging treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ between storage temperatures.
[2]AP: 21% O_2, 79% N_2; MAP1: 10 % CO_2 + 5 % O_2+85 % N_2; MAP2: 10 % CO_2+ 70 % O_2 + 20 % N_2.
[3] Values in the same column (A-D) and same row (a-d) with different superscripts are significantly different ($p < 0.05$).

Table 4. The effect of modified atmosphere packaging, storage time and temperature on firmness (N) of pomegranate arils

Treatments	Temperature (°C)	Day 0	Day 9	Day 12	Day 18	Day 30
AP	4	90.871 ± 6.855^{c}	$128.911\pm0.971^{a,A}$	$105.287\pm21.287^{bcd,A}$	$94.022\pm18.878^{cd,AB}$	$90.355.11\pm036^{c,AB}$
MAP1	4	90.871 ± 6.855^{c}	$116.961\pm8.145^{ab,B}$	$104.502\pm5.698^{bcd,A}$	$114.548\pm7.246^{ab,A}$	$104.067\pm34.633^{bcd,A}$
MAP2	4	90.871 ± 6.855^{c}	$112.235\pm6.745^{bc,B}$	$107.829\pm19.821^{bcd,A}$	$118.831\pm20.888^{ab,A}$	$98.053\pm6.181^{c,A}$
AP	8	90.871 ± 6.855^{c}	$110.199\pm17.972^{ab,AB}$	$93.977\pm2.976^{bc,AB}$	$86.199\pm4.418^{cd,B}$	$78.866\pm5.300^{d,AB}$
MAP1	8	90.871 ± 6.855^{c}	$113.185\pm6.284^{ab,B}$	$109.851\pm4.285^{bc,A}$	$96.355\pm5.263^{bc,AB}$	$88.407\pm6.623^{c,AB}$
MAP2	8	90.871 ± 6.855^{c}	$112.916\pm17.971^{b,AB}$	$106.628\pm15.197^{bcd,A}$	$91.961\pm7.680^{cd,B}$	$81.295\pm6.442^{d,AB}$

[1]For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among packaging treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ between storage temperatures.
[2]AP: 21% O_2, 79% N_2; MAP1: 10 % CO_2 + 5 % O_2+85 % N_2; MAP2: 10 % CO_2+ 70 % O_2 + 20 % N_2.
[3] Values in the same column (A-D) and same row (a-d) with different superscripts are significantly different ($p < 0.05$).

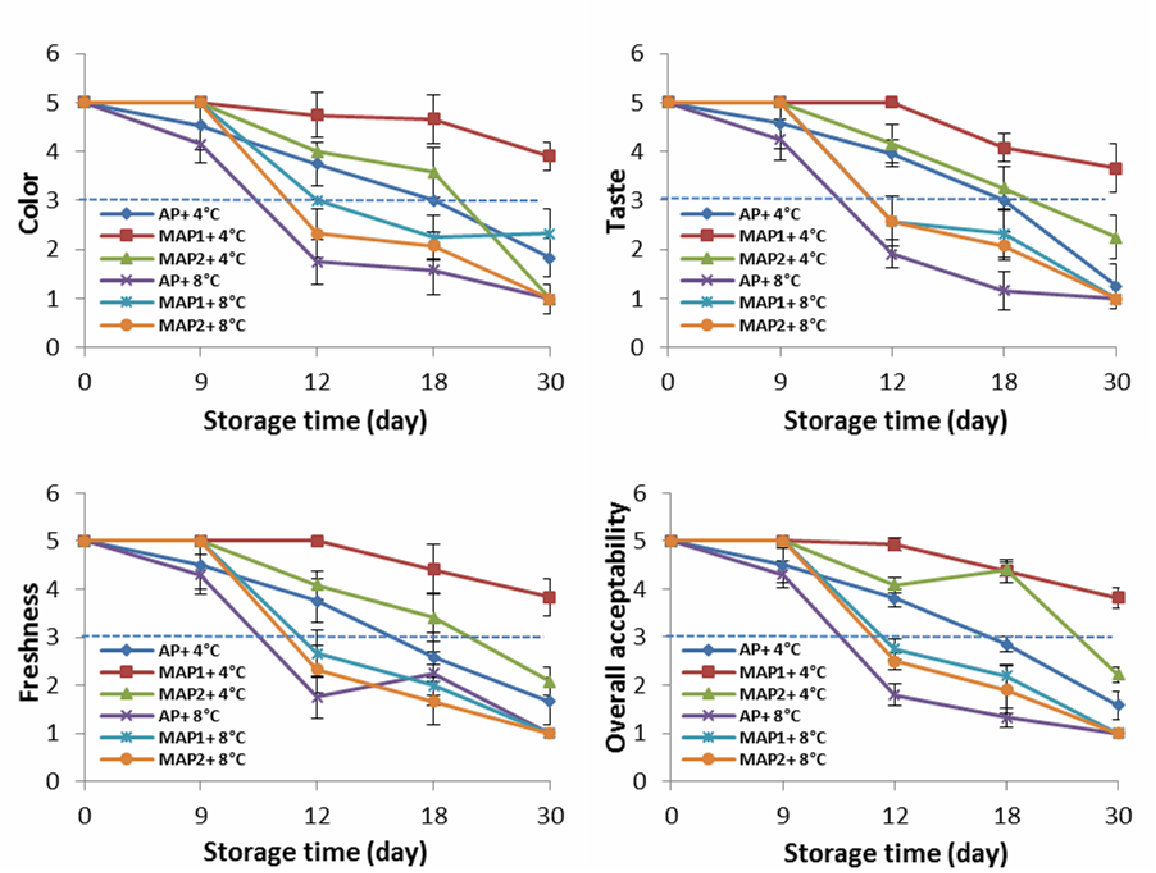

Figure 1. The effect of MAP, storage time and temperature on the sensory attributes of pomegranate arils during cold storage.

4. Conclusions

The shelf life of pomegranate arils of was suggested as 9 d under air, 12d under MAP2 (the enriched oxygen atmosphere), and 30d under MAP1 (the low oxygen atmosphere) at 4˚C storage. The low oxygen modified atmosphere at low storage temperature (4˚C) provided commercially acceptable shelf life of 30d, quality, and convenience for pomegranate arils sanitized with chlorine. This study demonstrated the effects of modified atmosphere packaging on the quality of pomegranate arils stored at 4 and 8˚C. The results showed that the pomegranate arils kept in MAP1 (low oxygen atmosphere) maintained their texture and appearance significantly ($p < 0.05$) better than those packaged under air and MAP2 (enriched oxygen atmospheres). The arils stored at 4˚C by MAP1 increased their post-harvest life from 9 to 30 days, without any attack of fungus or any change in their external appearance. At the end of storage time firmness significantly ($p < 0.05$) decreased during storage of arils in MAP2 and air packed, but intensely in arils stored at 4˚C and packaged by MAP1. Mold was detected in arils stored under air packaging after 9 days. However no mold growth was detected on day 30 of storage in the sample under MAP1 and stored at 4˚C.

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EXPERIMENTAL MODELLING THE APPLICATION OF PULSED ELECTRIC FIELD FOR ENHANCEMENT OF BETANIN AND JUICE EXTRACTION USING RSM TECHNIQUE

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ABSTRACT
A procedure based on Response Surface Modeling (RSM) is applied for identifying the set point of the juice extraction process using pulsed electric field pre-treatment. The experiments were carried out on a laboratory experimental bench of beet juice extraction, in which three factors of the process were analyzed: the electric field level E (kV/cm), the number of pulses n and the pulse duration T (µs). Three "one-factor-at-a-time experiments", followed by a composite design, were performed following a well defined experimental procedure. A set point was identified corresponding to the optimal values of the above-cited factors.

1. Introduction

PEF treatment can prove to be useful for the pasteurization of liquid foods (Maciej et al. 2005; Grahl and Markl 1996; Heinz et al.2002; Hulshegeret al.1981; Nabil et al. 2011) and the recovery of desired substances from plant cells without the use of chemical or thermal treatment (Schilling et al. 2008; Ade-Omowaye et al. 2001; Robert et al. 2009). Plant cell cultures producing secondary metabolites are permeabilized by electroporation by moderate high voltage pulsed electric field (1-5 kV/cm).

Traditional process of fruit juice production consists of mechanical expression by pressing or decanting combined with enzyme pre-treatment of the mash (Jia et al. 1999; Tanya et al. 2006). Such pre-treatment can damage cell walls of tissues. To obtain higher juice yield and to reduce the processing time, the manufacturers apply higher treatment temperatures at the mash stage. However, enzyme and thermal treatments are always accompanied by high energy consumption and degradation of juice quality, loss of the vitamins and changes in colour and flavor (Massimiliano et al. 2013).

The PEF-treated extraction process depends on a multitude of factors. In such application, the list of factors influencing the process includes the pulse duration, the electric field, the number of pulses, the average power and so on…. Thus, it's not simple to determine with precision the optimal values of the process factors. An experimental procedure for optimizing the extraction process was employed using a home-made experimental set-

up, comprising a pulse generator, a treatment chamber and a pressing machine. Three "one-factor-at-a-time" experiments, corresponding to three controllable factors, followed by a factorial design were performed based on a two steps strategy: fixing the variation domain of the input variables and searching the optimum set point.

2. Materials and methods

2.1. Pulsed electric field (PEF) for electroporation

Electroporation refers to the ability of electric fields to cause the formation of reversible or irreversible pores in the membranes of cells. Exposing a biological cell (plant, animal and microbial) to a high intensity electric field (kV/cm) using very short pulses (μs to ms) induces the formation of temporary or permanent pores on the cell membrane (Figure 1). This phenomenon causes the permeabilization of cell membrane i.e. an increase of its permeability and if the intensity of the treatment is sufficiently high, cell membrane disintegration occurs.

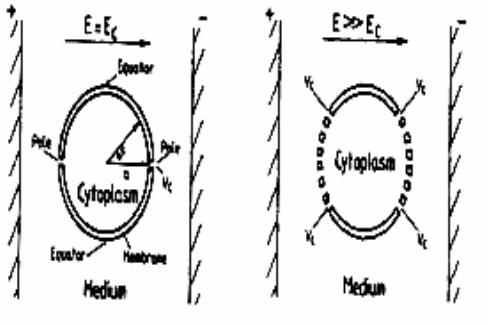


Figure 1. Schematic diagram of a cell exposed to electric field

2.2. Treatment chamber

Fresh beets were obtained at local market of fruits and vegetables. After sorting and cleaning operations, they were comminuted with a domestic food processor (Thomson, THMX05736 Model) for 5 min to obtain a homogenous mash. The obtained mash was then kept in a closed vessel to prevent evaporation prior to use.

The treatment chamber consisted of an insulated cylinder made of plastic (Teflon, PTFE) of length 140 mm and diameter 70 mm (Figure 2). The electrodes are constituted by a cylindrical plunger and a disc base of a same diameter 70 mm having a rigid structure for juice pressing operation, both made with stainless steel. Extracted juice was filtered through a stainless steel sieve placed on top of the perforated plunger. Juice extracted during pressing was collected in a plastic collector placed under the treatment chamber. The volume of the treatment chamber was 192.3 ml. For all experiments, the same treatment chamb was used for both pressing and pulsed electric field treatment steps.

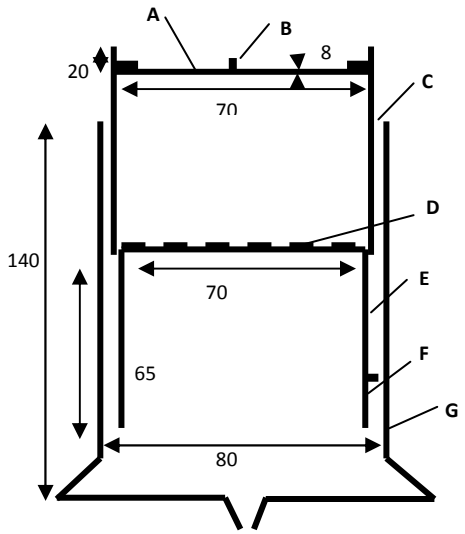


Figure 2. Schematic description of the treatment chamber (all dimensions are in mm). A- Stainless Steel disk (upper electrode), B – High voltage connection, C - Teflon cylinder, D – Stainless steel sieve, E - Perforated stainless steel plunger (lower electrode), F - Ground connection, G- Plastic container for the collection of extracted juice

The pressure was applied using a hydraulic pressing machine (Mega, 15 tons). Just after PEF treatment, the filled treatment chamber was pressed until a defined pressure of 100 kg/cm^2, and was then held at this pressure for 5 min. For all experiments, the thickness of the sample was equal to 2 cm, corresponding to a sample mass of 60 g.

An electronic balance of 0.1 g precision was used to weight the beet juice collected in flacon tubes.

2.3. Pulse generator

Pulsed electrical field treatment was achieved by using a PEF generator, represented in Fig. 3. The input voltage was regulated by the autotransformer. The voltage was delivered by a direct current high voltage supply (DC-HV) (35 kV, 30 mA), comprising a step-up transformer and a bridge of rectifying diodes. A variable autotransformer (AT) (Langlois ALT5A) was used to supply the desired voltage to the treatment chamber. A 10-kΩ resistor (R) was used to limit the current passing through the bank of capacitors used to store the energy. A spark-gap switch was used to discharge the energy stored in the condenser into the treatment chamber. Figure 4 represents the experimental setup used for this work.

2.4. Preliminary results of juice extraction with PEF treatment

PEF pre-treatment of beet tissue is followed by the application of a pressure of 100 kg/cm^2, at ambient temperature, for a duration of 5 min. PEF treatment resulted in a significant increase in the yield of juice. More juice was extracted from the treated mash as shown in Figure 5. Obtained results represented in Table 1 show that pulsed electric field treatment increases the quantity of extracted beet juice by more than 90% compared with untreated one.

The increase of the extracted juice is due to the new cellular structure of the plant as a result of the electroporation of the cell membrane because of an interaction with the pulsed electric field**.**

Table 1. Obtained results of beet juice extraction

Samples of Beet	Treatment parameters	Mass of extracted juice
PEF treated sample	3 kV/cm, 100 pulses, 30 µs, 100 kg/ cm^2	40.6 g
Untreated sample	100 kg/ cm^2	21.3 g

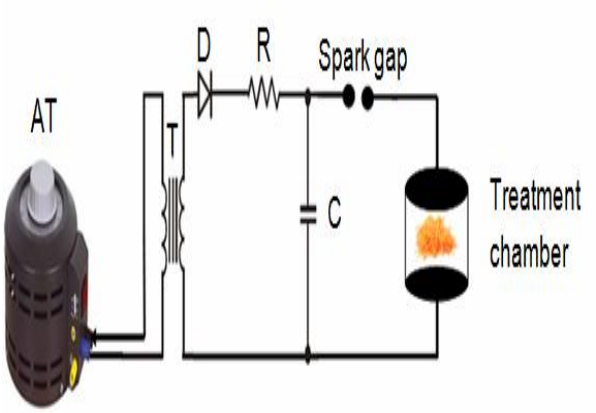


Figure 3. Electrical circuit of the pulse generator

Figure.4. The experimental setup
1. Auto-transformer- 2. DC-HV power supply- 3. Electrostatic voltmeter- 4. HV measuring probe 5. Charging resistors- 6. HV charging capacitor- 7. HV discharge switch- 8. Treatment chamber

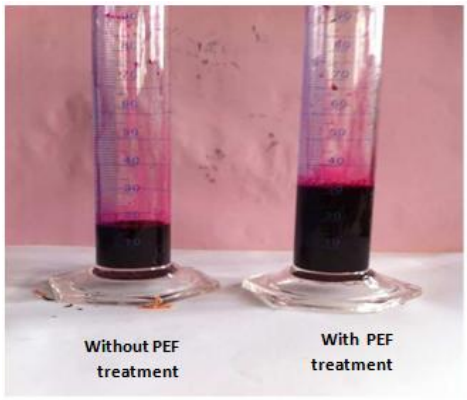


Figure 5. PEF treated and Untreated juice extraction

2.5. Experimental Designs Methodology

The Composite Centred Faces design (CCF), which gives quadratic models, was adopted. The quadratic dependence between the output function to optimize (response) and the input variables u_i (i = 1,.....,k) (factors) has the following expression (Eriksson et al. 2000; Frigon and Mathews, 1996; Taguchi, 1987):

$$y = f(u_i)c_0 + \sum c_i u_i + \sum c_{ij} C_{ij} + \sum c_{ii} u_i^2 \quad (1)$$

As Δu_i and u_{i0} are respectively the step of variation and the central value of factor *i*, reduced centred values of input factors may be defined by the following relation:

$$x_i = (u_i - u_{i0}) / \Delta u_i \quad (2)$$

With these new variables, we obtain:

$$y = f(x_i) = a_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2 \quad (3)$$

A Windows program dedicated to experimental designs was used (MODDE 5.0 software, Umetrics AB, Umea, Sweden) (MODDE 5.0).

2.6. Design of PEF juice extraction experiments

Classical "one-factor-at-a-time" experiments are carried out to identify the domain of variation of the three following factors:

1. Electric field level *E* (kV/cm);
2. Number of pulses *n*;
3. Pulse duration T (µs).

The pulse duration was varied by using appropriate values of the charging capacitor. As the voltage wave shape is bi-exponential, the pulse duration *T* corresponds to half of the amplitude (Fig.6).Thus, following values were obtained: T = 30 µs for C = 1 µF, T = 55 µs for C = 2 µF and T = 80 µs for C = 3 µF.

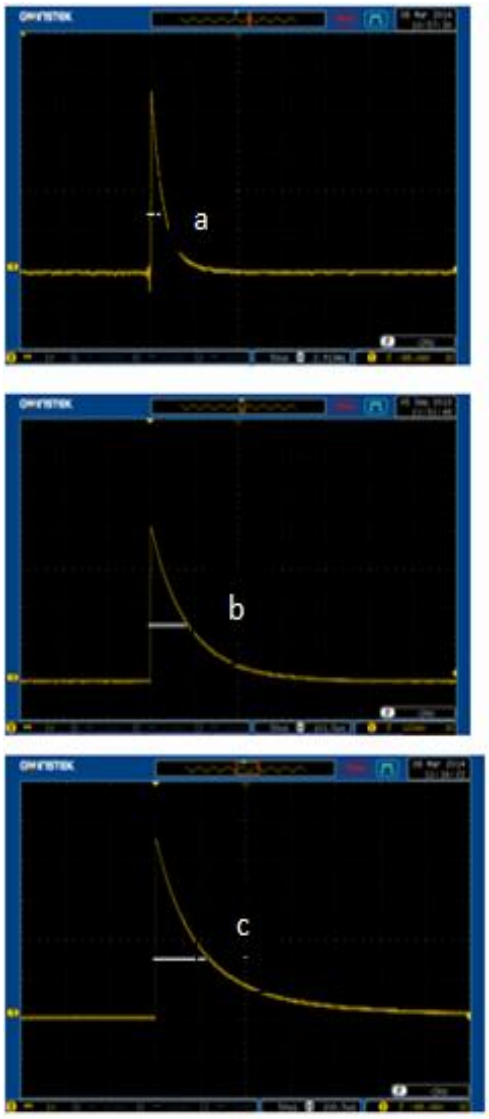


Figure 6. Current waveforms delivered by the pulse generator for different values of capacitance C
a. T= 30 µs (C = 1 µF), b. T= 55µs (C = 2 µF), c. T= 80 µs (C = 3 µF)

3. Results and discussions

The experimental procedure to obtain a mathematical model starts with following "one-factor-at-a-time" experiments.

Experiment 1: Variable pulsed electric field intensity E (1- 4 kV/cm), at constant values of n = 100 pulses and T = 30 µs.

Experiment 2: Variable number of pulses n (50-200), at constant values of E = 2,5 kV/cm and T = 30 µs.

Experiment 3: Variable pulse duration T (30-80 µs) of the pulse generator at constant values of E = 3 kV/cm and n = 100 pulses.

Obtain results in this section served to the definition of the domain of variation of E, n and T. Obtained results of Experiments 1–3 are represented in Figures 9 - 11. The mass of extracted juice and the absorbance were considered as significant for the evaluation of the process and represented as functions of the three control factors. Obtained results in this section served to define the domain of variation of E, n and T to indentify a mathematical model using MODDE 5.0 software.

First, the graph in Figure 9 shows that in the conditions of Experiment 1, the mass of extracted juice and the absorbance increase with increasing pulsed electric field intensity up to 3 kV/cm. Then they decrease for higher values of E. Thus, E_{min} = 2.5 kV/cm and E_{max}= 3.5 kV/cm were retained as the limit values for the electric field (PEF).

In the conditions of Experiment 2 (Figure 10), we noticed the same variation concerning the influence of of pulses number. The mass of extracted juice and the absorbance firstly increased with the pulses number up to n =100 pulses, then they decreased. Consequently, the domain of variation of this factor was defined as n_{min} = 50 and n_{max} = 150 pulses.

Furthermore, results of experiments 3 (Figure 11) obtained according to the pulse duration τ show that τ should not exceed 55 µs. Otherwise the mass of the juice will decrease, causing the diminution of the extraction efficiency. Indeed, when the pulse duration is higher the carrot cells receive a great amount of energy, causing the reverse effect. So, we opted for the T_{min} = 30 µs and T_{max} = 80 µs as limits of variation domain of T.

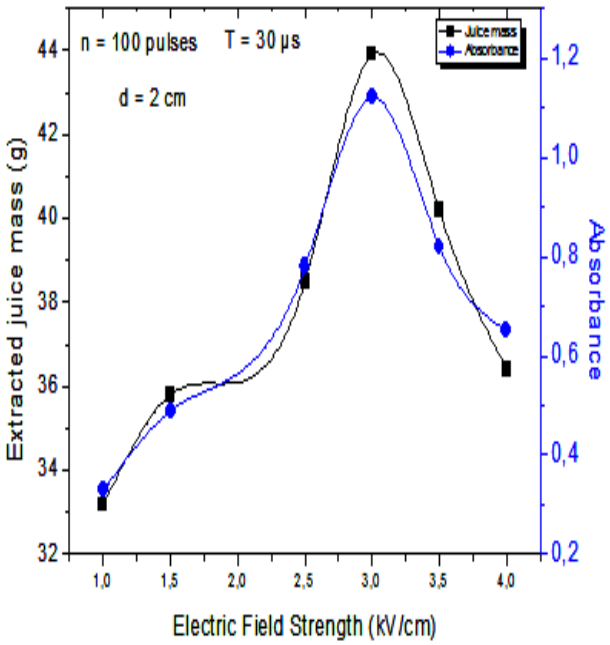


Figure 9. Evolution of extracted carrot juice mass and absorbance according to the voltage (n=100 pulses, T = 30 µs)

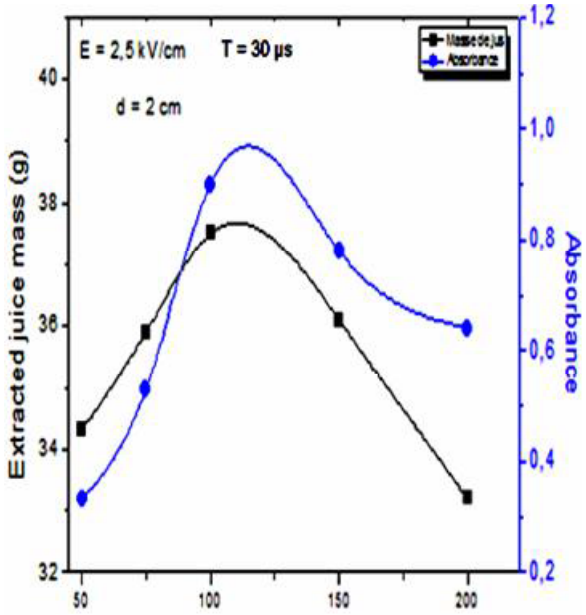


Figure 10. Evolution of extracted carrot juice mass and the absorbance according to the number of pulses (E = 2,5 kV/cm, T =30 µs)

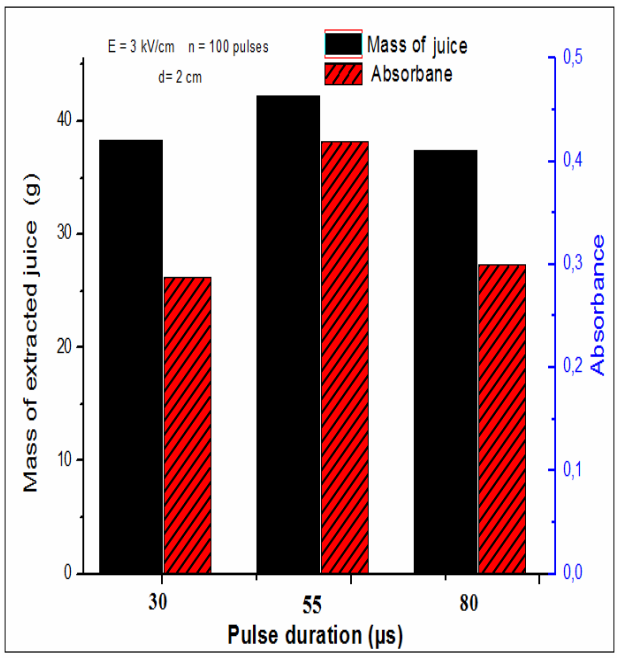


Figure 11. Evolution of extracted carrot juice mass and the absorbance according to the pulse duration
(E = 3 kV /cm, n = 100)

A central CCF design was carried out for identifying the set point (E_0, n_0 and T_0); the two levels "max" and "min" are the limits established in previous section for each of the three input variables (E_{min}, E_{max}), (n_{min}, n_{max}) and (T_{min}, T_{max}), the central point (E_c, n_c and T_c) being calculated as follows:

$$E_c = (E_{min} + E_{max})/2 = (2.5 + 3.5)/2 = 3kV/cm \quad (4)$$

$$n_c = (n_{min} + n_{max})/2 = (50 + 150)/2 = 100 Pulses \quad (5)$$

$$T_c = (T_{min} + T_{max})/2 = (30 + 80)/2 = 55\mu s \quad (6)$$

The results of all the experiments are given in Table 2. According to all of the experiments modeling software MODDE 5.0 gave us a mathematical model of juice extraction and the absorbance using pulsed electric field treatment. This mathematical model is very satisfactory because the coefficients R^2 and Q^2 are very close to 1 (Figure 12). MODDE 5.0 also gives the effect of each parameter on extracted juice yield (Figure 13).

Table 2. Results juice mass experience extract according to variation in treatment values

Exp.N°	E [kV/cm]	T [µs]	n	M [g]	Abs
1	2.5	30	50	39.61	1,031
2	3.5	30	50	36.08	1,287
3	2.5	80	50	40.31	1,215
4	3.5	80	50	34.28	0,71
5	2.5	30	150	40.31	0,488
6	3.5	30	150	38.2	0,577
7	2.5	80	150	39.41	1,21
8	3.5	80	150	35.78	0,52
9	2.5	55	100	41.89	0,996
10	3.5	55	100	36.72	0,714
11	3	30	100	40.82	0,639
12	3	80	100	39.86	0,621
13	3	55	50	40.04	1,349
14	3	55	150	40.28	0,765
15	3	55	100	40.88	0,703
16	3	55	100	40.88	0,703
17	3	55	100	40.88	0,703

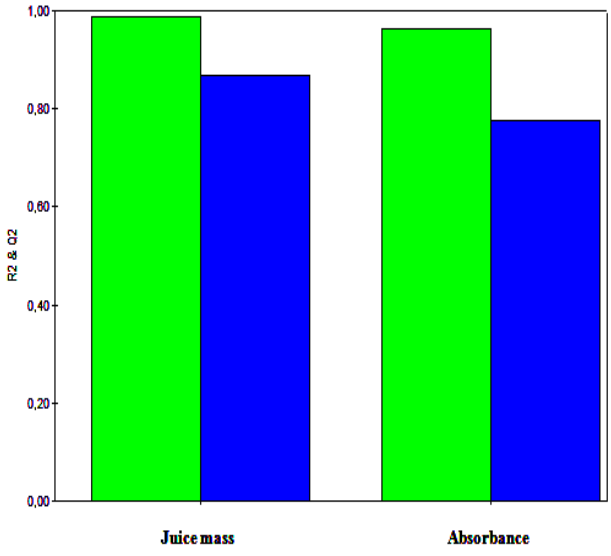


Figure 12. Representation of descriptive quality and predictive quality of mathematical model of juice extraction and the absorbance

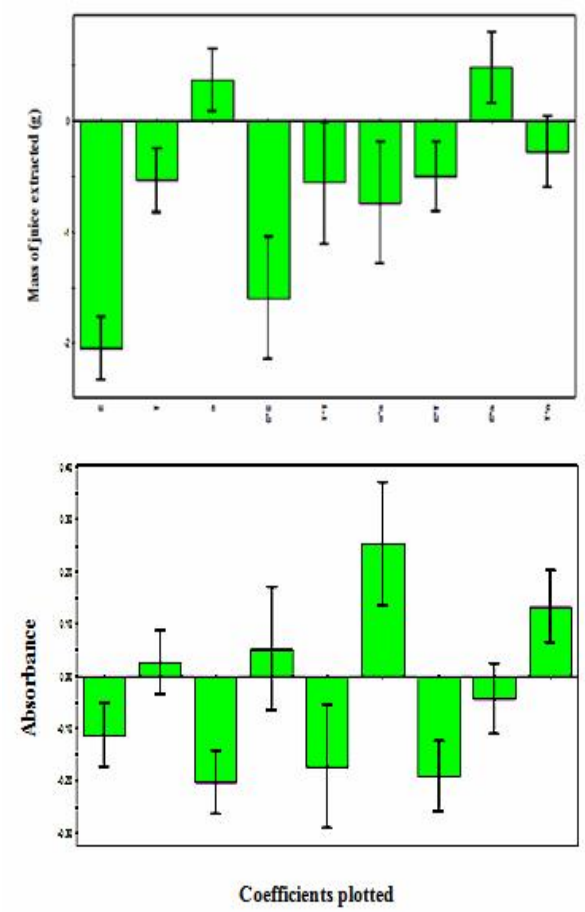


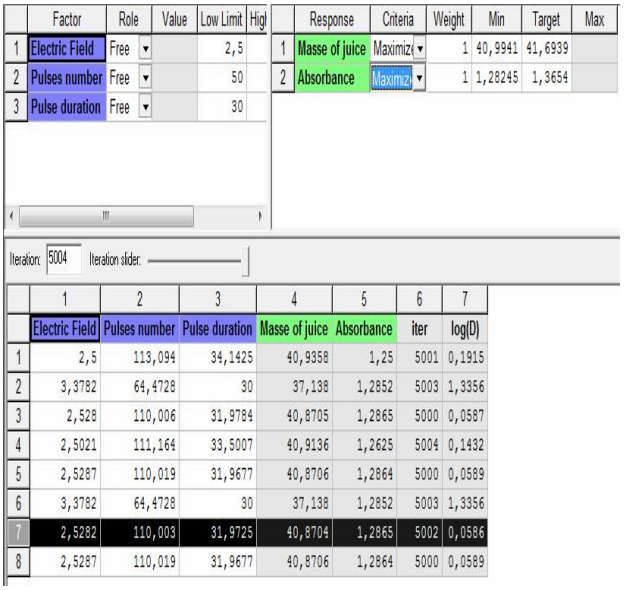
Figure 13. Plotted coefficients of the obtained model

The mathematical model of masse of extracted juice using pulsed electric field (PEF) proposed by modelisation software MODDE 5.0 is presented in equation bellow:

$$M = 40.89 - 2.05E + 0.37n - 0.54T - 1.59E^2 - 0.74n^2 - 0.56T^2 - 0.50E*T - 0.47E*n \quad (7)$$

$$Abs = 0.760 - 0.11E + 0.025n - 0.20T - 0.174n^2 - 0.256T^2 + 0.132n*T - 0.19E*n \quad (8)$$

According to this model, the optimum of the process (i.e. the greatest amount of carrot juice) (Figure 14) should be obtained for Electric field E_0=2.5 kV/cm, number of pulses n_0= 110 and pulse duration T_0 = 31 µs.



	Factor	Role	Value	Low Limit	Hig
1	Electric Field	Free		2,5	
2	Pulses number	Free		50	
3	Pulse duration	Free		30	

	Response	Criteria	Weight	Min	Target	Max
1	Masse of juice	Maximiz	1	40,9941	41,6939	
2	Absorbance	Maximiz	1	1,28245	1,3654	

Iteration: 5004 Iteration slider:

	1	2	3	4	5	6	7
	Electric Field	Pulses number	Pulse duration	Masse of juice	Absorbance	iter	log(D)
1	2,5	113,094	34,1425	40,9358	1,25	5001	0,1915
2	3,3782	64,4728	30	37,138	1,2852	5003	1,3356
3	2,528	110,006	31,9784	40,8705	1,2865	5000	0,0587
4	2,5021	111,164	33,5007	40,9136	1,2625	5004	0,1432
5	2,5287	110,019	31,9677	40,8706	1,2864	5000	0,0589
6	3,3782	64,4728	30	37,138	1,2852	5003	1,3356
7	2,5282	110,003	31,9725	40,8704	1,2865	5002	0,0586
8	2,5287	110,019	31,9677	40,8706	1,2864	5000	0,0589

Figure 14. Subroutine of MODDE.05 representing the set point

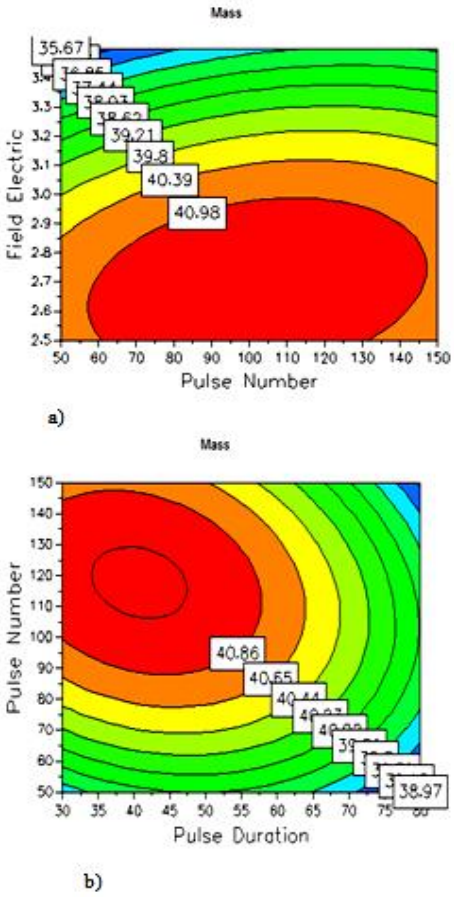


Figure 15. Response contour plots for middling.

Figure 15 shows the iso-response contours obtained with the present model; figure 15. represents the dependency of the middling mass of PEF extracted juice according to the variation of both treatment unit factors (i.e., the electric field level, the pulses number and pulse duration or pulse with).

3.1. Analysis of betanin concentration

Three samples were analyzed with a spectrophotometer: a control sample, a random point (1.5 kV/cm, 30 µs and 50 pulses) and a sample treated with the optimal values (E_0 = 2.5 kV/cm, n_0 = 110 and T_0 = 31 µs).

Fresh extracted samples were filtered through two layer cheese cloths andthen have been put in 90% methanol (50 ml for each gram). The samples were centrifuged using the Eppendorf 5804R model centrifuge at 3000 rpm for ten minutes. The supernatant was separated and the absorbance was measured at λ= 537 nm and λ= 600 nm on Specord 200 plus spectrophotometer (Sükran et al., 1998). An obtained result represented in Figure 16 clearly shows a significant increase of betanin concentration for the sample pre-treated with optimal values.

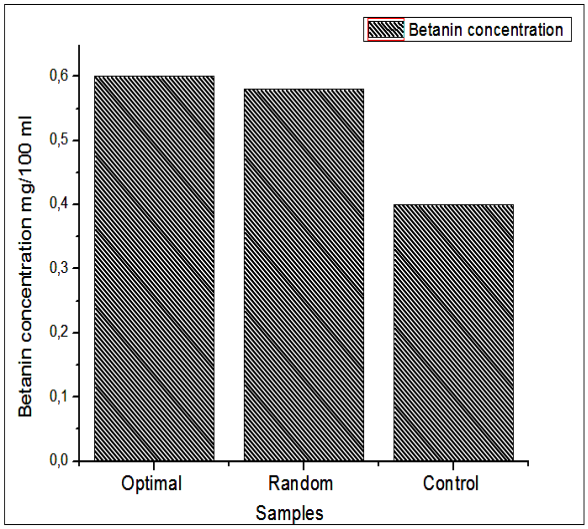


Figure 16. Concentration of betanin of the three samples

The physico-chemical analysis of different extracts juice have shown that there are significant differences in the concentrations of pigments between the sample treated with PEF and the sample untreated (Figure 12). The results showed that treatment with PEF increases the quality of juice extracted by increasing the concentration of substances in the juice. Moreover, the juice obtained without treatment (control sample) remains less quality than juice treated with PEF.

4. Conclusions

Today's juice extraction industry needs to increase the quantity of production while maintaining the same quality of the juice. Application of PEF would assist the industry. From the conducted experiments and analysis we can conclude that PEF treatment resulted in the breakdown of the cell membrane which in turn enhanced juice extraction more than the untreated sample. PEF treatment increased the quantity of substances contained in juice extracted which increased the quality of juice extracted using a pulsed electric field.

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TAPIOCA RESISTANT STARCH PRODUCTION AND ITS STRUCTURAL PROPERTIES UNDER ANNEALING AND PLASMA TREATMENTS

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ABSTRACT
Structure of tapioca starch (raw) was reorganized under annealing treatment during 12-72 h. Then, raw and annealed starches were modified by Argon-plasma treatment (P sample) under atmospheric pressure at constant parameters (137.5 V, 1 A, 10 min). Treated samples were characterized degree of cross-linking (DCL), degree of relative crystallinity (DRC), and *in vitro* digestibility using Fourier Transform Infrared Spectroscopy, X-ray difractometry, respectively. Results showed that DCL increased during both annealing and plasma treatments. Besides, DRC of annealed starches was higher than that of raw, but it was slightly reduced under plasma treatment. Furthermore, A-type crystal pattern of samples was remained during treatments. Annealing resulted in a significant increase of resistant starch (RS) fraction and further plasma treatment continuously enhanced RS content from 30.5 % of raw starch to around 71.0 % of 72h-P (dual treated) sample. After boiling, RS fraction of the starch was (decreased by 4%) 67%. Thus, dual treatment (annealing and plasma) is a novel and potent method of both RS and boiled RS production.

1. Introduction

Starch, which is one of a major component in plant foods, is a main dietary source of carbohydrate in human nutrition. Tapioca root has a high content of starch with a wide range of applications. Tapioca starch is remarkable for food industry because of its odorless, paste clarity, and stickiness. However, raw tapioca starch has some disadvantages for industrial applications and in nutritional aspects such as insolubility in water, loss of viscosity, and especially high content of rapid digestible starch (RDS) under cooking process. These shortcomings of raw tapioca starch could be improved by various modification methods which include chemical or physical routes (Deeyai et al., 2013).

There are three fractions of starch: (a) rapidly digestible starch (RDS) consists mainly of amorphous and dispersed starch, found in high amounts in starchy food cooked by moist heat; (b) slowly digestible starch (SDS) is completely digested in the small intestine; and (c) resistant starch (RS) escapes digestion in the small intestine. RS fractions fermented by the colonic flora, resulting in short-chain fatty acids. RS has some beneficial effects such as a component of dietary fiber, prevention of colonic cancer, hypoglycemic effects, hypocholesterolaemic effects, a prebiotic,

reduction of gallstone formation and inhibition of fat accumulation (Sajilata, 2006)

Annealing treatment is a "physical modification of starch slurries in water at temperature below gelatinization". Annealed starch could be more difficult hydrolyzed by amylase enzymes than raw starch (Collado and Corke, 1999; Tester and Debon, 1999). Besides, plasma treatment is a physical treatment which is used to modify starch properties. In the plasma environment, energetic particles (electrons, atoms, molecules, ions and free radicals) attach to starch molecules and induce changes in properties. Previous publications reported the formation of cross-linking and resistant starch (RS) under argon-plasma treatment (Deeyai et al., 2013; Trinh et al., 2014). There are previous studies of starch modification focusing on structural properties and digestibility under annealing or plasma treatment (Tester and Debon, 1999; Trinh et al., 2014). However, there is a lack of reporting on dual treated (annealing and argon-plasma) starch for RS production. In this study, tapioca starch was treated by annealing and argon-plasma. Structure and *in vitro* digestibility changes of starches were investigated.

2. Materials and methods

2.1. Annealing treatment of starch

Starch was suspended in distilled water (1:3, $^{w}/_{w}$) and then incubated at 50^{o}C for 12-72 hours (Tester and Debon, 1999) Starch suspension was subsequently dried at 40^{o}C for 24 hours to reach the final moisture of around 11 %.

2.2. Plasma treatment of starch

Starch sample (5.0 g) was spreaded (and blended regularly) on a glass plate which was put on an electrode inside a DBD plasma divice (Figure 1). The DBD (Dielectric Barrier Discharge) plasma device was used to generate the plasma environtment throughout our experiments. Plasma was generated in an Argon gas-phase (flow rate of 5 ml/min) by applying a high potential difference between two electrodes. Starch was treated following the previous methods [1,5] under atmospheric pressure at constant parameters (137.5 V, 1 A, 10 min).

2.3. Fourier Transform Infrared Spectroscopy (FTIR) and ratio of ordered/amorphous structure

FTIR spectra were recorded using a FTIR-8400S (Shimadzu, Japan). The absorbance spectra were collected from 400 to 4000 cm^{-1} at room temperature, and at a resolution of 2 cm^{-1} (in at least triplicate) (Deeyai et al., 2013).

The ratio of ordered (α-helix)/amorphous structure was identified by the ratio of the height of the bands at 1039/1014 (Jeroen et al., 1995)

2.4. X-ray diffractometry

XRD was determined using a powder X-ray diffractometer (Model D8 Advance, Bruker, Germany). The operating conditions were 40 kV and 40 mA with Cu-Kα radiation of 0.15406 nm (Nickel filter; time constant, 4 s). Each scan was performed from 3 to 30◦ (2θ) (Trinh et al., 2014) DRC was calculated using the equation DRC = *Ac*/ (*Ac* + *Aa*), where *Ac* is the area of crystalline portion and *Aa* is the area of amorphous portion, according to the method of Nara and Komiya, 1983, with peak-fitting software (Originversion 7.5, OriginLab, Northampton, Mass., U.S.A.).

2.5. In vitro digestibility

Two gram of Pancreatin (Sigma-Aldrich) was dissolved in 24 ml of ditilled water (D.W) and was stirred for 10 min. Enzyme suspension was centrifuged (10 min, 1500×g) and then 20 ml of supernatant was mixed with 3.6 ml of D.W and 0.4 ml of 300 L AMG (amyloglucosidase, Novozymes). This solution was kept in waterbath (37 ^{o}C) for 15 min. Starch (30 mg) was put in a 2-ml microtube with a glass bead. Next, 0.75 ml of sodium acetate buffer (pH5.2) was added and the tube was stored in a shaking incubator (37 ^{o}C, 10 min, 240 rpm). After adding 0.75 ml of the prepared enzyme solution, the microtube was

shaken continuously. The enzymatic reaction was stopped after 10 or 240 min by boling for 10 min and then sample was left. GOD-POD kit (BCS, Anyang, Korea) was used to determine the glucose content in the supernatant obtained by centrifugation (5 min, 5000×g). Starch *in vitro* digestibility was determined according to the method of Brumovsky and Thompson (2011).

3. Results and discussions

3.1.Fourier Transform Infrared Spectroscopy (FTIR) and ratio of ordered / amorphous structure

FTIR spectra of starches were shown on Figure 2. Between samples, there were not significant changes in shape of spectra and position of peaks. However, the height of peaks significantly changed by both annealing and plasma treatments indicating the stronger vibration of chemical groups. Besides, peak at 1039 cm^{-1} was sensitive to the amount of ordered (α-helix) starch and the wavelength of 1014 cm^{-1} was the characteristic of amorphous starch. Thus, α-helix/amorphous structure ratio (ORD) (Table 1) was identified by the proportion of the height of the wavelength at 1039 to 1014 cm^{-1} (Jeroen et al., 1995). In the presentstudy, this ratio was not significantly different between samples treated by annealing and plasma technique. The similar result was reported by Deeyai et al. 2013.

FTIR spectra (400-4000 cm^{-1}) of starches (Figure 2) in four main regions were as follows: the fingerprint region (600-1500 cm^{-1}), the double-bond region (1500-2000 cm^{-1}), the triple-bond region (2000-2500 cm^{-1}), and the X-H stretching region (2500-4000 cm^{-1}. It was not easy to (?) assign the exact band at the 600-1500 cm^{-1} because of highly overlapping and complex spectra. The skeletal modes of pyranose ring were observed at around 537 cm^{-1}. The peak at 764 cm^{-1} was the C-C stretching. The skeletal modes of α-1,4 glycosidic linkage (C-O-C) were found at around 930 cm^{-1} (Ramzan et al. 2002). The C-O-H bending occured at 1094 cm^{-1}. The absorption peak at 1163 cm^{-1} was due to the coupling mode of C-O and C-C stretching. The absorption band at 1241 cm^{-1} was attributed to the CH_2OH (side chain) related mode. The C=C and C=O stretching were observed in the 1500-2000 cm^{-1} region (Deeyai et al., 2013).

Depending on the type of C=O bond, carbonyl stretching occurred in the 1650-1830 cm^{-1}. C=C stretching was in around 1650 cm^{-1} but this band was often absent for symmetry or dipole moment reasons. The region above 2000 cm^{-1} was the vibration of metal carbonyls (Deeyai, et al. 2013).

The peak at 1635 and 3300 cm^{-1} reflected a tight bond and a weak absorption of water molecules. The degree of cross-linking (DCL) could be identified from the relative intensity of these two peaks with the C-O-H peak at around 993-1094 cm^{-1} (Deeyai et al., 2013).

In this study, during annealing treatment, DCL of treated starch continuously increases compared with raw. Moreover, further argon-plasma treatment significantly enhances DCL of raw and annealed starches to reach the maximum of 71h-P sample (both DCLa and DCLb values). Thus, both treatments caused the increase of DCL in samples (Table 1).

3.2. X-ray diffractometry (XRD) and degree of relative crystallinity (DRC)

XRD patterns of starch samples were presented in Figure 3. The diffractograms of the raw, annealing treated and plasma treated samples werenot significantly different. All samples showed strong diffraction at 2 theta of about 15.4 and 23.6^o and unresolved doublet at 16.6 and 18.3^o, which was close to the A-type structure (Table 1) (Bogracheva , 2001) DRC of starches was shown in Table 1. Annealing treatment did not change DRC of the starch. However, under plasma treatment, intensity of peaks was weakened, especially in the case of 16.6 and 18.3^o peaks (Figure 3), leading the disruption of crystalline structure. In a previous study, Deeyai P. (2013) stated that the change of DRC is rather small under plasma treatment and it is not easy to be detected by XRD (Deeyai et al., 2013).

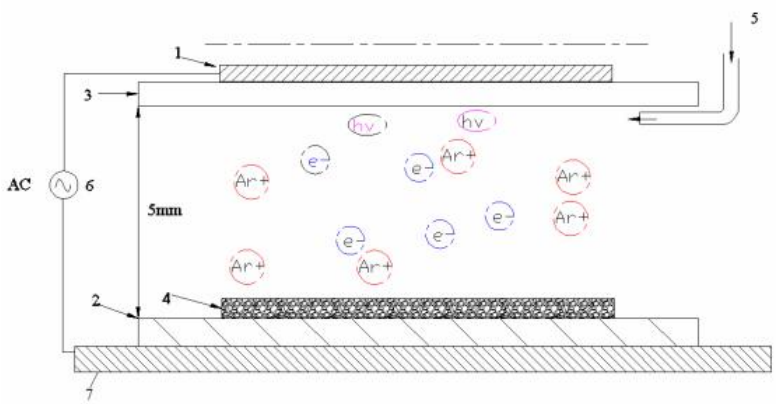

Figure 1. DBD plasma device: 1. Cathode, 2. Glass plate, 3. Dielectric material, 4. Starch sample, 5. Argon input, 6. Plasma environment, 7. Anode

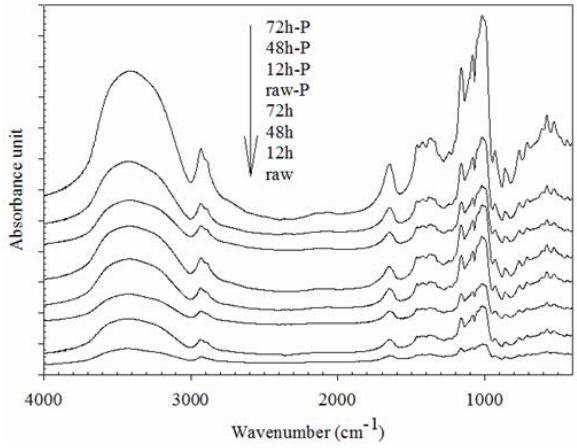

Figure 2. FTIR spectra of starches[*]; [*]12h, 48, 72h was annealed starches, which was incubated at 50 ^{o}C for 12-72 hours; P: plasma treated starch

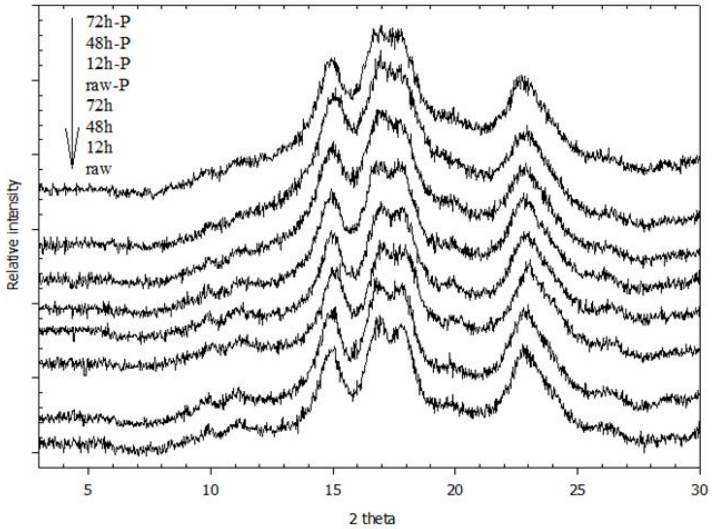

Figure 3. X-ray diffractograms of starches; [*]12h, 48, 72h was annealed starches, which was incubated at 50 ^{o}C for 12-72 hours; P: plasma treated starch

Table 1. Degree of cross-linking (DCL), α-helix/amorphous ratio (ORD), degree of relative crystallinity (DRC), and crystal pattern of starches*.

Sample	DCL		ORD	DRC	Crystal pattern
	997-1649 (DCLa)	997-3421 (DCLb)			
raw	0.08 ± 0.01^{h}	0.03 ± 0.00^{g}	0.89 ± 0.03^{a}	38.88 ± 0.6^{f}	A
12h	0.20 ± 0.05^{g}	0.07 ± 0.01^{f}	0.88 ± 0.02^{b}	45.67 ± 0.9^{c}	A
48h	0.27 ± 0.03^{e}	0.08 ± 0.01^{e}	0.86 ± 0.02^{c}	50.90 ± 0.7^{b}	A
72h	0.37 ± 0.06^{d}	0.17 ± 0.04^{b}	0.86 ± 0.00^{c}	57.21 ± 0.7^{a}	A
raw-P	0.37 ± 0.03^{d}	0.10 ± 0.03^{d}	0.88 ± 0.01^{b}	32.86 ± 0.8^{h}	A
12h-P	0.40 ± 0.06^{c}	0.16 ± 0.01^{c}	0.88 ± 0.02^{b}	34.62 ± 0.5^{g}	A
48h-P	0.54 ± 0.05^{b}	0.17 ± 0.02^{b}	0.85 ± 0.03^{c}	41.33 ± 0.7^{e}	A
72h-P	1.08 ± 0.06^{a}	0.36 ± 0.02^{a}	0.86 ± 0.02^{d}	43.64 ± 0.7^{d}	A

*superscipt in each column indicate the significant difference (P<0.05).

Table 2. In vitro digestibility* of non-boiled and boiled** starches.

Sample	Non-boiled			Boiled		
	RDS (%)	SDS (%)	RS (%)	bRDS (%)	bSDS (%)	bRS (%)
raw	46.1 ± 1.4^{a}	23.3 ± 1.5^{g}	30.6 ± 0.2^{h}	48.9 ± 0.2^{a}	44.6 ± 0.5^{a}	6.5 ± 0.3^{h}
12h	17.0 ± 0.1^{f}	39.6 ± 0.4^{a}	43.3 ± 0.5^{f}	32.5 ± 0.3^{b}	30.9 ± 0.6^{d}	36.6 ± 0.8^{f}
48h	19.7 ± 1.5^{c}	34.1 ± 1.0^{d}	46.3 ± 0.8^{e}	31.2 ± 0.5^{b}	28.9 ± 0.7^{e}	40.0 ± 0.9^{e}
72h	18.1 ± 0.5^{e}	25.7 ± 0.4^{f}	56.2 ± 0.3^{b}	30.7 ± 0.9^{b}	27.9 ± 0.4^{g}	41.5 ± 0.7^{d}
raw-P	20.2 ± 0.2^{b}	38.1 ± 1.4^{b}	42.8 ± 0.2^{g}	26.3 ± 0.8^{b}	37.5 ± 1.1^{b}	36.1 ± 0.4^{g}
12h-P	14.3 ± 0.3^{h}	37.7 ± 0.7^{c}	47.8 ± 0.4^{d}	21.3 ± 0.8^{c}	35.6 ± 0.9^{c}	43.1 ± 0.5^{c}
48h-P	18.3 ± 0.4^{d}	27.2 ± 0.8^{e}	54.5 ± 0.6^{c}	24.8 ± 0.1^{b}	28.6 ± 0.5^{f}	46.4 ± 0.6^{b}
72h-P	15.0 ± 0.6^{g}	14.2 ± 0.7^{h}	71.0 ± 0.3^{a}	13.3 ± 0.1^{c}	19.8 ± 0.4^{h}	66.9 ± 0.3^{a}

*superscipt in each column indicate the significant difference (P<0.05); **after stop reaction, starch sample was boiled for 15 min before digestibility measurement.

3.3. In vitro digestibility

In vitro digestibility was presented in Table 2. During annealing treatment, RDS reduced while SDS and RS levels increased. Furthermore, sample 72h-P contained 56.2% of RS, which was 25.6% higher than that of the raw. Some previous authors reported the crystalline "perfection" and the increaseof crystallinity was found in annealed starch (Trinh et al., 2014). Hyun-Jung Chung et.al. (2009) reported that annealing decreased SDS whereas the treatment increased RDS and RS levels in granular starches. By contrast, annealing decreased RDS and increased SDS, RS levels in gelatinized starches (Hyun-Jung Chung et al., 2009). In that study, the authors

concluded that a superior crystalline structure caused the resistance to enzymatic hydrolysis. Under plasma treatment, RDS was decreased while RS was increased significantly. The sample 72h-P reached RS content of 71.0 %, which was 14.8 and 40.4 % higher than that of 72h and raw samples, respectively.

Such an increase in RDS content and reduce in RS content were generally observed in boiled starches because the boiling destroyed the semi-crystalline structure of raw starch granules (Eliasson, and Gudmundsson, 2006) indicates the boiling-stable of this starch fraction. In the present study, boiled-RS of 72h-P was more than 60 % higher than that of raw. Hence, the dual treatment (annealing and plasma treatment) resulted in boiling-stable RS formation.

In this study, the relationship between RS-DCL and bRS-DCL were presented in these equations:

(a) RS= 35.961×DCLa + 34184 (R^2= 0.8429);
(b) bRS= 47.85×DCLa + 19.84 (R^2= 0.7633);
(c) RS= 110.36×DCLb + 33.336 (R^2= 0.9002);
(d) bRS= 14.63×DCLb + 19.456 (R^2= 0.7583).

Obviously, both RS and bRS levels correlate to the degree of cross-linking (DCL).

4. Conclusions

Under annealing and plasma conditions, structural properties of starch were changed, especially in the increase of DCL and DRC. These changes resulted in the increase of resistant starch fraction in the treated samples. Positive effects of dual treatment surpassed those induced by single treatment.Our results also showed that the physically dual modification was a useful and efficient route for RS and boiling-stable RS formation.

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EXPERIMENTAL ANALYSIS OF MONOAXIAL AND BIAXIAL PULSED ELECTRIC FIELD TREATMENT CHAMBERS FOR FOOD PROCESSING

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ABSTRACT

Except for the "Co-field" electrodes, the majority of PEF treatment chambers, used in food processing experiments, are constituted of two parallel metal electrodes energized by a pulsed voltage. The electric field lines which are perpendicular to the electrodes are monoaxial and oriented almost in only one direction. The objective of the present work is an experimental comparative analysis between such treatment chamber (TC1) to a similar one but comprising four identical metal plates placed on the side walls of a square treatment chamber (TC2). For this latter, each pair of the adjacent metal plates form one electrode, the electric field lines being biaxial and oriented in two directions. The chamber made of Plexiglas has a square paralelipedic shape of dimensions 6x10x10 cm^3, in which are placed either two (TC1) or four (TC2) vertical stainless steel electrodes, of dimensions 6x10 cm^2. The experimental analysis was made using a classic pulse voltage generator, by measuring the mass of PEF pretreated extracted juice from beet and the amount of betanine using a spectrophotometer. The obtained results, using the methodology of experimental designs, have shown that the TC2 model is more efficient, because higher quantities of juice and betanine were obtained up to 25% more with an energy saving of nearly 90%.

1. Introduction

Pressing and pulsed electric field (PEF) treatments are widely combined for extraction of juice. Several studies confirmed the positive effect of PEF treatment on both juice yield and its quality (Akinlaja, 1998; Bazhal, 2003; Gachovska, 2006; Grishko, 1991; Nabil, 2010). Moreover, inactivation of microorganisms and acceleration of the drying process using PEF technique were demonstrated (Qian, 2015; Raluca, 2010). Furthermore, the extraction rates of many compounds were increased in the food industry (El-Belghiti, 2005).

Food product is placed in the treatment chamber, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. There are two types of treatment chambers: static and dynamic. In both cases, high voltage electrical pulses are applied to the electrodes, which then conduct the high intensity electrical pulse to the product placed between the two electrodes, to achieve membrane electroporation (Humberto, 2007; Maged, 2012). In the static chambers only a given volume can be processed at once, for experimental applications. A dynamic chamber enables continuous processing, in accordance with the requirements of industrial application (Ratna, 2010).

While the parallel plate treatment chamber consists of two stainless steel plate electrodes and an insulator, the cofield chambers have two stainless steel tubes separated by an insulator, the electric field and the food flow concurrently (Lebovka, 2002). The parallel plate treatment chamber allows the uniformity of the electric field, and thus a homogeneous treatment, while co-field electric chambers which are more commonly used provide better fluid dynamics characteristics. The coaxial chamber is basically composed of an inner cylinder surrounded by an outer annular cylindrical electrode that allows food to flow between them. Although the coaxial treatment chambers are simpler, their cross-section which permits the flow of the food is narrower, and since some foods contain solid particles, this may cause blockage of the flow. This is not the case with co-field chambers which allow a continuous flow without risk of blocking even for foodstuffs with large particles (Kambiz, 2009; Nicolas, 2006).

For classic PEF treatment chambers with two parallel metal electrodes, the electric field lines which are perpendicular to the electrodes are monoaxial and oriented in only one direction. The objective of the present work is an experimental comparative analysis between such treatment chamber to a similar one but comprising four identical metal plates placed on the side walls of a square treatment chamber. For the latter, each pair of the adjacent metal plates forms one electrode.

2. Materials and methods

2.1. Experimental setup

The experimental setup used in the present work is composed of several components, comprising a high DC voltage source, an energy storage capacitor, a spark gap switch and a treatment chamber (Figure 1). A DC high voltage supply (Spellman 40 kV, 9 mA) charges the bank of capacitors until producing the spark gap's breakdown, causing an abrupt voltage (shock) applied to the load (treatment chamber where the sample is disposed). The storage element is composed of three sets of five series capacitors (2 µf, 2 kV), with the possibility to reach a maximum voltage of 10 kV and a total capacitance of 1.2 µF (Figure 2).

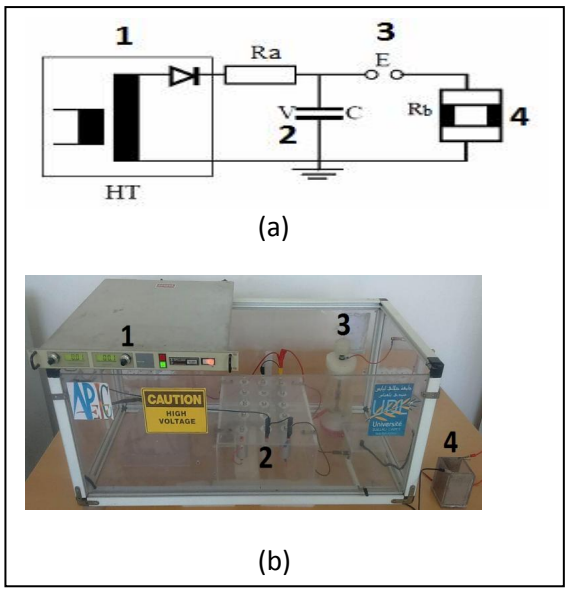


Figure 1. The pulse generator.
a) Descriptive schematic of the setup;
b) The photography of the setup
1- HV DC power supply, 2-Set of capacitors,
3- Spark gap switch, 4-Treatment chamber

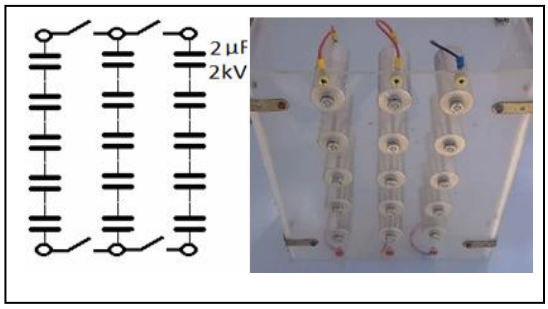


Figure 2. The bank of capacitors.
a) Descriptive schematic,
b) The photography of the capacitors bank

Two square parallelipedic treatment chambers made of Plexiglas, of dimensions 6x6x10 cm^3, in which are placed vertical stainless steel electrodes, were used in this work. The model TC1 comprises two parallel and opposite electrodes of dimensions 6x10 cm^2, while the model TC2 is constituted of four electrodes (Figure 3). For this latter, each pair of the adjacent metal plates form one electrode.

The volume of both treatment chambers is 192.3 ml.

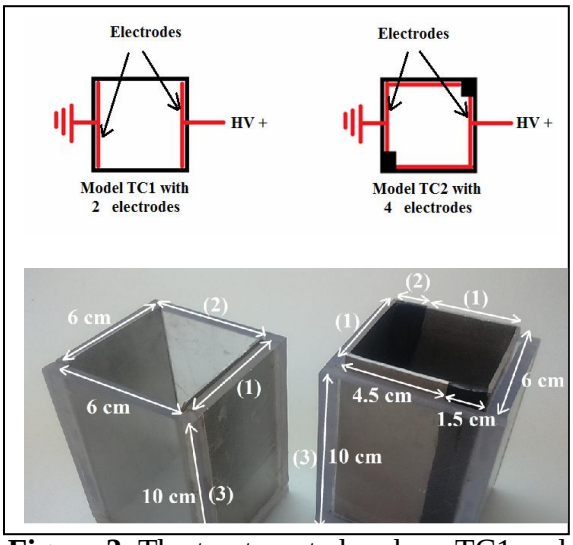


Figure 3. The treatment chambers TC1 and TC2; 1: Electrode 2: Insulating 3: Plexiglas

2.2. Materials and method

Beets were crushed with a household robot to obtain a homogeneous leg. The sample was held in a closed container to prevent evaporation before use. A beet paw sample of mass 80 g was used for each experiment. After PEF treatment, an extraction step was achieved using an extraction chamber and a hydraulic pressing machine (Mega, 15 tons). The PEF treated extracted juice was then analyzed by measuring both its mass using an electronic balance of 0.1 mg precision and the betanine amount using a spectrophotometer (Optizen *200 plus*) for λ= 530 nm.

All experiments were performed while maintaining following factors at constant values: pulse repetition frequency *f* = 1 Hz, extraction pressure P = 50 kg/cm^2, total pressing duration t = 300 s and the inter-electrodes gap d= 60 mm.

The methodology of the experimental designs makes it possible to determine the number of experiments to be achieved according to a well-defined objective, to analyze several factors simultaneously, to reduce dispersion related to measurements, to appreciate the effects of coupling between factors and finally to evaluate the respective influence of the factors and their interactions (Frigon, 1996; Taguchi, 1987). Before starting the experiments, we need to opt for the most suitable design which can model the process with maximum precision. The Composite Centered Faces design (CCF), which gives quadratic models, was adopted in the present work.

3. Results and discussions

The three following factors were considered in this work: the applied voltage V (kV), the number of pulses (n) and the pulse duration T (µs). The pulse duration is determined by the corresponding value of the capacitance, as shown in Table 1. The pulse duration was deduced from the corresponding oscillogram for a determined value of the capacitance, as shown for example in Figure 4 for C= 0.8 µF. The three following factors were considered in this work: the applied voltage V (kV), the number of pulses (n) and the pulse duration T (µs). The pulse duration is determined by the corresponding value of the capacitance, as shown in Table 1. The pulse duration is calculated at 37% of the amplitude of the electric field and deduced from the corresponding oscillogram for a determined value of the capacitance, as shown for example in Fig.4 for C= 0.8 µF.

Table 1: Values of the pulse duration according to corresponding capacitance

C (µF)	0.2	0.4	0.5	0.8	1.2
T (µs)	8	20	24	40	60

Moreover, the mass of extracted juice m (g), the amount of betanine expressed in terms of absorbance and the energy (W = ½ n C V^2) were considered significant to be considered as the response of the model. Absorbance (Abs) is the measurement of the amount of light absorbed by a given material for a determined wavelength using a spectrophotometer which is proportional to the coloration rate. Higher is the absorbance greater is the concentration of betanine substance.

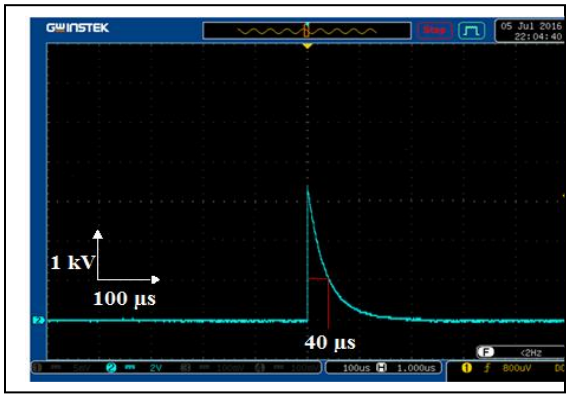


Figure 4. Pulse shape of duration T= 40 µs obtained for C=0.8 µF

A set of three preliminary experiments was performed. For each experiment, a factor was varied while the others were kept constant. The purpose of these preliminary experiments is to determine the variation domain for each factor in view of the following step by performing a CCF experimental design, which should enable the modeling and optimization of the PEF treated extraction process.

The obtained results of the preliminary experiments are plotted in Figs.5-7, representing the variation of the extracted juice mass *m* as function of the voltage *V*, the pulses number *n* and the pulse duration *T* respectively. Moreover, the variation of the absorbance is shown in Figs.8-10 for both treatment chambers.

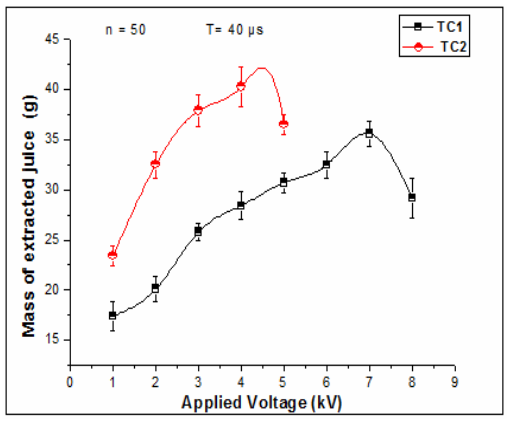


Figure 5. Evolution of the extracted beet juice mass according to the voltage (n=50, T=40µs)

According to the obtained results, following variation domains of each factor were selected, corresponding to the best limits giving maximum outcome:

Model TC1: V_{min}= 6 kV &V_{max}= 8 kV; n_{min}= 80 & n_{max}= 120; T_{min}= 20 µs & T_{max}= 60 µs

Model TC2: V_{min}= 3 kV &V_{max}= 5 kV; n_{min}= 60 & n_{max}= 100; T_{min}= 8 µs & T_{max}= 40 µs

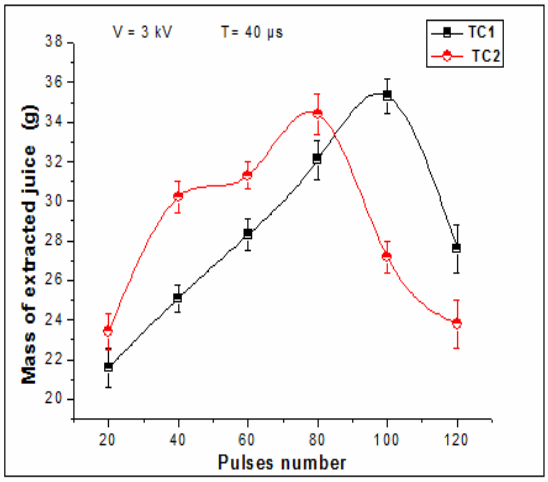


Figure 6. Evolution of extracted beet juice mass according to the pulses number (V=3kV, T= 40 µs)

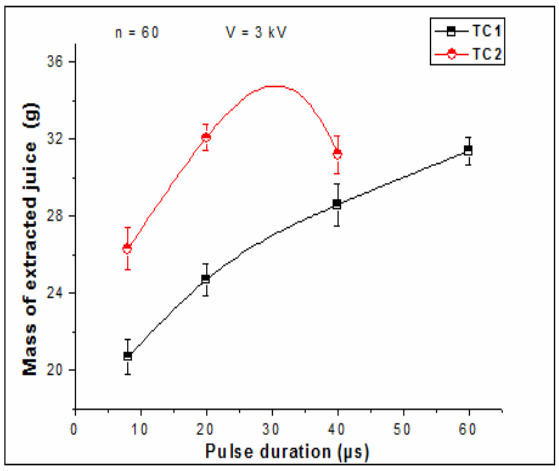


Figure 7. Evolution of extracted beet juice mass according to the pulse duration (V=3kV, n=60)

The following step is the modeling of the process using the design of experiments methodology, by performing two CCF designs

for both TC1 and TC2 chambers (Tables 2 and 3).

Predictive and descriptive quality of the models are satisfactory since values of criteria $Q^2$and R^2 are close to the unit, leading to validated mathematical models for both chambers.

The mathematical model of the responses considered for optimization, which are the mass of extracted juice, the absorbance and the consumed energy were obtained with MODDE 5.0 (MODDE. 1999), as shown in Table 4.

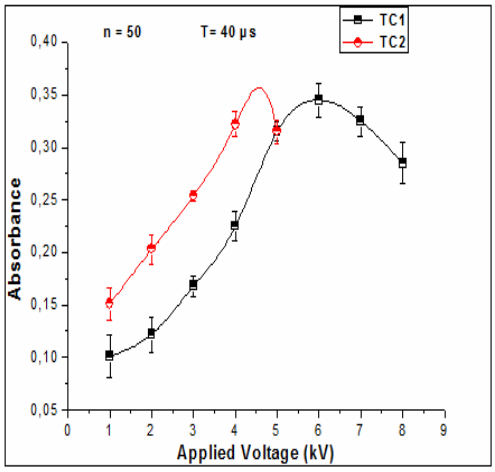


Figure 8. Evolution of the juice absorbance according to the voltage (n=50, T= 40 µs)

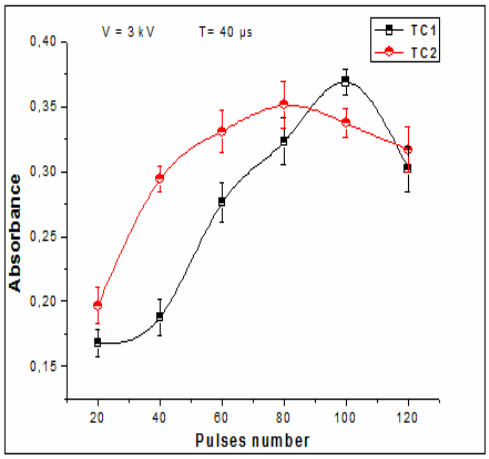


Figure 9. Evolution of the juice absorbance according to the pulses number (V=3kV, T= 40 µs)

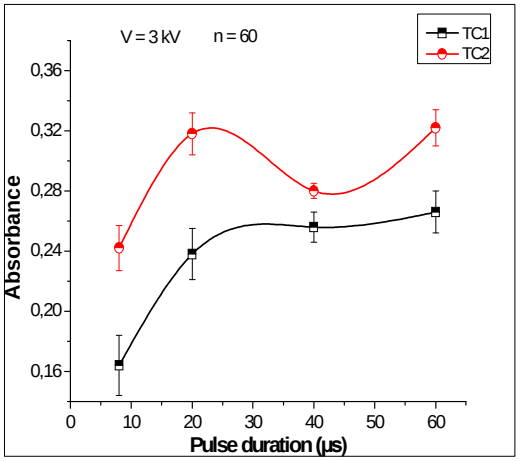


Figure 10. Evolution of the juice absorbance according to the pulse duration (V=3kV, n=60)

Table 2. Results of the CCF design experiment (TC1)

Exp N°	V (kV)	n	T (µs)	m (g)	Abs	W (J)
01	6	80	20	21.3	0.317	576
02	8	80	20	29.3	0.384	1024
03	6	120	20	24.7	0.364	864
04	8	120	20	30.3	0.395	1536
05	6	80	60	27.4	0.389	1728
06	8	80	60	32.7	0.457	3072
07	6	120	60	28.5	0.377	2592
08	8	120	60	32.8	0.383	4608
09	6	100	40	25.2	0.386	1440
10	8	100	40	32.8	0.415	2560
11	7	80	40	31.3	0.371	1568
12	7	120	40	31.4	0.373	2352
13	7	100	20	26.6	0.355	980
14	7	100	60	29.9	0.388	2940
15	7	100	40	30.5	0.385	1960
16	7	100	40	30.5	0.382	1960
17	7	100	40	30.5	0.384	1960

Table 3. Results of the CCF design experiment (TC2)

Exp N°	V (kV)	n	T (µs)	m (g)	Abs	W (J)
01	3	60	8	34.3	0.376	54
02	5	60	8	39.1	0.482	150
03	3	100	8	36.2	0.456	90
04	5	100	8	39.5	0.515	250
05	3	60	40	38.4	0.488	216
06	5	60	40	40.1	0.522	600
07	3	100	40	39.2	0.492	360

08	5	100	40	39.5	0.495	1000
09	3	80	24	37.7	0.474	180
10	5	80	24	41.5	0.543	500
11	4	60	24	35.9	0.404	240
12	4	100	24	36.8	0.422	400
13	4	80	8	37.7	0.53	128
14	4	80	40	39.8	0.577	512
15	4	80	24	37.6	0.449	320
16	4	80	24	37.2	0.471	320
17	4	80	24	37.4	0.459	320

Table 4. Mathematical model of the responses

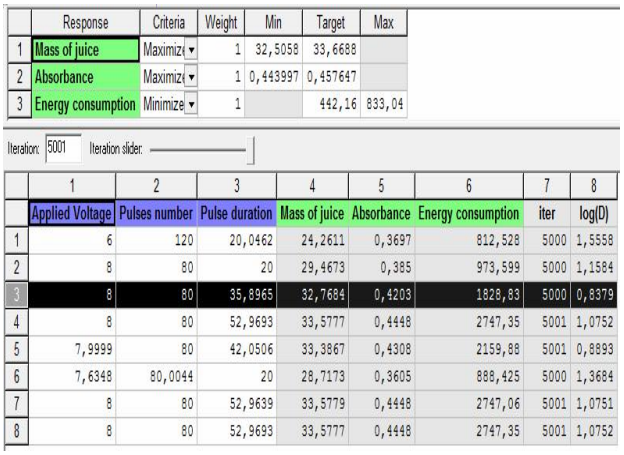
	TC1			TC2		
	Mass (g)	**Abs**	**W (J)**	**Mass (g)**	**Abs**	**W (J)**
Constant	30.3	0.382	1960	37.76	0.47	320
V	3.08	0.020	560	1.39	0.02	160
n	0.56	-0.00	398.4	0.34	0.01	84
T	1.91	0.01	996	1.02	0.02	201.6
V*V	-1.19	0.02	40	1.56	0.01	20
n*n	1.15	-0.01	0	-1.68	-0.07	0
T*T	-1.94	-0.01	0	0.71	0.06	0
V*n	-0.42	-0.01	112	-0.36	-0.01	40
V*T	-0.49	-0.09	280	-0.76	-0.01	96
n*T	-0.4	-0.01	200	-0.26	-0.01	51

The software offers the possibility to identify the optimal values of the factors which should give the highest amount of extracted juice m and Absorbance Abs for the smallest power consumption. It contains an optimization routine that is capable of simultaneously processing several responses, affected by different weighting coefficients. MODDE.05 has an optimizer tool, which proposes the optimal values of factors by maximizing both m and Abs and minimizing the energy.

According to this model, the optimum of the process (i.e., maximizing the mass m and the absorbance Abs and minimizing the energy) should be obtained:

For model TC1: V= 8 kV, n = 80 and T = 35.9 µs corresponding to m = 32.7 g, Abs= 0.48 and P =1828.8 J (Figure 11).

For Model TC2: V = 5 kV, n = 70 and T = 8 µs corresponding to m = 40.68 g, Abs= 0.5 and P =167.9 J (Figure 12).

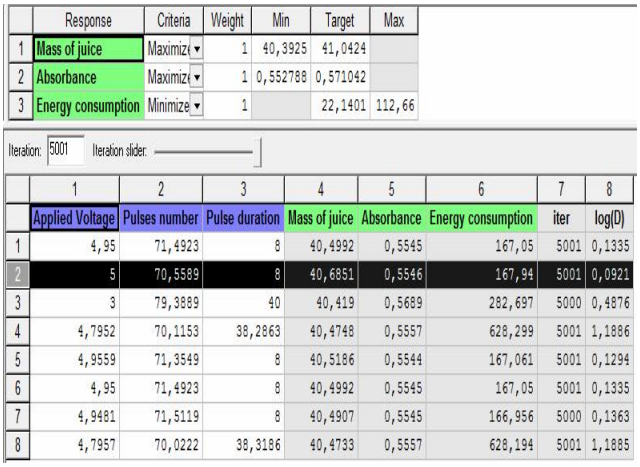


	Response	Criteria	Weight	Min	Target	Max
1	Mass of juice	Maximize	1	32,5058	33,6688	
2	Absorbance	Maximize	1	0,443997	0,457647	
3	Energy consumption	Minimize	1		442,16	833,04

Iteration: 5001 Iteration slider:

	1	2	3	4	5	6	7	8
	Applied Voltage	Pulses number	Pulse duration	Mass of juice	Absorbance	Energy consumption	iter	log(D)
1	6	120	20,0462	24,2611	0,3697	812,528	5000	1,5558
2	8	80	20	29,4673	0,385	973,599	5000	1,1584
3	8	80	35,8965	32,7684	0,4203	1828,83	5000	0,8379
4	8	80	52,9693	33,5777	0,4448	2747,35	5001	1,0752
5	7,9999	80	42,0506	33,3867	0,4308	2159,88	5001	0,8893
6	7,6348	80,0044	20	28,7173	0,3605	888,425	5000	1,3684
7	8	80	52,9639	33,5779	0,4448	2747,06	5001	1,0751
8	8	80	52,9693	33,5777	0,4448	2747,35	5001	1,0752

Figure 11. Optimal values of the factors proposed by MODDE 5.0 for model



	Response	Criteria	Weight	Min	Target	Max
1	Mass of juice	Maximize	1	40,3925	41,0424	
2	Absorbance	Maximize	1	0,552788	0,571042	
3	Energy consumption	Minimize	1		22,1401	112,66

Iteration: 5001 Iteration slider:

	1	2	3	4	5	6	7	8
	Applied Voltage	Pulses number	Pulse duration	Mass of juice	Absorbance	Energy consumption	iter	log(D)
1	4,95	71,4923	8	40,4992	0,5545	167,05	5001	0,1335
2	5	70,5589	8	40,6851	0,5546	167,94	5001	0,0921
3	3	79,3889	40	40,419	0,5689	282,697	5000	0,4876
4	4,7952	70,1153	38,2863	40,4748	0,5557	628,299	5001	1,1886
5	4,9559	71,3549	8	40,5186	0,5544	167,061	5001	0,1294
6	4,95	71,4923	8	40,4992	0,5545	167,05	5001	0,1335
7	4,9481	71,5119	8	40,4907	0,5545	166,956	5000	0,1363
8	4,7957	70,0222	38,3186	40,4733	0,5557	628,194	5001	1,1885

Figure 12. Optimal values of the factors proposed by MODDE 5.0 for model TC2

The obtained results show that the second model with four electrodes is more efficient, because better results in terms of juice mass and absorbance were obtained with less energy. This is explained by the increase of the number of field lines for the model TC2, thus the food product is exposed to more electric field lines than the simple configuration of the model TC1. However, simulation and electric field calculation for the two configurations should be useful for understanding the difference between the two models.

4. Conclusions

The present paper describes an experimental comparative analysis between two square treatment chambers of same dimensions but having either two or four metal electrodes placed on the side walls. For this latter, each pair of the adjacent metal plates form one electrode. The experimental analysis was made by measuring the mass of PEF pretreated extracted juice from beet and the amount of betanine using a spectrophotometer. The obtained results, using the methodology of experimental designs, have shown that the second model with four electrodes is more efficient, because higher quantities of juice and betanine were obtained up to 25% more with an energy saving of nearly 90%.

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EFFECT OF UTILIZATION PARTIAL DRYING BY HOT AIR ON THE CHEMICAL COMPOSITION, RHEOLOGICAL AND MICROBIAL PROPERTIES OF JAMEED

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ABSTRACT

Eleven treatments of jameed were made from sheep butter milk and from goat and cow skim milk. After shaping the jameed paste into balls, control balls (made from sheep butter milk and from goat and cow skim milk) were sun dried for 15 days whereas other balls of goat and cow skim milk jameed were partially dried using hot air at 45ºC/12h, 60ºC/10h, 75ºC/8h and 90ºC/6h then jameed drying process was completed in sun till moisture content of jameed reached to ~20%. Utilization of partially drying after shaping of jameed paste reduced the time of solar drying period. During storage, acidity ratios of sun dried jameed were higher whereas total solids, fat, total protein, ash, salt, water soluble nitrogen and non-protein-nitrogen contents were lower than those of partial dried treatments. Manufacturing of jameed by traditional method (solar drying) increased the numbers of the total viable bacterial count, lactic acid bacteria and proteolytic bacteria as compared with partially dried jameed. Using of high temperatures in partial draying of jameed increased wettability while decreased syneresis values. Also, jameed samples treated with higher temperature of partial drying had the highest levels hardness, cohesiveness, gumminess and chewiness and the lowest levels of springiness. The micrograph images of partially dried jameed treatments showed that protein matrices characterized by little aggregates, plates structure, more open protein network and high fusion. These properties were more obvious with higher temperature heat treatments and in goat skim milk jameed.

1. Introduction

Jameed is a milk product widely used in rural and desert areas in some Arabian countries, such as Jordan, Syria, Northern Saudi Arabia and the western part of Iraq, and it forms a major component of the family diet. This product is also known as *Marees* or *Afig* (Yagil, 1982). Jameed is a dried fermented milk product (a hard cheese-like product), usually prepared from sheep buttermilk. However, buttermilk from other sources such as goat, cow and camel (Yagil, 1982) can also be utilized for preparation of jameed. Normally, jameed is reconstituted by dissolving in seven times its weight of water before consumption.

Drying methods of jameed include a reduction in moisture content to decrease or inhibit growth of pathogenic microorganisms. Salt is added in order to increase the shelf life. Numerous factors associated with environmental and manufacturing conditions play an important role in the shelf life and safety of drying jameed, including high salt content, low water activity, high level of lactic

acid and low pH value (less than 4.0). The presence of lactic acid and salt in jameed reduces and inhibits the growth of pathogenic microorganisms (Alu'datt et al., 2015). The sun drying technique was usually used in jameed drying. It improves the quality of jameed, such as aroma, color, flavor and texture, due to the growth of microorganisms such as mold and bacteria on jameed through the drying technique. Nevertheless, undesirable chemical, physical and biological changes in functional properties may happen during the process of drying jameed. In addition to the traditional sun-drying, other methods can be used. Freeze drying results in 8.9% less moisture in the end product compared to sun drying (Al Omari et al., 2008), and was preferred by consumers (Mazahreh et al., 2008). Therefore, the aim of this work was to investigate the possibility of acceleration of jameed drying process by using hot air and study effect of this technic on the chemical composition, rheological and microbial properties of produced jameed.

2. Materials and Methods

2.1. Milk and Starter Culture

Fresh sheep's, goat's and cow's milks were obtained from El-Serw Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A commercial classic yoghurt starter containing *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) (Chr. Hansen's Lab A/S Copenhagen, Denmark) was used. Starter cultures were in freeze-dried direct-to-vat set form and stored at –18°C until used.

2.2. Jameed manufacture

Eleven treatments of Jameed were made from sheep buttermilk (control) and from goat and cow skim milk according to the traditional method described by Quasem et al., (2009). After shaping the jameed paste into balls, control balls were sun dried for 15 days whereas balls of goat and cow skim milk jameed were placed on trays and partially dried using hot air in electricity oven at different temperatures and times, then jameed drying process was completed in sun till moisture content of jameed reached to ~20%. Generally, jameed samples were as follow:

-Treatment A: Jameed made from sheep butter milk (control) with sun drying (traditional method).
-Treatment B: Jameed made from goat skim milk with sun drying (traditional method).
-Treatment C: Jameed made from goat skim milk and dried at 45ºC/12h.
-Treatment D: Jameed made from goat skim milk and dried at 60ºC/10h.
-Treatment E: Jameed made from goat skim milk and dried at 75ºC/8h.
-Treatment F: Jameed made from goat skim milk and dried at 90ºC/6h.
-Treatment G: Jameed made from cow skim milk with sun drying (traditional method).
-Treatment H: Jameed made from cow skim milk and dried at 45ºC/12h.
-Treatment I: Jameed made from cow skim milk and dried at 60ºC/10h.
-Treatment J: Jameed made from cow skim milk and dried at 75ºC/8h.
-Treatment K: Jameed made from cow skim milk and dried at 90ºC/6h.
-The dried jameed balls were packaged in cloth bags which were put in plastic containers and stored at room temperature for six months. Samples were analyzed when fresh (jameed curd) and after 15, 30, 60, 90, 120, 150 and 180 days of storage period.

2.3. Chemical analyses

Jameed yield was calculated by two means as follows:

Yield-1 (%) = Weight of jameed at the end of storage /Weight of milk used to make jameed x 100 (1)

Yield-2 (%) = Weight of jameed at the end of storage /Weight of jameed paste (before drying) x 100 (2)

Total solids, fat, total nitrogen and ash contents of samples were determined according

to (AOAC, 2000). Titratable acidity in terms of % lactic acid was measured by titrating 10g of sample mixed with 10ml of boiling distilled water against 0.1 N NaOH using a 0.5% phenolphthalein indicator to an end point of faint pink color (Parmar, 2003). pH of the sample was measured at 17 to 20°C using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Water soluble nitrogen (WSN) and non-protein-nitrogen of jameed were estimated according to Ling (1963). The Volhard's method as described by Richardson (1985) was used to determine the salt content of jameed. Salt in moisture percentage of the cheese was estimated as follow:

(Salt percentage x 100) / (Moisture percentage + Salt percentage) (3)

2.4. Microbiological Analyses

Jameed samples were analyzed for total viable bacterial count (TVBC), lactic acid bacteria (LAB), coliform, proteolytic bacteria, moulds and yeast counts according to the methods described by the American Public Health Association (1992).

2.5. Rheological Properties

Force and torque measurements of jameed treatments stored for six months were measured using a Texturometer model Mecmesin Emperor ^{TM}Lite 1.17(USA). Mechanical primary characteristics of hardness, springiness, gumminess and cohesiveness and also the secondary characteristic of chewiness (hardness x cohesiveness x springiness) were determined from the deformation Emperor ^{TM}Lite Graph. Because jameed samples were very hard, they were soaked in distilled water for 6h at room temperature before measurements.

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2.6. Wettability (Diffusability) test

A cube weighing ca. 45 g of jameed was cut using a hand saw from a whole jameed ball; 315 ml water were added to the piece placed in 500 ml cup and soaked for 24 h (Quasem et al., 2009). The excess free water was carefully decanting weighed to calculate the soaked amount as follows:

Absorbed water (%) = 315 ml water-X /Weight of cubs (g) x 100 (4)

where; X: the weight of excess water (g).

2.7. Syneresis (whey separation) test

The soaked cube (45 g) was mixed with (315 ml water) for two minutes using electrical hand mixer (Hinari, model FM2, China) with the whipping accessory. The dispersed jameed was transferred to a 100 ml graduated cylinder and the clear zone was measured after 1h and 24h (Quasem et al., 2009). Syneresis (whey separation) was calculated, as follow:

Syneresis (%) = X /Y x 100 (5)

where; X: The height of the clear zone. Y: Total height of jameed dispersion.

2.8. Scanning Electron Microscopy (SEM) Examination

Jameed samples were prepared for SEM according to the method of Brooker and Wells (1984). The specimens were viewed in a scanning electron microscope (JXA-840A Electron Probe Microanalyzer-JEOL-Japan) after dehydrated using Critical Point Dried instrument and coating with gold using S150A Sputter Coater-Edwards England.

2.9. Statistical Analysis

The obtained results were statistically analyzed using a software package (SAS, 1991) based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan (1955) for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

3. Results and Discussion

3.1. Chemical composition of milk used in jameed manufacture

Data in Table 1 show the differences in chemical properties of milk used in jameed manufacturing. Because of fermentation process, sheep butter milk had the highest acidity content and the lowest pH values. On the contrary, total solids (TS) and solids-not-fat (SNF) levels were higher in goat and cow skim milk than that of sheep butter milk. Because fat globules of goat's milk don't easily separate by separator as occurred in cow's milk, fat concentration of goat skim milk was the highest as compared with those found in sheep buttermilk or cow skim milk. Sheep butter milk is richer in protein than goat or cow skim milk.

Table 1. Chemical composition of milk used in jameed manufacture

Treatments	Acidity %	pH values	TS %	Fat %	Total Protein %	SNF %
Sheep buttermilk	0.99^{a}	5.92^{b}	7.81^{b}	0.7^{a}	5.10^{a}	6.50^{b}
Goat skim milk	0.16^{b}	6.61^{a}	9.88^{a}	0.9^{a}	3.12^{b}	8.98^{a}
Cow skim milk	0.18^{b}	6.58^{a}	9.40^{a}	0.3^{b}	3.01^{b}	9.10^{a}

^{abcde} Letters indicate significant differences between milk treatments

Table 2. Moisture content of jameed samples during solar drying process

Treatments	Solar drying process (days)							
	Fresh	3	5	7	9	11	13	15
A	51.33^{c}	43.72^{c}	37.37^{c}	32.68^{b}	28.89^{b}	24.16^{b}	21.56^{b}	18.00^{b}
B	68.11^{a}	56.68^{a}	49.14^{a}	42.02^{a}	37.16^{a}	31.56^{a}	27.47^{a}	24.21^{a}
C	33.20^{d}	30.08^{d}	27.90^{d}	25.42^{cd}	23.25^{c}	21.30^{c}	19.04^{c}	-
D	31.00^{e}	28.33^{e}	25.00^{e}	22.08^{d}	20.10^{e}	-	-	-
E	28.77^{f}	26.07^{f}	24.98^{e}	21.15^{de}	19.37^{e}	-	-	-
F	27.00^{g}	25.73^{fg}	22.06^{f}	20.07^{e}	-	-	-	-
G	65.86^{b}	54.12^{b}	47.88^{b}	41.67^{a}	36.53^{a}	31.23^{a}	26.76^{a}	23.76^{a}
H	32.60^{de}	30.66^{d}	28.05^{d}	26.34^{c}	22.74^{d}	19.58^{d}	-	-
I	30.03^{ef}	27.80^{e}	24.77^{e}	21.03^{de}	19.94^{e}	-	-	-
J	27.64^{fg}	25.33^{fg}	23.96^{ef}	20.69^{e}	-	-	-	-
K	26.33^{g}	24.05^{g}	22.11^{f}	20.08^{e}	-	-	-	-

^{abcde} Letters indicate significant differences between milk treatments

Table 3. Effect of using partial drying on jameed yield

Treatments	Yield-1	Yield-2
A	16.03[a]	54.66[a]
B	7.57[b]	39.33[b]
C	7.22[b]	37.69[c]
D	7.19[b]	37.56[c]
E	7.18[b]	37.50[c]
F	7.14[b]	37.29[c]
G	6.80[d]	40.84[b]
H	6.61[c]	39.58[b]
I	6.60[c]	39.47[b]
J	6.57[c]	39.33[b]
K	6.55[c]	39.17[b]

[abcde] Letters indicate significant differences between milk treatments

Table 4. Effect of using partial drying on some physicochemical properties of jameed

Properties	Treatments	Storage period (days)								Means
		Fresh	15	30	60	90	120	150	180	
Acidity %	A	2.05	3.48	4.11	4.46	4.78	5.01	5.25	5.36	4.31[a]
	B	1.82	2.78	3.27	3.58	3.88	4.07	4.30	4.40	3.51[ab]
	C	1.91	2.75	3.24	3.55	3.81	4.03	4.22	4.33	3.48[ab]
	D	1.83	2.61	3.05	3.32	3.54	3.77	3.98	4.08	2.98[b]
	E	1.71	2.42	2.77	3.01	3.21	3.40	3.59	3.70	2.82[b]
	F	1.66	2.27	2.60	2.84	3.04	3.25	3.42	3.54	3.25[b]
	G	1.72	2.60	3.10	3.38	3.62	3.83	3.96	4.08	3.29[b]
	H	1.78	2.58	3.07	3.34	3.57	3.73	3.92	4.02	3.03[b]
	I	1.73	2.47	2.84	3.08	3.30	3.45	3.62	3.71	2.92[b]
	J	1.68	2.39	2.71	2.97	3.18	3.35	3.51	3.60	2.78[b]
	K	1.60	2.25	2.52	2.75	2.97	3.16	3.31	3.42	2.75[b]
	Means	1.77[D]	2.60[CD]	3.02[CB]	3.25[CAB]	3.48[CAB]	3.68[AB]	3.87[AB]	3.97[A]	
pH values	A	4.98	4.43	4.19	3.91	3.70	3.55	3.41	3.36	3.94[a]
	B	5.32	4.61	4.45	4.32	4.11	4.05	3.96	3.87	4.34[a]
	C	5.23	4.64	4.49	4.36	4.18	4.11	4.01	3.92	4.37[a]
	D	5.29	4.73	4.57	4.42	4.30	4.23	4.14	4.01	4.46[a]
	E	5.41	4.84	4.68	4.59	4.47	4.39	4.31	4.25	4.62[a]
	F	5.47	4.91	4.76	4.64	4.55	4.49	4.40	4.30	4.69[a]
	G	5.41	4.71	4.49	4.38	4.32	4.23	4.18	4.06	3.94[a]
	H	5.33	4.77	4.55	4.41	4.36	4.29	4.23	4.11	4.50[a]
	I	5.39	4.81	4.62	4.55	4.44	4.36	4.28	4.24	4.59[a]
	J	5.45	4.88	4.67	4.60	4.50	4.41	4.36	4.31	4.65[a]
	K	5.53	4.95	4.77	4.69	4.60	4.54	4.45	4.40	4.74[a]
	Means	5.34[A]	4.77[AB]	4.59[AB]	4.46[AB]	4.34[B]	4.26[B]	4.18[B]	4.10[B]	
TS %	A	48.67	82.00	84.95	86.12	87.08	87.87	88.58	89.06	81.79[e]
	B	31.89	75.79	78.14	79.10	81.11	82.15	82.21	82.95	74.17[f]
	C	66.80	81.75	83.24	84.30	84.74	84.90	85.17	85.44	82.04[de]
	D	69.00	81.94	83.60	84.57	84.87	85.06	85.24	85.75	82.50[dce]
	E	71.23	82.55	83.95	84.85	85.23	85.41	85.68	85.89	83.09[cb]
	F	73.00	83.02	84.19	85.34	85.62	85.95	86.11	86.36	83.69[ab]
	G	34.14	76.24	79.12	80.26	81.33	82.46	82.97	83.78	75.04[f]
	H	67.40	81.82	83.79	84.90	85.11	85.31	85.47	85.69	82.43[dce]
	I	69.97	82.88	83.96	85.14	85.31	85.49	85.72	85.94	83.00[dcb]
	J	72.36	83.38	84.08	85.25	85.56	85.74	85.97	86.23	83.57[ab]
	K	73.67	83.82	84.79	85.72	85.91	86.15	86.34	86.59	84.12[a]
	Means	68.01[E]	82.57[D]	84.06[C]	85.13[B]	85.49[AB]	85.76[AB]	86.03[AB]	86.27[A]	

Fat %	A	3.85	10.40	10.64	10.87	11.05	11.14	11.23	11.35	10.06^{ced}
	B	4.19	11.36	11.57	11.71	11.84	11.98	12.20	12.35	10.90^{cadb}
	C	7.30	10.98	11.03	11.33	11.51	11.61	11.74	11.80	10.90^{cadb}
	D	7.43	11.05	11.28	11.45	11.50	11.65	11.78	11.84	11.00^{cab}
	E	7.50	11.14	11.42	11.50	11.56	11.70	11.84	11.91	11.07^{ab}
	F	7.71	11.19	11.51	11.58	11.64	11.72	11.90	12.01	11.16^{a}
	G	3.17	9.90	9.95	10.19	10.31	10.40	10.49	10.60	9.75^{e}
	H	6.35	10.05	10.25	10.32	10.40	10.49	10.56	10.62	9.88^{e}
	I	6.50	10.10	10.37	10.40	10.43	10.52	10.60	10.66	9.95^{ed}
	J	6.64	10.18	10.44	10.47	10.54	10.55	10.62	10.71	10.0^{ced}
	K	6.83	10.27	10.51	10.60	10.64	10.69	10.70	10.75	10.1^{cedb}
	Means	6.68^{B}	10.59^{A}	10.82^{A}	10.94^{A}	11.03^{A}	11.11^{A}	11.21^{A}	11.29^{A}	

^{abcde} Letters indicate significant differences between jameed treatments
^{ABCD} Letters indicate significant differences between storage times

Table 5. Effect of using partial drying on some chemical properties of jameed

Properties	Treatments	Storage period (days)								Means
		Fresh	15	30	60	90	120	150	180	
Total protein %	A	29.55	51.13	53.05	53.16	53.31	53.61	53.70	53.81	50.17^{e}
	B	14.95	43.91	47.01	47.34	47.45	47.66	47.78	47.92	43.00^{g}
	C	43.78	51.36	52.50	52.90	53.05	53.15	53.25	53.32	51.66^{d}
	D	45.98	51.48	52.77	53.21	53.33	53.46	53.56	53.60	52.17^{cd}
	E	48.62	52.10	53.20	53.48	53.62	53.69	53.77	53.82	52.79^{cab}
	F	50.46	52.45	53.38	53.62	53.75	53.84	53.97	54.04	53.19^{ab}
	G	17.24	47.11	49.23	49.54	49.69	49.87	49.99	50.28	45.37^{f}
	H	44.89	52.11	53.10	53.23	53.37	53.53	53.61	53.67	52.19^{cab}
	I	48.30	52.61	53.36	53.54	53.67	53.74	53.80	53.88	52.79^{cab}
	J	50.11	52.24	53.57	53.70	53.84	53.96	54.11	54.18	53.21^{a}
	K	51.74	52.64	53.79	53.92	54.08	54.17	54.29	54.37	53.63^{a}
	Means	45.94^{C}	52.01^{B}	53.13^{A}	53.42^{A}	53.56^{A}	53.68^{A}	53.78^{A}	53.85^{A}	
Ash %	A	11.50	14.87	14.95	15.38	15.59	15.81	16.04	16.14	15.03^{a}
	B	10.14	13.57	13.81	13.97	14.31	14.47	14.60	14.74	13.70^{b}
	C	12.33	14.38	14.45	14.52	14.65	14.83	14.98	15.13	14.40^{a}
	D	12.37	14.49	14.52	14.61	14.80	14.97	15.17	15.29	14.52^{a}
	E	12.40	14.54	14.60	14.66	14.84	15.04	15.23	15.30	14.57^{a}
	F	12.50	14.62	14.68	14.72	14.93	15.16	15.31	15.42	14.67^{a}
	G	9.97	13.30	13.57	13.69	13.94	14.27	14.49	14.64	13.48^{b}
	H	12.38	14.40	14.44	14.50	14.66	14.73	14.97	15.14	14.40^{a}
	I	12.40	14.53	14.55	14.65	14.79	14.93	15.20	15.31	14.54^{a}
	J	12.45	14.58	14.65	14.70	14.87	15.06	15.30	15.37	14.62^{a}
	K	12.56	14.66	14.72	14.80	14.98	15.21	15.41	15.49	14.72^{a}
	Means	12.32^{B}	14.56^{A}	14.61^{A}	14.72^{A}	14.90^{A}	15.08^{A}	15.29^{A}	15.39^{A}	
Salt %	A	7.02	10.23	10.58	10.62	10.78	10.87	10.95	11.07	10.27^{b}
	B	6.40	9.72	10.11	10.25	10.39	10.45	10.51	10.57	9.80^{b}
	C	8.80	10.81	11.03	11.25	11.40	11.47	11.51	11.55	10.98^{ab}
	D	9.09	11.25	11.33	11.40	11.48	11.53	11.58	11.70	11.17^{ab}
	E	9.20	11.39	11.49	11.55	11.60	11.67	11.71	11.79	11.30^{a}
	F	9.35	11.57	11.69	11.75	11.79	11.84	11.87	11.94	11.47^{a}
	G	5.88	9.61	9.70	10.07	10.12	10.20	10.33	10.42	9.54^{b}
	H	8.84	10.94	11.18	11.40	11.48	11.57	11.62	11.68	11.08^{ab}
	I	9.13	11.37	11.49	11.59	11.65	11.70	11.73	11.81	11.31^{a}
	J	9.33	11.50	11.66	11.74	11.80	11.82	11.86	11.93	11.46^{a}
	K	9.55	11.71	11.87	11.91	11.95	11.98	12.04	12.14	11.64^{a}
	Means	8.92^{B}	11.19^{A}	11.36^{A}	11.46^{A}	11.54^{A}	11.60^{A}	11.65^{A}	11.73^{A}	
	A	12.03	36.23	41.27	43.34	45.48	47.26	48.94	50.29	40.60^{dc}
	B	8.59	28.65	31.62	33.01	35.48	36.93	37.14	38.27	31.21^{f}
	C	20.95	37.19	39.69	41.74	42.76	43.16	43.69	44.23	39.18^{e}

Salt in moisture %	D	22.67	38.39	40.85	42.48	43.14	43.55	43.96	45.08	40.02^{de}
	E	24.22	39.49	41.72	43.25	43.98	44.44	44.98	45.52	40.95^{dc}
	F	25.72	40.52	42.50	44.49	45.05	45.73	46.07	45.72	41.98^{b}
	G	8.20	28.80	31.72	33.79	35.41	36.78	37.75	39.11	31.44^{f}
	H	21.33	37.56	40.82	43.01	43.53	44.05	44.43	44.94	39.96^{de}
	I	23.31	39.90	41.73	43.92	44.22	44.63	45.09	45.65	41.05^{bc}
	J	25.23	40.89	42.27	44.31	45.48	45.32	45.80	46.42	41.96^{b}
	K	26.62	41.98	43.83	45.47	45.89	46.38	46.85	47.51	43.06^{a}
	Means	22.45^{G}	39.12^{F}	41.63^{E}	43.55^{D}	44.38^{CD}	44.94^{CD}	45.53^{AB}	46.15^{A}	

^{abcde} Letters indicate significant differences between jameed treatments
^{ABCD} Letters indicate significant differences between storage times

Table 6. Effect of using partial drying on some nitrogen fractions of jameed

Properties	Treatments	Storage period (days)								Means
		Fresh	15	30	60	90	120	150	180	
WSN %	A	0.468	1.401	1.435	1.463	1.478	1.492	1.510	1.521	1.353^{a}
	B	0.450	1.187	1.194	1.222	1.237	1.245	1.260	1.272	1.133^{ab}
	C	0.884	1.263	1.284	1.308	1.319	1.330	1.342	1.350	1.264^{a}
	D	0.871	1.255	1.275	1.294	1.304	1.315	1.324	1.331	1.246^{a}
	E	0.865	1.247	1.269	1.284	1.291	1.301	1.311	1.320	1.242^{a}
	F	0.858	1.240	1.255	1.270	1.277	1.284	1.293	1.301	1.227^{a}
	G	0.441	1.159	1.177	1.193	1.206	1.217	1.230	1.242	1.108^{b}
	H	0.867	1.249	1.266	1.284	1.297	1.305	1.314	1.320	1.238^{a}
	I	0.855	1.241	1.259	1.272	1.280	1.284	1.291	1.301	1.236^{a}
	J	0.847	1.235	1.250	1.263	1.268	1.271	1.280	1.286	1.214^{a}
	K	0.842	1.224	1.237	1.250	1.257	1.260	1.266	1.272	1.213^{a}
	Means	0.81^{A}	1.27^{A}	1.28^{A}	1.29^{A}	1.30^{A}	1.31^{A}	1.33^{A}	1.33^{A}	
WSN/TN %	A	10.10	17.49	17.26	17.56	17.70	17.76	17.95	18.04	16.73^{a}
	B	19.23	17.25	16.20	16.44	16.55	16.67	16.82	16.94	17.01^{a}
	C	12.88	15.68	15.62	15.77	15.87	15.96	16.09	16.16	15.50^{b}
	D	12.09	15.57	15.41	15.51	15.61	15.71	15.78	15.84	15.19^{bc}
	E	11.35	15.28	15.23	15.32	15.36	15.46	15.57	15.65	14.90^{bc}
	F	10.86	15.08	15.01	15.11	15.16	15.23	15.30	15.36	14.66^{bc}
	G	16.33	15.70	15.24	15.37	15.48	15.56	15.70	15.76	15.64^{b}
	H	12.33	15.30	15.21	15.39	15.51	15.55	15.64	15.69	15.08^{bc}
	I	11.29	15.06	15.05	15.16	15.21	15.32	15.31	15.41	14.72^{bc}
	J	10.78	15.09	14.89	15.02	15.04	15.05	15.09	15.14	14.51^{bc}
	K	10.39	14.83	14.67	14.79	14.84	14.84	14.89	14.92	14.27^{c}
	Means	11.34^{B}	15.37^{A}	15.49^{A}	15.51^{A}	15.59^{A}	15.65^{A}	15.76^{A}	15.80^{A}	
NPN %	A	0.089	0.271	0.280	0.290	0.298	0.310	0.317	0.325	0.272^{a}
	B	0.078	0.240	0.249	0.256	0.261	0.269	0.273	0.278	0.238^{a}
	C	0.141	0.256	0.262	0.266	0.271	0.277	0.284	0.288	0.255^{a}
	D	0.133	0.244	0.248	0.252	0.257	0.263	0.266	0.273	0.245^{a}
	E	0.125	0.235	0.240	0.246	0.256	0.261	0.265	0.270	0.237^{a}
	F	0.108	0.221	0.225	0.230	0.237	0.242	0.247	0.252	0.220^{a}
	G	0.077	0.250	0.259	0.266	0.273	0.279	0.285	0.293	0.248^{a}
	H	0.132	0.250	0.256	0.260	0.266	0.271	0.274	0.278	0.245^{a}
	I	0.121	0.234	0.237	0.240	0.245	0.250	0.253	0.256	0.229^{a}
	J	0.110	0.230	0.240	0.244	0.247	0.253	0.257	0.260	0.230^{a}
	K	0.102	0.210	0.213	0.219	0.222	0.226	0.229	0.234	0.206^{a}
	Means	0.11^{B}	0.23^{A}	0.24^{A}	0.25^{A}	0.26^{A}	0.26^{A}	0.27^{A}	0.27^{A}	
NPN/TN %	A	1.92	3.38	3.36	3.48	3.56	3.69	3.76	3.85	3.38^{a}
	B	3.33	3.49	3.38	3.45	3.51	3.60	3.64	3.70	3.51^{a}
	C	2.05	3.18	3.19	3.20	3.26	3.32	3.40	3.44	3.13^{a}
	D	1.84	3.02	2.99	3.02	3.07	3.14	3.17	3.25	2.94^{a}
	E	1.64	2.87	2.88	2.93	3.04	3.10	3.14	3.20	2.91^{a}
	F	1.36	2.68	2.69	2.73	2.81	2.87	2.92	2.97	2.63^{a}

	G	2.59	3.39	3.35	3.42	3.50	3.57	3.64	3.72	3.40^{a}
	H	1.87	3.06	2.70	2.75	2.83	2.88	2.94	2.99	2.75^{a}
	I	1.59	2.83	2.83	2.86	2.91	2.98	3.00	3.03	2.75^{a}
	J	1.40	2.81	2.86	2.90	2.93	2.99	3.03	3.06	2.75^{a}
	K	1.25	2.54	2.52	2.59	2.62	2.66	2.69	2.74	2.45^{a}
	Means	1.65^{B}	2.89^{A}	2.93^{A}	2.94^{A}	3.06^{A}	3.07^{A}	3.11^{A}	3.17^{A}	

^{abcde} Letters indicate significant differences between jameed treatments

^{ABCD} Letters indicate significant differences between storage times

Table 7. Effect of using partial drying on some microbial groups of jameed

Properties	Treatments	Storage period (days)								Means
		Fresh	15	30	60	90	120	150	180	
TVBC ($x\ 10^3$)	A	67	35	29	22	18	15	13	10	26.12^{a}
	B	58	25	20	15	14	13	9	7	20.12^{b}
	C	63	31	25	18	14	10	7	5	21.62^{b}
	D	49	28	17	13	11	7	4	2	16.38^{d}
	E	27	15	12	10	8	5	2	0.8	9.98^{e}
	F	12	8	6	5	2	0.9	0.5	0.14	4.32^{g}
	G	50	18	17	15	10	8	6	5	16.12^{d}
	H	57	27	21	13	11	6	3	0.90	17.36^{c}
	I	37	21	11	5	3	0.9	0.6	0.3	9.85^{e}
	J	18	12	10	3	2	0.9	0.5	0.2	5.83^{f}
	K	10	7	3	0.9	0.6	0.4	0.1	0.08	2.76^{h}
	Means	37.7^{A}	20.4^{B}	14.89^{C}	9.98^{D}	7.73^{E}	5.12^{F}	3.41^{G}	2.15^{H}	
Lactic acid bacteria ($x\ 10^3$)	A	55	28	21	16	13	10	9	8	20.0^{a}
	B	44	20	15	10	9	8	6	3	14.4^{c}
	C	53	27	22	15	11	8	5	2	17.8^{b}
	D	32	23	15	10	9	6	3	0.9	12.3^{d}
	E	25	12	10	7	6	4	1	0.6	8.20^{e}
	F	9	6	5	3	0.9	0.6	0.3	0.08	3.11^{g}
	G	38	16	13	10	9	5	3	0.6	11.8^{d}
	H	49	23	17	10	8	4	2	0.7	14.2^{c}
	I	29	19	10	4	1	0.7	0.3	0.09	8.01^{e}
	J	14	10	8	0.8	0.6	0.6	0.1	0.08	4.27^{f}
	K	8	5	1	0.6	0.3	0.1	0.07	0.04	1.82^{h}
	Means	30.4^{A}	17.0^{B}	12.1^{C}	7.31^{D}	5.53^{E}	3.78^{F}	2.30^{G}	1.39^{H}	
Proteolytic bacteria ($x\ 10^3$)	A	6	0.9	0.7	0.3	0.10	0.08	0.05	0.05	1.06^{a}
	B	4	0.6	0.4	0.07	0.06	0.04	0.03	0.01	0.65^{cab}
	C	5	1	0.6	0.3	0.09	0.07	0.03	0.01	0.89^{a}
	D	4	0.8	0.4	0.1	0.06	0.04	0.01	0.007	0.68^{cab}
	E	2	0.5	0.2	0.08	0.04	0.01	0.008	0.007	0.36^{cdb}
	F	2	0.3	0.1	0.05	0.02	0.008	0.006	0.005	0.31^{cd}
	G	3	0.2	0.09	0.08	0.05	0.04	0.03	0.01	0.44^{cdb}
	H	4	1	0.5	0.2	0.07	0.03	0.009	0.005	0.73^{ab}
	I	2	0.6	0.3	0.1	0.04	0.03	0.008	0.006	0.39^{cdb}
	J	1	0.6	0.2	0.07	0.04	0.008	0.006	0.003	0.24^{d}
	K	1	0.4	0.09	0.05	0.01	0.006	0.004	0.002	0.19^{d}
	Means	3.00^{A}	0.68^{B}	0.34^{BC}	0.13^{C}	0.05^{C}	0.04^{C}	0.03^{C}	0.01^{C}	
Mould & Yeast ($x10^3$)	A	0	0	0	0	0.3	0.4	0.7	0.9	0.29^{a}
	B	0	0	0	0	0.1	0.3	0.6	0.8	0.22^{b}
	C	0	0	0	0	0.2	0.3	0.4	0.7	0.20^{b}
	D	0	0	0	0	0.2	0.2	0.4	0.5	0.16^{b}
	E	0	0	0	0	0	0.1	0.2	0.3	0.08^{c}
	F	0	0	0	0	0	0.1	0.1	0.2	0.05^{c}
	G	0	0	0	0	0.09	0.2	0.3	0.5	0.14^{b}
	H	0	0	0	0	0.1	0.3	0.4	0.6	0.18^{b}
	I	0	0	0	0	0.1	0.3	0.4	0.6	0.18^{b}

	J	0	0	0	0	0	0.1	0.2	0.3	0.08^{c}
	K	0	0	0	0	0	0.1	0.2	0.2	0.06^{c}
	Means	0.00^{E}	0.00^{E}	0.00^{E}	0.00^{E}	0.10^{D}	0.21^{C}	0.33^{B}	0.48^{A}	

^{abcde} Letters indicate significant differences between jameed treatments
^{ABCD} Letters indicate significant differences between storage times

Table 8. Effect of using partial drying on wettability and syneresis of jameed

Properties	Treatments	Storage period (days)							Means
		15	30	60	90	120	150	180	
Wettability (%)	A	210.85	220.73	225.22	227.12	228.79	230.91	233.34	225.28^{a}
	B	196.48	201.36	210.89	215.78	218.33	219.14	221.97	210.71^{cd}
	C	190.67	194.76	197.55	199.87	201.90	202.45	204.30	198.79^{e}
	D	203.98	209.12	211.96	214.45	215.33	216.85	218.17	212.83^{cb}
	E	213.00	219.33	222.09	223.89	225.89	227.01	228.55	222.82^{ab}
	F	220.71	226.65	228.33	229.56	230.98	231.87	233.49	227.31^{a}
	G	191.85	202.88	210.04	212.87	217.23	218.20	219.09	210.31^{cd}
	H	177.43	182.63	186.55	188.94	189.76	190.21	191.72	186.74^{f}
	I	180.88	188.89	194.09	197.55	200.62	201.98	203.30	195.33^{ef}
	J	188.67	195.33	199.07	204.77	206.03	207.74	208.48	201.44^{ed}
	K	197.43	203.63	208.55	213.95	214.72	215.64	216.88	210.11^{cd}
	Means	197.07^{C}	204.56^{BC}	208.16^{AB}	211.12^{AB}	212.67^{AB}	213.85^{AB}	215.31^{A}	
Syneresis % (after 1h of mixing with water)	A	39.84	47.87	48.03	51.97	54.67	55.84	57.22	50.78^{c}
	B	42.74	47.87	50.45	53.14	56.49	57.66	58.49	52.40^{c}
	C	44.48	50.04	51.00	55.80	59.93	61.12	63.66	55.00^{b}
	D	41.66	45.25	47.90	53.54	55.93	58.34	60.06	51.81^{c}
	E	37.24	43.33	46.94	52.24	54.72	55.98	58.97	49.20^{d}
	F	32.04	37.10	40.80	48.93	50.66	53.47	57.89	45.62^{e}
	G	44.31	50.80	52.01	55.13	57.24	57.99	59.24	53.82^{c}
	H	53.44	56.08	57.82	59.09	61.83	63.75	65.90	59.55^{a}
	I	51.34	54.89	56.70	59.13	60.97	62.15	63.40	58.36^{a}
	J	47.21	50.90	53.07	55.67	59.81	61.56	62.22	55.78^{b}
	K	42.09	45.88	46.03	51.87	56.42	57.96	59.33	51.36^{c}
	Means	43.26^{G}	47.81^{F}	49.81^{E}	54.14^{D}	56.54^{C}	58.96^{B}	60.85^{A}	
Syneresis % (after 24h of mixing with water)	A	42.42	50.00	50.11	54.65	57.14	60.12	63.16	54.08^{cab}
	B	44.43	51.62	52.30	54.31	57.27	60.14	63.38	54.79^{cab}
	C	46.90	51.30	53.79	57.88	60.30	62.54	64.11	56.68^{ab}
	D	44.89	48.00	52.23	56.68	58.32	60.21	63.80	54.80^{cab}
	E	40.13	45.87	50.81	53.92	56.10	58.37	60.22	52.20^{cb}
	F	34.30	40.79	44.88	49.30	52.11	54.37	59.89	47.95^{c}
	G	47.15	53.25	54.23	56.97	59.41	61.20	64.24	56.63^{ab}
	H	54.98	57.24	58.21	60.91	63.15	64.88	66.11	60.78^{a}
	I	52.03	55.18	57.00	59.56	60.99	63.56	65.46	59.11^{ab}
	J	50.78	53.54	55.84	56.06	59.98	62.74	63.88	57.55^{ab}
	K	44.75	50.48	51.97	53.34	57.70	59.21	61.00	53.97^{cab}
	Means	45.79^{D}	50.27^{CD}	52.69^{BCD}	55.76^{ABC}	58.42^{AB}	60.67^{A}	63.07^{A}	

^{abcde} Letters indicate significant differences between jameed treatments
^{ABCD} Letters indicate significant differences between storage times

Table 9. Textural properties of jameed at the end of storage period

Treatments	Hardness (N)	Cohesiveness (B/A area)	Springiness (mm)	Gumminess (N)	Chewiness (N/mm)
A	22.10^{f}	0.309^{a}	1.497^{a}	6.846^{g}	4.573^{d}
B	14.72^{g}	0.153^{b}	0.757^{f}	3.266^{h}	6.410^{cd}
C	25.30^{e}	0.347^{a}	1.124^{b}	7.464^{gf}	4.809^{d}
D	33.00^{d}	0.389^{a}	0.875^{d}	10.256^{ef}	5.875^{cd}
E	37.40^{c}	0.420^{a}	0.824^{e}	14.421^{cd}	6.325^{cdb}
F	41.90^{ab}	0.522^{a}	0.523^{h}	17.621^{ab}	9.493^{ab}
G	15.66^{g}	0.172^{b}	0.628^{g}	4.371^{h}	6.860^{cd}
H	30.31^{d}	0.361^{a}	0.956^{c}	11.160^{ed}	5.470^{d}
I	37.45^{c}	0.410^{a}	0.830^{e}	14.338^{cd}	6.306^{cd}
J	39.60^{cb}	0.452^{a}	0.612^{g}	16.545^{cb}	8.963^{cab}
K	43.00^{a}	0.531^{a}	0.499^{h}	19.942^{a}	9.966^{a}

^{abcde} Letters indicate significant differences between jameed treatments

3.2. Changes in moisture content of jameed during solar drying process

After partially drying of jameed treatments at different temperatures and times in electricity oven, drying process was completed in sun till moisture content of jameed reached to ~20%. To determine the end of solar drying stage, the moisture contents were followed in jameed paste and after 3, 5, 7, 9, 11, 13 and 15 days of solar drying period. Results are shown in Table 2. Moisture values of jameed made from goat or cow skim milk and dried in sun (samples B and G respectively) were higher than jameed prepared from sheep butter milk with sun drying (sample A). As expected, moisture levels at the beginning and within drying period of samples A, B and G were higher than other treatments. Utilization of partially drying after shaping of jameed paste significantly ($P<0.05$) reduced moisture concentrations. The reduction rates were proportional with increasing of drying temperatures. Consequently, samples of goat skim milk jameed dried at 90ºC/6h (sample F) reached to ~20% moisture at the seventh day of drying period while samples dried at 75ºC/8h, 60ºC/10h and 45ºC/12h reached after 9, 9 and 13 days of drying respectively. The same trend but with faster levels was observed in cow skim milk jameed. Treatments dried at 90ºC/6h, 75ºC/8h, 60ºC/10h and 45ºC/12h (samples K, J, I, and H respectively) recoded 20% moisture after 7, 7, 9 and 11 days of drying stage. This main that the time of solar drying period reduced by 53.33, 53.33, 40.00 and 26.67% for the mentioned above samples respectively. Moisture levels were a little bit lower in cow skim milk jameed than that of goat skim milk one. Generally, the highest levels of moisture losing were noticed in the first five days of drying process.

3.3. Yield of jameed

Cheese yield is defined as the amount of cheese, expressed in kilograms, obtained from 100 kg of milk. It is a very important parameter: the higher the recovered percentage of solids, the greater is the amount of cheese obtained and therefore gains in economic terms (Abd El-Gawad and Ahmed, 2011). Data of the obtained yield (Table 3) show that very higher differences could be detected between jameed treatments made from sheep butter milk and goat or cow skim milk using solar drying (samples A, B and G respectively). The values of yield -1 or 2 of sample A were significantly ($P<0.05$) higher than those of samples B and G which may be due to the high total protein content of sheep butter milk. Hilali (2001) and Park et al., (2007) stated that proteins account for approximately 96% of the total N in sheep milk, with 4% being non-protein nitrogen (NPN). The level of NPN in goat milk is double that of sheep milk. This difference affects cheese yield which is higher in sheep milk.

The yield-1 values of partial draying jameed were similar to that of solar drying whereas levels of yield-2 were slightly higher in the latter than the former. Also, no remarkable changes were noted in jameed yield between various heat treatments of partial drying. Goat skim milk jameed possessed slightly higher yield-1 values than jameed manufactured from cow skim milk. An altogether opposite trend was observed for yield-2. Yield-1 values of samples B, C, D, G, H and I were 7.57, 7.22, 7.19, 6.80, 6.61 and 6.60% respectively. Respective values of yield-2 were 39.33, 37.69, 37.56, 40.84, 39.58 and 39.47% respectively.

3.4. Chemical composition of jameed during storage period

The changes in the titratable acidity (% lactic acid), pH, total solids (TS) and fat contents during storage of jameed are presented in Table 4. The values of titratable acidity gradually increased during storage of all samples of jameed. The greatest increasing levels were occurred in the first month of storage. The results of the pH values followed an opposite trend to that observed for titratable acidity measurements, i.e., as the acidity increased, the pH decreased. This may be due to fermentation of lactose, which produces lactic and acetic acid during fermentation and storage period. Because of higher acidity of sheep butter milk, it is normal that jameed prepared from it had the highest acidity values comparing with jameed made from goat or cow skim milk. Moreover, the rises rates in titratable acidity or drop in pH during storage were higher in control jameed (sheep butter milk) than that observed in goat or cow skim milk jameed. On the other hand, goat skim milk jameed contained slightly higher acidity values than cow skim milk jameed. The acidity values of treatments A, B, C, G and H after 15 days of storage were 3.48, 2.58, 2.75, 2.50 and 2.58% respectively.

In respect of the impact of partial drying on acidity and pH levels, it is shown from results of Table 4 that after finishing of partial drying (fresh samples) the acidity values of goat and cow skim milk jameed dried at 45ºC/12h and 60ºC/10h (samples C, D, H and I respectively) were slightly higher while dried at 75ºC/8h and 90ºC/6h (samples E, F, J and K respectively) were slightly lower than the acidity levels of fresh jameed paste made from goat and cow skim milk (samples B and G respectively). On the fifteenth day and during storage, acidity ratios of sun dried jameed were significantly ($P<0.05$) higher than those of partial dried treatments. With increasing of temperature and decreasing of time of heat treatments, acidity levels of jameed lowered while pH values increased. After 60 days of storage, acidity contents of cow skim milk jameed samples (H, I, J and K) dried at 45ºC/12h, 60ºC/10h, 75ºC/8h and 90ºC/6h were 3.57, 3.30, 3.18 and 2.97% respectively. These outcomes may be attributed to the influence of heat treatment of partial drying on bacterial activity in jameed. As it is well known, high temperatures inhibit bacterial growth and activity.

It can easily be observed from Tables 4 and 5 that there is a substantial effect of the partial drying on TS, fat, total protein and ash contents of jameed especially in fresh treatments. After partial drying of jameed past, significant increases in TS, fat, total protein and ash contents were obtained as compared with control. As well the increases in these components more rose with increasing of temperature of heat treatment. Total solids contents of fresh A, B, C, G and H samples were 48.67, 31.89, 66.80, 34.14 and 67.40% respectively. Of course, this due to higher moisture evaporation at higher heat treatment temperature. At the end of solar drying period (after 15 days) and through storage, the findings radically varied. Jameed manufactured from sheep butter milk using solar drying possessed the highest levels of TS and ash whereas goat and cow skim milk jameed treatments dried by sun had the lowest. Generally, TS and ash values of goat skim milk jameed were close to their counterparts of jameed made from cow skim milk. Samples of goat skim milk jameed had the highest fat concentrations followed by sheep butter milk

and cow skim milk jameed. Total protein values of sun dried jameed treatments prepared from goat and cow skim milk were lower than partial dried samples. Overall, total protein was the predominant content of TS in various jameed samples. Jameed has high ash content due to the salt added to jameed paste before shaping and drying.

Regardless of milk type or drying method applied, TS, fat, total protein and ash contents of different jameed treatments increased with the advancing of storage period. The largest percentages of increases were found at the end of sun drying period (after 15 days). Jism, (1997) stated that the chemical composition of jameed differs because of numerous factors, including the stage of milk production (i.e., lactation cycle), milk sources, animal feeds and processing method. From the viewpoint of quality, moisture content in jameed should not be more than 15% in order to reduce microbial spoilage and to stop any undesirable chemical and physical changes from taking place during storage **(**Krokida and Marinos-Kouris 2003 and Koç et al., 2008). Mazahreh et al., (2008) reported a fat content up to 31.7%, which indicate the low efficiency churning in traditional Jameed processing methods.

Data of salt and salt in moisture of jameed samples during drying and storage periods are tabulated in Table 5. With progressive of storage period, salt and salt in moisture ratios increased in different jameed treatments. Jameed made from goat and cow skim milk and partially dried by hot air had slightly higher salt contents than jameed manufactured from sheep butter milk or goat and cow skim milk with sun drying. As a result of moisture evaporation during partial drying, salt levels were slightly higher in jameed samples treated with this technic than control. Salt values of samples A, B, C, G and H at the end of storage period were 11.07, 10.57, 11.55, 10.42 and 11.68% respectively. Irrespective of drying method applied, salt and salt in moisture results were almost similar in both jameed treatments made from goat and cow skim milk.

3.5. Changes in some nitrogen fractions of jameed

In jameed paste (fresh), utilization of partial drying increased WSN and NPN levels than control (Table 6). This may be due to increasing of total solids and total protein. During storage period, results of WSN and NPN reflected while sheep butter milk jameed dried by sun had the highest levels followed by that prepared from goat and cow skim milk using partial drying while goat and cow skim milk jameed dried in sun had the lowest levels. On the other side, using of low temperature with prolongation of heat treatment time through partial drying of jameed paste significantly increased ($P<0.05$) WSN and NPN values. These results could be interpreted on the basis of stimulation of jameed paste bacteria by low temperature (45ºC/12h) thus more proteolysis was done. Inversely, high temperature (90ºC/6h) inhibited jameed bacteria thus decreased WSN and NPN contents. Values of WSN of samples C, D, E and F after 30 days of storage were 1.284, 1.275, 1.269 and 1.255% respectively. When comparing results of WSN and NPN between goat and cow skim milk jameed, it was clear that these contents were higher in the former than the latter. These results are in line with those reported by Ismail (2010), he reported that WSN/TN and NPN/TN values of Halloume cheese made from goat's milk were higher than that made from cow's milk.

3.6. Changes in microbial counts of jameed during storage

Using of various types of drying or milk impacted on the microbial numbers of fresh jameed and during storage period. It could be viewed form Table 7 that jameed made from sheep butter milk by traditional method (completely sun drying) had higher numbers of total viable bacterial count (TVBC) than jameed made from goat or cow skim milk and dried by sun or hot air. Conversely, loss of viability ratios of TVBC during storage period highly increased in partial dried jameed than control one. Values of loss of viability for

samples A, B, C, G and H were 85.07, 87.93, 92.06, 90.00 and 98.42% respectively. As it is expected, raising of temperature in heat treatments of partial drying significantly ($P<0.05$) decreased TVBC of jameed. Numbers of TVBC is samples C, D, E and F after 90 days of storage were 14, 11, 8, and 2 x 10^3 CFU/g respectively. The TVBC increased in jameed made from goat skim milk as compared with that made from cow skim milk jameed.

Manufacturing of jameed by traditional method (samples A, B and G) significantly ($P<0.05$) increased the numbers of lactic acid bacteria in fresh product and within storage period. As TVBC reduced in jameed samples treated with partial drying, also lactic acid bacteria decreased in these samples. With increasing of temperature of partial drying, lowering levels increased. Using goat skim milk increased the counts of lactic acid bacteria than using cow skim milk in jameed production. In addition to this, goat skim milk jameed possessed lower levels of survival loss during storage than cow skim milk jameed.

It is quite apparent from the results reported in Table 7 that small numbers of proteolytic bacteria were found in fresh jameed samples and during storage period. Numbers of these bacteria exhibited the same behavior of TVBC and lactic acid bacteria regarding the effect of applied drying method, partial drying temperature and milk type. Supported to the effect of drying method of jameed on bacterial numbers, Al Omari et al., (2008) cleared that the bacteria counts of the freeze-dried samples are generally higher than solar dried ones. This may demonstrate the lethal effect of the prolonged heat (50ºC) on certain lactic acid bacteria in solar drying of this product.

In all jameed treatments, there were significant ($p<0.05$) reduction in TVBC, lactic acid bacteria and proteolytic bacteria through storage period which due to the accumulation of acids, reduction of moisture and increasing of salt contents.

After 90 days of storage, moulds and yeasts were detected in some jameed samples while at 120, 150 and 180 days, they found in all treatments. Their numbers increased in jameed made by traditional method and lowered by higher temperature of partial drying and increased in goat skim milk jameed than that prepared from cow skim milk. Because of high hygienic conditions of the manufacture, coliform bacteria were not detected in fresh jameed samples and during storage period.

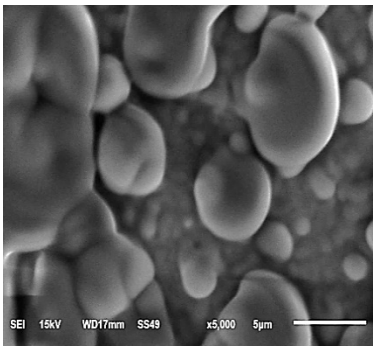
3.7. Changes in solubility of jameed during storage

An important criterion for quality assessment of jameed treatments is the solubility, so the wettability and syneresis are an important characteristic that determines the acceptability of the product for reconstitution. Results of Table 8 illustrate the influence of using partial drying and different types of milk on the wettability and syneresis of jameed during storage. From 15 till 180 days of storage, goat skim milk jameed partially dried at 90ºC/6h had the greatest wettability levels as compared with other treatments. Cow skim milk jameed partially dried using various heat treatments possessed the lowest wettability values whereas control jameed was at an intermediate position. In both goat and cow skim milk jameed, increasing of temperature and decreasing of time of heat treatments of partial drying significantly ($p<0.05$) increased the wettability levels. The wettability levels of samples A, F and K after 120 days of storage were 228.79, 230.98 and 214.72% respectively.

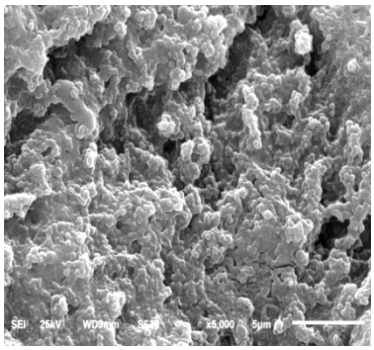
Quite the contrary, values of syneresis determined after 1 or 24 h of mixing with water were higher in cow skim milk jameed than those of goat skim milk and control jameed. Raising of temperature and lowering of time of heat treatments of partial drying significantly ($p<0.05$) reduced the values of syneresis. In different jameed treatments, syneresis levels increased after 24 hours of mixing with water. However wettability values always inversely proportional with syneresis, but both of them gradually increased during storage stage in various jameed treatments.

3.8. Changes in textural characterizes of jameed at the end of storage period

Rheological studies are widely used in food products to understand their texture and microstructure. Small strain dynamic rheological methods are nondestructive. They conduct within the linear viscoelastic region, and determine the elastic and viscous nature of cheese. Large strain rheological methods determine rheological properties that occur outside of the linear viscoelastic region and characterize the nonlinear and fracture properties of the material (Rahimi et al., 2013). Table 9 shows the textural properties of jameed after 180 days of storage. Using of partial drying in jameed manufacturing increased values of hardness, cohesiveness, gumminess and chewiness values and decreased values of springiness comparing with control which made using sun drying. Olson and Johnson (1990) indicated that relative amounts of water, protein, and fat were the dominant factors electing cheese hardness. Fat and moisture act as the filler in the casein matrix of cheese texture (Madadlou et al., 2005), giving it lubricity and softness.



Sample A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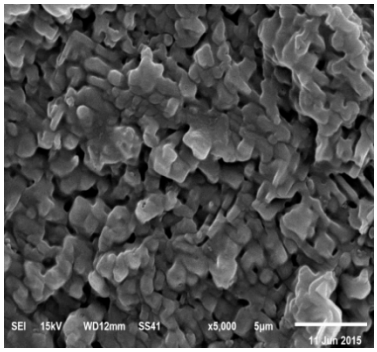
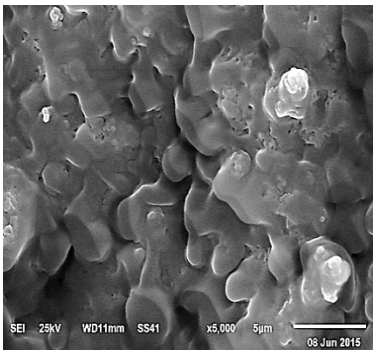
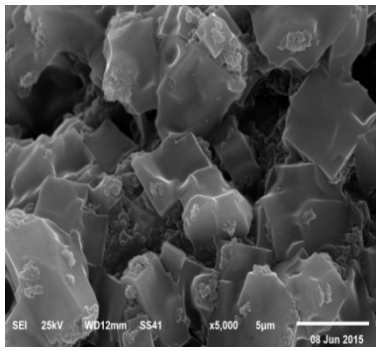


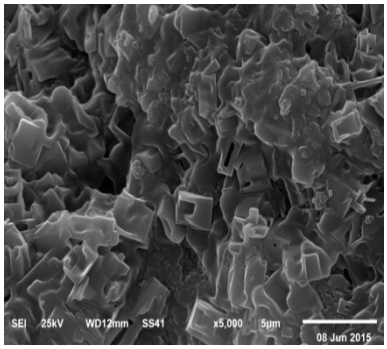
Sample C



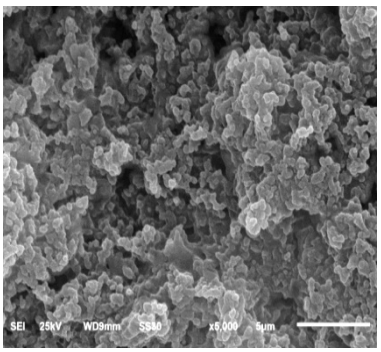
Sample D

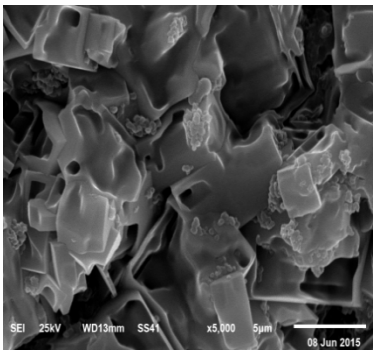


Sample E

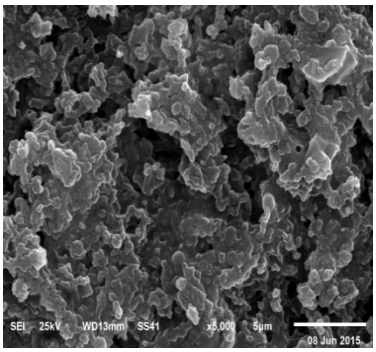


Sample F

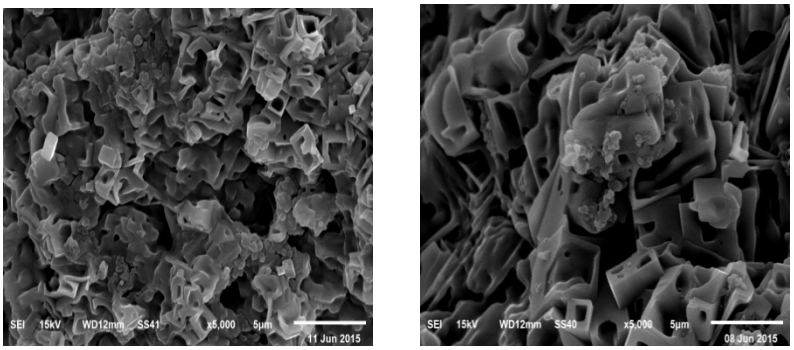

Sample G



Sample H



Sample I



Sample J Sample K

Figure 1. Scanning electron micrographs of jameed at the end of storage period

On the other hand, jameed samples treated with higher temperature of partial drying had the highest levels hardness, cohesiveness, gumminess and chewiness and the lowest levels of springiness. This may be due to increasing of TS, ash and salt at higher temperatures. These results are in agreement with those found by Kaminarides et al., (2006) who reported that with increasing the salt and ash contents in blend Halloumi cheese, the hardness of the resulting processed cheese increased. Also Desouky et al., (2013) showed that the change in the apparent viscosity of Labneh made from camel milk was linear with the increase in the thermal treatment of milk, where the highest treatment (95°C/30 min) increase in the solids concentration led to highest apparent viscosity. As appeared from Table 9, jameed made from cow skim milk had higher significant ($P<0.05$) values of hardness, cohesiveness, gumminess and chewiness and lower significant ($P<0.05$) values of springiness than jameed made from goat skim milk.

3.9. Microstructure of jameed at the end of storage period

Microstructure has a major impact on the texture and other physical properties of acid milk gels (Desouky et al., 2013). Thus, scanning electron microscopy (SEM) was performed for control and high thermally treated jameed treatments.

The microstructure of the jameed samples using SEM is shown in Figure 1. There was clear distinction between the microstructure of the jameed treatments. Protein matrices composed of casein micelle chains and clusters were entirely different among jameed samples. The scanning electron microscopy showed that the protein matrices of sheep butter milk jameed dried in sun (sample A) appeared to be relatively more intensive than other treatments and spaces in it were very small and filled by the fat globules. In this treatment, the casein micelles were predominantly linked by particle fusion into big aggregates, rather than by particle to particle attachment in chains with comparatively small interspaced voids. In goat and cow skim milk jameed dried in sun (samples B and G), protein matrices characterized by coarse structure and no casein micelle fusion especially in goat skim milk jameed. Void spaces occupied by the fat globules uniformly scattered within the jameed matrix.

The micrograph images of partially dried jameed treatments showed that protein matrices characterized by little aggregates, plates structure, more open protein network and high fusion. These properties were more obvious with higher temperature heat treatments and in goat skim milk jameed. The SEM observations of our study are relatively close to those reported by Desouky et al., (2013) who cleared that for control of Labneh made from camel milk, protein structure characterized by short casein micelles chains and no appreciable casein micelle fusion were observed. The

control exhibited a more open, loose and less dense protein network than thermal treatments.

4. Conclusions

Using of partial drying technique at 75ºC/8h and 90ºC/6h saved the cost production of jameed by reducing time of solar drying to half. Jameed made from goat or cow skim milk using this technique exhibited good chemical, microbial and rheological properties during storage period for six months.

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ANALYSIS OF PHYSICOCHEMICAL AND MICROBIOLOGICAL MEASUREMENTS OF FOOD PREPARED USING DIFFERENT STOVES

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ABSTRACT
This research aims to analyze the physicochemical and microbiological measurements of food prepared using different stoves as liquefied petroleum gas (LPG), wood, charcoal, electric resistance and induction stoves. The results of the analysis showed how the stove could affect the thermal treatment of the food. The most significant results observed were the total bacteria concentration in the boiled milk in the wood and charcoal stove did not meet the requirements after the cooking process and is not fit to consume. In addition, the preservation of protein and fat was greater in induction stoves in grilled beef baked beans and steamed salmon after the cooking process. Furthermore, the micronutrients of vitamin C lost in similar quantities in electric, LPG and induction stoves for boiled spinach, boiled potatoes and apple cider. A greater loss of vitamin C was observed in wood and charcoal stoves.

1. Introduction

Poor diet quality is associated with obesity and diet related diseases. In particular, it has prompted the attention to the need to improve cooking skills among the general public. The literature favors the idea of cooking for good health. Frequently, cooking at home is assoc with consumption of a healthier diet particularly among households with high socioeconomic status (SES), (U.S. Department of Labor, 2004; Caraher et al., 2004). In addition, cooking at home is increasingly promoted as an obesity prevention measure (Masera et al., 2005; Hastings et al., 2003). Meanwhile, the consumption more food away carries the reduction of prevalence and use of cooking knowledge and skills in the general population. Typically food away carries both energy dense and of lower nutritional value (Bathgate & Begley, 2011; Brooks & Begley, 2014). The nutritional value of including more commercially prepared foods has been found to be inferior to home-made foods (Allirot et al., 2012; Allirot et al., 2014). Home-cooked meals tend to be healthier with fewer calories, and less saturated fat, cholesterol and sodium on a per calorie basis while providing more fiber, calcium, and iron (Nestle, 2013).

The ingredients are also directly sourced, so there is control over authenticity, taste, and nutritional value. The superior nutritional quality of home-cooking could therefore play a role in preventing chronic disease (World Health Organization, 2003).

Meanwhile, in the societies with low socioeconomic status (LES), the International

Energy Agency observed that globally 2.6 billion people do not have access to clean cooking facilities (World Health Organization, 2009). The World Health Organization (WHO) estimates that 1.5 million premature deaths a year are directly associated to air pollution from the use of solid fuels for cooking activities (World Health Organization, 2009). Up to 85% of these deaths (about 1.3 million) are attributed to the use of biomass, the rest to coal (World Health Organization, 2009). This problem is especially harmful to children, old people and women who spend more time near the kitchens stove (International Energy Agency, 2006). In addition, not having clean cooking facilities carries the risk of food borne illness.

Prior to consumption, most dietary components are cooked using different methods according to the recipes and the culinary traditions of various countries (U.S. Department of Labor, 2004; Caraher et al. 2004). Cooking is an important part of daily food preparation in commercial and residential settings. Cooking techniques and ingredients vary widely across the world, from the use of Liquefied Petroleum Gas (LPG), electric coil and induction stoves in SES societies to wood, charcoal, dung and wood stove, characterized by open fire in LES societies.

Induction stoves present a few advantages when they are compared to traditional liquid petroleum gas (LPG), biomass or electric coil based stoves, which can be listed below: i) increased energy efficiency, as the magnetic field is induced in the cookware and there is absence of calorific focus of high temperature, which reduces heat losses to the environment; ii) a higher speed in heating, because the ferromagnetic material of the base of the pan has the ability to attract and pass electricity through the magnetic fields, as soon as it flows through the coil, which causes that the cookware is heated directly; iii) higher safety because there is no risk to get burned when using the kitchen, or explosions, as no flame is produced; iv) more hygienic and easy to clean, while having a smooth surface of vitroceramic; v) easy to operate with digital controls. On the contrary, the main disadvantages are: i) a more sophisticated technology than electrical coil and LPG stoves; ii) fragile use, as vitroceramic can get scratches (Villacís et al., 2015; Martínez-Gómez et al., 2016).

The ways or types of cooking also depend on the skill and type of training an individual cook has. Cooking can also occur through chemical reactions, usually with the presence of heat (U.S. Department of Labor, 2004).

Although, cooking is only one aspect of food consumption, it is essential for the safety of food products and contributes to the digestion, micronutrients and acceptability of food (Nesbakken and Strom, 1993, Pimentel and Pimentel, 2008). Cooking minimizes the risk of food borne illness and the application of microbiological risk assessment for the management of food safety is well established. Food has been demonstrated to have been the vehicle for transmission in a number of foodborne outbreaks of infection (Tood et al., 2007) Risks from microbiological hazards as viruses, parasites and pathogenic bacteria are a serious concern to food safety and human health (Yepiz-Gomez et al., 2006). For this reason, cooking is used to prevent many foodborne illnesses that would otherwise occur if the food is eaten raw. When heat is used in the preparation of food, it can kill or inactivate harmful organisms, such as bacteria and viruses, as well as various parasites such as tapeworms and Toxoplasma Gondii (Pimentel and Pimentel, 2008). Food poisoning and other illness from uncooked or poorly prepared food may be caused by bacteria such as pathogenic strains of Escherichia coli, Salmonella typhimurium and Campylobacter, viruses such as noroviruses, and protozoa such as Entamoeba histolytica Parasites may be introduced through salad, meat that is uncooked or done rare and unboiled water (Thompson, 2009)

The sterilizing effect of cooking depends on temperature, cooking time, and technique used. However, some bacteria such as Clostridium botulinum or Bacillus cereus can form spores

that survive boiling, which then germinate and regrow after the food has cooled. It is therefore recommended that cooked food should not be reheated more than once to avoid repeated growths that allow the bacteria to proliferate to dangerous level.

Cooking also increases the digestibility of some foods because many foods, such as grains, are inedible when raw, and some are poisonous (Tiffany, 2013). The application of heat alters the composition of food products to enhance taste, texture, digestibility and shelf-life (Lund, 1975). The biological, physical and chemical modifications that occur during cooking lead to sensory, nutritional and textural changes of food that may be beneficial or detrimental to human health (Lund, 1975). For example, cooking inactivates the microorganisms and anti-nutritional factors and enhances the digestibility of food and the bioavailability of nutrients (Yeung & Morris, 2001) Additionally, cooking is involved in the formation of structural and desirable compounds, such as those that confer crispiness, flavor, antioxidants and coloring agents to the food (Magnússon et al. 2012). Domestic cooking also induces adverse effects, such as the loss of certain nutrients and the formation of undesired compounds (e.g., acrylamide or other toxic molecules) (Griffith, 2006).

In this paper it has been studied the reduction in the number of pathogenic and spoilage microorganisms after heat treatment between samples made with different cooking techniques (stoves), in order to analyze the effect of the stove in the microbiological food safety management. Furthermore, it has been analyzed the denaturation of food to know if people are taking the necessary nutrients degraded or are taking food without proper nutritional values and the stove influence in it.

2. Materials and methods

No sources of pollution or contamination were found nearby of the stoves. The studied hood, range and appliances are the typical used in a restaurant kitchen and it has a volume flow rate of 1.2 m^3/s. The geometric data of this case-study kitchen are listed in Figure 1. To perform these tests, exterior windows and interior door in the kitchen remained closed in order to avoid pollution sources.

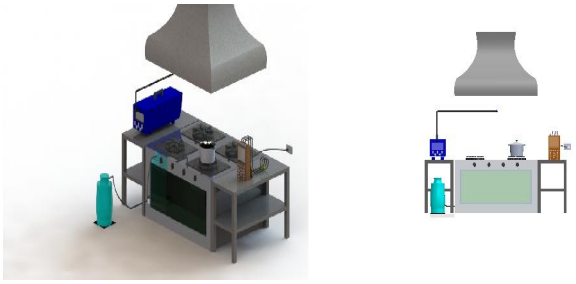

Figure 1. Layout of the of the case-study kitchen.

The dishes were cooked using four kind of stoves described as follow:

1. An electric induction stoves of three induction zones of 28 cm, 18 cm and 14,5 cm of diameter. The nominal power of this device is 7400 W and 3,2 kW for the highest zone. However, for the test only one induction zone was used.
2. An industrial LPG cooker SILKO ECG74E of 15000 W. As this LPG cooker is industrial equipment, during the test the power of the cooker burners were limited to 3200 W in order to simulate a domestic cooker.
3. The electric cooker used for tests has two burners with a nominal power of 500 W. The cooker characteristics have a direct influence on the test duration, although it was not taken into account when changing any parameter within standards applied to food.
4. The wood and charcoal stove used for the tests was a large plate steel stove with outstanding heating capabilities. This unit heated 2,500 sq. ft. of your home with up to 112,000 BTUs. The stove has an elegant, large, arched glass door with a built in air wash feature that helps keep the glass clean and the fire viewable.

The following experiments were conducted using the observational method applied to one kind of induction pot and one induction pan made of AISI 304 stainless steel in their body and AISI 430 stainless steel in their bottom

which is the normal configuration of induction cookware. Each cookware was covered with an AISI 304 stainless steel lid during the tests.

The wood selected for the study was almond wood. Almond wood generated a hot and long burn and for this reason were selected. This hardwoods took a long time to get lit, but once they did, the embers they produced a super-hot and ignited practically anything you throw on their afterwards. The charcoal used in the experiment was composed by coal purchased in a local supermarket.

2.1. Case-study dishes and site selection

The dishes prepared in this study were chosen considering by the chemical composition (macro and micronutrients), microbiological safety and easy to prepare. In addition, these dishes are representatives of the dietary of many countries. The cooked dishes were: grilled beef, milk, baked beans, steamed salmon, boiled Spinach, boiled potatoes and apple cider. The essential information of ingredients and cooking process of each dish is presented in Table 1. The quantity of food was considered for one portion, for this reason, the amounts used was measured. Achiote oil as cooking oil was used. All ingredients and condiments were purchased from a local supermarket to have the food as fresh as possible. In order to have the best control of consistency the experiments were repeated three times for each dish.

Table 1. Information of the cooking processes and the ingredients of the different dishes

Dishes	Ingredients	Quantity	Treatment	Picture	Cooking process
Grilled beef	Oil Beef Salt	40 ml 400 g 5 g	Fillets		Step 1: Heat up the oil. Step 2: Fry the beef fillets.
Milk	Milk	250 ml			Step 1: Heat the milk to 72°C for 15 s.
Baked beans	Water Beans	500 ml 150 g			Step 1: Turn on the stove and heat water until it boils. Step 2: Add the beans in the pot. Step 3. Boils for 90 minutes

Steamed Salmon	Water Salmon Salt	1 l 400 g 5 g	Fillets		Step 1: Turn on the stove and heat the water, salmon until 75 °C for 5 minutes.
Boiled Spinach	Water Spinach Salt	1 l 10 5 g			Step 1: Turn on the stoves and heat the water until it boils. Step 2: Boil the Spinach for 1 minute.
Apple cider	Water Apple Cinnamon Sugar	1 l 3 pieces 1 g 15 g			Step 1: Turn on the cooker and heat the water, the apple for 25 minutes. Step 2: Simmer for 2 hours Step 3: Strain apple mixture though a fine mesh sieve. Discard solids. Drain cider again though a cheesecloth lined sieve.
Boiled potatoes	potatoes	75 g			Step 1: Turn on the stove and heat water until it boils. Step 2: Add the potatoes in the pot. Step 3. Boils for 25 minutes

2.2.Physicochemical and microbiological measurements

Food samples of the different stoves were taken as soon as the cooking process finished for making proximate and microbiological analysis. After the cooking process three sample of 30 g and 100 ml for each dish were collected. These samples were analyzed by the accredited laboratory with certify number OAELE C 09-008. The results were compared with values established in national and international standards and references in Table 2.

The physicochemical changes produced by thermal treatment in food are described in Table 3. The nutrients studied in the analysis were proteins, lipids, carbohydrates, vitamins A and C, minerals and ash. In table could be observed how the temperature of the cooking process could be affected the chemical changes.

Table 2. Preparation processes of the dishes and references for the procces.

Dish	Process	Reference
Grilled beef	Fry beef until it gets to an internal temperature of 72 °C	(McGee, 2007; NTE INEN 2346:2010, 2010)
Milk	Heat milk to 75°C for 15 s	(McGee, 2007; NTE INEN 0010:2012)
Baked beans	Boil the beans for 90 minutes.	(McGee, 2007; NTE INEN 2390:05, 2014)
Steamed Salmon	Heat water until 75 °C and cook for 5 minutes.	(McGee, 2007; NTE INEN 0183:2013, 2013).
Boiled Spinach	Boil the Spinach for 1 minute.	(McGee, 2007)
Apple cider	Boil the apples until they become soft and simmer for 2 hours	(McGee, 2007)
Boiled potatoes	Boil the potatoes until they become soft.	(McGee, 2007)

Table 3. Chemical and physical changes produced by thermal treatment in food (Verdú, 2013; Badui Dergal, 2006; Martínez et al. 2005)

Physical	Chemical	
Volume	Nutrient	Effect
Color	Proteins	Coagulation of colloidal solutions (70-80°C). Protein hydrolysis and breakdown of muscle fibers when excessive heating. Denaturation but not casein coagulation (in milk) . Enzymes inactivation. Digestibility improvement (<100°C).
Consistency	Lipids	Foundry and partial abandonment. Formation of acrolein (>180°C), a substance with unpleasant taste.
Organoleptic characteristics	Carbohydrates	Solubilization. Gelatinization of complex carbohydrates in a moist environment (60-70°C). Dextrinization, change of color and flavor in dry environment. Carbonization (>170°C).
	Vitamins	Solubilization Oxidation.
	Minerals	Solubilization.

3. Results and discussions

The microbiological and physicochemical changes produced by cooking process in food are described in Table 4. The used references were found in national standards, books of the matter as table 4 presents (Verdú, 2013; Badui Dergal, 2006; Martínez et al. 2005; NTE INEN 2346:2010, 2010; NTE INEN 0010:2012, 2012; NTE INEN 2390:05, 2014; NTE INEN 0183:2013, 2013). It was found that milk, beef and the steamed salmon need a thermal treatment before consumption. Milk, beef and the steamed salmon, as feedstock, are above of the allowed concentrations during total recount of bacteria. After the cooking process the total bacteria concentration in the boiled milk in the wood and charcoal stove did not meet the requirements. The other samples of microbiological load of spoilage and pathogenic decreases to concentrations, that is not harmful for human health. The reason of these results must likely a contamination during sampling and/or analysis, which might have changed was determinant to modify the results. This contamination could be possible to the kind of fuels. The results of table 4 showed a preservation of the protein in induction stoves in grilled beef, milk, baked beans and steamed salmon after the cooking process. The % of proteins in feedstock beef was 25,4 %, when the beef was grilled in the induction stove it presented a % of protein of 24,06 %, while the % of proteins were reduced in electric and LPG stoves until 21,65 % and 21,94 %, respectively. Meanwhile, the wood and charcoal stove presented the highest reduction in the percentage of protein between the cooked and feedstock beef, beans and salmon. In case of beef grilled the % of proteins was reduced until 20,38 % and 20,25 % in wood and charcoal stove respectively. In case of milk, the % of proteins was 3,63 % in feedstock. When the milk was pasteurized in the induction stove it presented a % of protein of 3,61 %, meanwhile the % of proteins were reduced in the rest of the stoves until 2,85 % in electric stove, 2,52 % in LPG stove, 2,19 % in wood stove and 2,08 % in charcoal stove. The same trend could be observed with the % of proteins in the steamed salmon and baked beans.

Similar tendency of the % proteins were observed in the quantity of fat was greater after cooking in induction stoves the grilled beef, milk, baked beans and steamed salmon than electric, LPG, wood and charcoal stoves. The % of fat in feedstock beef was 13,1 %. After the cooking in the induction stove it presented a % of fat of 12,79 %, meanwhile the % of fat after the thermal treatment were reduced in electric and LPG stoves until 11,71 % and 11,84 %, respectively. In case of, the wood and charcoal stove the beef grilled a fat % of 10,25 % and 10,22 % was observed. For the steamed salmon, the % of fat was 13,78 % in feedstock salmon. When the salmon was cooked in the induction stove it presented a % of fat of 12,69 %. For the rest of the stoves, the % of fat were reduced until 10,72 % in electric stove, 10,78 % in LPG stove, 9,81 % in wood stove and 9,82 % in charcoal stove. The same trend could be observed with the % of proteins in the milk and baked beans.

The analyzed micronutrients were vitamin C and vitamin A in boiled spinach, boiled potatoes and apple cider. The thermal treatment of the stove affected the loss of micronutrients. After the cooking, it was observed that vitamin C lost in similar quantities in electric, LPG and induction stoves. The quantity in vitamin C in feedstock potatoes was 19,8 mg per 100 g. After the thermal process the quantities in vitamin C for induction, electric and LPG stoves were 16.24 mg per 100 g, 15.11 mg per 100 g and 14.68 mg per 100 g. Meanwhile, a greater loss of vitamin C was observed in wood and charcoal stove, the quantities in vitamin C, after the cooking process were 11,12 mg per 100 g, and 12,08 mg per 100 g in wood and charcoal stove. Similar tendency was observed in boiled spinach and apple cider. The vitamin A lost in less quantity when using the induction stoves. The vitamin A lost was reduced by induction cooker between 64 % and 366 % less than the other stoves.

Table 4. Microbiological and physiochemical changes produced by thermal treatment in food (Verdú, 2013; Badui Dergal, 2006; Martínez et al. 2005; NTE INEN 2346:2010, 2010; NTE INEN 0010:2012, 2012; NTE INEN 2390:05, 2014; NTE INEN 0183:2013, 2013).

		Meat pulp	Feedstock	Induction	Electri	LPG	Wood	Charcoa
Grilled beef	Total bacterial count (CFU/g)	1,0E+02 - 1,0E+03	1,2E+05	<10	<10	<10	<10	<10
	Total coliform count (CFU/g)		7,8E+04	<10	<10	<10	<10	<10
	E. coli count (CFU/g)	1,0E+02 -	<10	<10	<10	<10	<10	<10
	S. aureus count (CFU/g)	1,0E+02 -	<10	<10	<10	<10	<10	<10
	Salmonella spp/ (Detection/25g)	Absence	Absence	Absence	Absen ce	Absen ce	Absenc e	Absence
	Protein (%)	20-30	25,4	24,06	21,65	21,94	20,38	20,25
	Humidity (%)	50-70	61,5	60,99	60,16	60,86	58,17	57,21
	Fat (%)	10-20	13,1	12,79	11,71	11,84	10,25	10,22
	Ash (%)	0,5-2,5	1,0	1,16	1,38	1,36	1,57	1,68

		Global chemistr	Pasteurized	Feedstock	Induction	Electric	LPG	Wood	Charcoal
Milk	Total bacterial count (CFU/ml)	1,5E+06 maximu	3,0E+04 (maximum)	1,2E+08	3,4E+02	7,2E+02	1,3E+ 03	3,7E+ 05	4,6E+05
	Total coliform count		< 1	6,8E+05	<10	<10	<10	<10	<10
	Mold count (CFU/ml)			<10	<10	<10	<10	<10	<10
	Leaven count (CFU/ml)			2,9 E+04	<10	<10	<10	<10	<10
	E. coli count (CFU/ml))		< 10	<10	<10	<10	<10	<10	<10
	S. aureus count			3,3E+02	<10	<10	<10	<10	<10
	Salmonella spp (Detection/25ml)		Absence	Absence	Absence	Absence	Absen ce	Absen ce	Absence
	Protein (%)	2,9 - 4	2,9	3,63	3,61	2,85	2,52	2,19	2,08
	Humidity (%)	85 - 90		88,87	88,56	88,51	88,73	86,53	88,77
	Fat (%)	2,5 - 5	3	3,7	3,65	2,39	2,42	2,28	2,13
	Ash (%)	0,7 - 1	0,65 - 0,80	0,75	0,62	0,64	0,65	0,89	0,91
	Carbohydrates (%)	4 - 5,5		4,93	4,78	4,41	4,71	4,22	4,16

		Debittering	Feedstoc	Inductio	Electric	LPG	Wood	Charcoal
Baked beans	Total bacterial count (CFU/g)	18,0E+02 - 1,0E+03	1,3E+07	<10	1,2E+01	3,2E+01	3,7E+ 01	4,2E+01
	Total coliform count (CFU/g)	10 - 10,0E+02 NMP/g	1,4E+05	<10	<10	<10	<10	<10
	Mold count (CFU/g)	0 - 5E+02	<10	<10	<10	<10	<10	<10
	Leaven count (CFU/g)		5,2E+03	<10	<10	<10	<10	<10
	E. coli count (CFU/g)	Absence	<10	<10	<10	<10	<10	<10
	Protein (%)	15 - 25	19,7	19,24	18,62	18,81	17,54	17,77
	Humidity (%)	60- 70	66,39	67,21	66,83	66,95	67,83	67,95
	Fat (%)	0,5 - 2	1,72	1,68	1,39	1,32	1,09	1,02
	Ash (%)	0 - 1	0,62	0,66	0,67	0,63	0,87	0,93
	Crude fiber (%)	10 - 20	13,63	12,86	12,56	12,33	11,56	11,33

Steamed Salmon		Fresh, refrigerated, frozen	Feedstock	Induction	Electric	LPG	Wood	Charcoal
	Total bacterial count (CFU/g)	5,0E+05 maximum	2,0E+05	<10	<10	<10	<10	<10
	Total coliform count (CFU/g)		1,0E+02	<10	<10	<10	<10	<10
	E. coli count (CFU/g)	10 maximum	<10	<10	<10	<10	<10	<10
	S. aureus count (CFU/g)	100 maximum	<10	<10	<10	<10	<10	<10
	Salmonella spp/ (Detection/25g)	Absence	Absence	Absence	Absence	Absence	Absence	Absence
	Protein (%)	15-25	21,38	20,72	18,24	19,01	19,74	19,56
	Humidity (%)	60-75	66,28	67,53	68,38	69,09	68,23	67,21
	Fat (%)	10-15	13,78	12,69	10,72	10,78	9,81	9,82
	Ash (%)	0-2	1,35	1,08	1,18	1,12	1,51	1,63

Boiled Spinach		Consumable part	Feedstock	Induction	Electric	LPG	Wood	Charcoal
	Protein (%)	1,5-3,5	2,59	2,39	2,12	2,08	2,02	1,98
	Humidity (%)	90-95	92,27	92,75	93,74	93,59	93,85	93,95
	Fat (%)	0-1	0,51	0,64	0,63	0,44	0,41	0,39
	Ash (%)	0-2	0,84	0,61	0,53	0,65	0,63	0,62
	Crude fiber (%)	1,5-2,5	1,88	1,99	2,01	1,91	1,98	1,97
	Carbohydrates (%)	2-5	2,92	2,85	2,65	2,54	2,36	2,41
	Vitamin A (UI/100g)	8500-10000	9382	8750	5320	5127	3985	1868
	*Vitamin C	25-35	28,3	27,51	24,62	24,85	22,35	22,64

The quantity in vitamin A for feedstock spinach was 9382 UI per 100 g. After boiling the spinach in the induction stove the quantity in vitamin A for induction was 8750 UI per 100 g. In case of the quantities in vitamin A for the rest of the stoves after the cooking process 5320 UI per 100 g in electric stove, 5127 UI per 100 g in LPG stove, 3985 UI per 100 g in wood stove and 1868 UI per 100 g in charcoal stove were observed.

The nutritional quality of any kind of food depends on the thermal treatment used. The induction stove present a higher speed in heating due a direct heating in the cookware which appears to influence in the cooking process and preserve the quantity of proteins and fats in the food. In addition the results presented and increment in the quantity of ass in wood and charcoal stoves. The reason of these results must likely a contamination during the cooking process.

5.Conclusions

The food components do not supply energy to human body, but they are essential in every chemical reaction that produces energy. In this context cooking is essential for heathy, and safety of food; it keeps the micronutrients and macronutrients products. In addition, it contributes to the digestion and acceptability of food and it minimizes the risk of food borne illness. For this reason, in this article was studied the physical-chemical and microbiological measurements in liquefied petroleum gas (LPG), wood, charcoal, electric resistance and induction stoves for different dishes, in order to demonstrate how the stove affect to the cooking process.

The chemical composition of any kind of food can be modified for changes in weather conditions, soil type (in vegetables) and feeding type (in animals). A change in these conditions could have modified the chemical composition

of analyzed food. It is for this reason that some products are outside the reference range.

The physicochemical analysis showed preservation of the protein and in induction stoves in grilled beef, baked beans and steamed salmon after the cooking process. Meanwhile, the wood and charcoal stove presented the highest reduction in the percentage of protein and fat between the cooked and feedstock beef, beans and salmon.

The microbiological analysis showed that the pathogens and spoilage present in the food cooked in the five types of cooker are reduced to concentrations no harmful to human health. The vitamin A lost in less quantity in food cooked in induction stoves than electric, LPG, wood and charcoal stoves. Vitamin C lost in similar quantities when cooking in induction, electric, LPG stoves. Meanwhile a greater loss of vitamin C was observed in wood and charcoal stoves.

The thermal treatment used in the three cookers decreased the microbiological load to the acceptable levels, except for milk boiled in the wood and charcoal based cooker, where the total bacterial count is higher than the quality requirement.

The induction stove present a higher speed in heating due a direct heating in the cookware which appears to influence in the cooking process and preserve macronutrients and influence in the microbiology. The study concluded that the positive aspects in induction cookers are the food cooked is more acceptable, less quantity of vitamin A losses, high food safety. For this reason the programs on clean cooking should be directed to use new technologies of cooking as induction stoves.

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HEAT STABILITY OF FATTY ACIDSOF SELECTED BLENDED PALM OILS DURING POTATO FRYING

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ABSTRACT
The study aimed to determine the hydrolytic stability of free fatty acid (FFA) and fatty acid composition (FAC) of selected palm oil (PO) blended with corn oil (POCO), sesame oil (POSO), and rice bran oil (PORBO). The blended POs were tested for their stability and fatty acid changes of the unheated oils after 10 and 20 times of potato frying. The FAC was determined using gas chromatography. As the oils were being heat treated for 0-20 times, the most significant changes were the increased in SFA and MUFA levels, and the reduction of PUFA that observed in the blended POs. The blended oils also had increased FFA contents after 20 times of potato frying. POCO was the most stable blended oil in terms of hydrolytic stability. The findings suggest that there is an improve in the quality of PO after blended with vegetable oils, especially its FAC.

1. Introduction

Fats and oils are important sources of energy for humans. They are concentrated sources of energy (9 kcal per gram) that provide essential fatty acids to human body. These fatty acids are building blocks for hormones needed for regulation of body systems and as carriers for fat-soluble vitamins A, D, E, and K. They also enhance taste and flavor of foods we eat by providing smooth texture and mouth feel, and contribute to satiety after eating (ISEO, 2007).

Fats and oils are composed of "triglyceride" that resulting from a combination of one unit of glycerol and three units of fatty acids. Fats and oils embrace a broad variety of chemical substances. Mono- and diglycerides,triglycerides, phosphatides, terpenes,sterols, fatty alcohols, fat-soluble vitamins and other compounds are also found in fats and oils.

Malaysian Dietary Guidelines recommended fat intake of 20-30% of total energy intake (NCCFN, 2005). Fat intake should reduce in saturated fatty acid (SFA) by <10% of total calories (kcal), increase in monounsaturated fatty acid (MUFA) by 12-15% of total kcal, and polyunsaturated fatty acid (PUFA) by 3-7% for omega-6 and 0.3-1.2% of total kcal for omega-3. Although a high PUFA diet can be considered as favorable, high intake of PUFA may increase susceptibility to fat oxidation (Hu et al., 1997).

High intake of dietary SFA from vegetable oil becomes a great concern in regard to cardiovascular health (FAO, 2007). Besides SFA, other fatty acids that include C12:0, C14:0, C16:0, and trans-9 C18:1 (Grundy,

1997) have been shown to elevate plasma total cholesterol and low-density lipoprotein cholesterol. Thus increase risk of cardiovascular disease (CVD). CVD is the leading cause of death in Malaysia, where the prevalence of CVD in Malaysia increased by 14.6%, from 96,000 cases in 1995 to 110,000 cases in 2000 (Dhanoa, 2004). Among the vegetable oils, palm oil is the major source of fat used in Malaysian diet. Food Balance Sheets for Malaysia indicated that the per capita consumption of palm oil was approximately 17 g per head in a day (146 kcal). It is probably the highest level of fat intake in the world (FAO, 2007). Although palm oil has high SFA, it does involve in physiological metabolism and it has been found to reduce blood cholesterol (Edem, 2002). It is related to intrinsic quality of palmitic acid, a fatty acid that different from the other fatty acids of other saturated vegetable oils.

Although most of the Malaysian consumed palm oil every day, the prevalence of cardiovascular diseases (CVD) is still high. Statistically, 30% of the total numbers of medically certified deaths in government hospitals in Malaysia are attributed to CVD (MHF, 2007). From the 137266 cases (6.6 %) of CVD that admitted to government hospitals in 2004, 8998 cases of mortality are due to CVD (DPH, 2007). Therefore, the use of edible oil in our daily diets should follow the recommendation by World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations that emphasized the importance of a balanced proportion of fatty acids in oil.

Previous studies revealed the importance of blended vegetable oils with a new fatty acid profile that may improve blood lipid level of individuals (Frankel and Huang, 1994; Noor Lida et al., 2002). Oils that contain a high composition of PUFA are more vulnerable to oxidative changes and need to be hydrogenated for stabilizing them before deep frying or for food processing. Hydrogenation of oils high in PUFA is not recommended since the process converts some of the PUFA into trans fatty acids. Trans fatty acids possess undesirable metabolic side effects, such as alteration of biological cell membranes permeability that interfere with normal cellular metabolism and normal cell function (O'Holohan, 1997). Hence, palm oil is the best oil with a wide versatility in various food applications. Besides that, fatty acid balance and antioxidants content of three blended vegetable oils have been reported byAzrina et al. (2009), where the blended oils were prepared by mixing 50:50 (w/w) palm oil (liquid portion) with corn oil, sesame oil, and rice bran oil, respectively. For that reason, we aimed to perform a study for comparing frying performance of the three blended oils after deep-fat frying of potato slices.

2. Materials and methods

2.1. Samples and sample preparation

Palm oil (PO), corn oil (CO), sesame oil (SO), and rice bran oil (RBO) were purchased from local supermarkets in Selangor, Malaysia. The oil samples were commercially packed in 1.5 L plastic bottles. Preparation of the blended oil mixtures were based on our preliminary work (Azrina et al., 2009). Briefly, four types of vegetable oil, PO, CO, SO, and RBO were sampled using four different beakers and the blended oils were prepared by mixing PO with the vegetable oils (CO, SO, and RBO) in a ratio of 50:50 (v/v). The oils were mixed and swirled for one hour at room temperature (25°C) before using them for potato frying. Homogenous mixing was carried out by using a glass rod in a 2 L beaker.

Preparation of food sample (potato slices) was adapted from the previous work reported by Azrina et al. (2009). Fresh potatoes were peeled and washed under running tap water before use and then sliced into discs of 0.5 cm thick and 2.5 cm in diameter using a mechanical slicer. When the oil temperature reached 175°C, a batch of 100 g potato was deep-fried in the boiling oil. The temperature during frying was maintained at 175 ± 5°C.

Deep frying of potatoes was carried out using a deep-fryer (Elba®, Malaysia). After each frying, the oil was left to cool at room temperature and stored in a plastic container. Potato frying was continuously performed daily until the required frequency of frying achieved. Three times frying were performed for each oil sample to obtain a representative data.

2.2. Estimation of free fatty acids content

Free fatty acids (FFAs) content of the blended oil samples was estimated using the method described by Nielsen (1994) with slight modification. Briefly, 7.00 ± 0.01 g of oil sample was weighed into a 250 ml Erlenmeyer flask. Exactly 75 ml of neutralized ethanol was then added and preheated to 60-65°C before addition of 1 ml of phenolphthalein. The flask was swirled for proper mixing. After that, 0.25 N sodium hydroxide solution was used for titration until the colour change to a permanent pink colour. The solutions were vigorously swirled throughout the titration. Amount of sodium hydroxide solution used was recorded for calculation of FFAs content.

2.3. Preparation of fatty acid methyl esters

Procedure for preparation of fatty acid methy esters (FAMEs) was adapted from the method described by David et al. (2002). Briefly, 100.0 mg sample was weighed into a 20 ml test tube. The sample was then dissolved in 10 ml hexane. Then 100 µl of 2 N KOH in methanol was added to the test tube. All tubes were capped and vortexed for 30 sec. The mixture was centrifuged at 2500 rpm for 5 min, and the clear supernatant was transferred into a 2-ml autosampler vial before injection into GC system.

2.4. Gas chromatography analysis of fatty acids

FAC of oil samples weredetermined using a gas chromatography (GC) method described by Vickers (2007). An Agilent™ 6890 GC system (CA, USA) equipped with a split-splitless injector and a Hewlett Packard™ EL-980 flame ionization detector (FID) was used to quantify FAME component. The stationary phase was DB-23 column (60 m × 0.25 mm, I.D., 0.15 µm polyethylene glycol film). The oven temperature was set at 50°C for 1 min, increased to 175°C at 4°C per min, and finally increased to 230°C. The temperature was then held for another 5 min. The temperatures for injector and detector were set at 250°C and 280°C, respectively. Oil sample (1 µl) was injected with a split ratio of 1:50 at column temperature of 110°C. Carrier gases used were helium gas (1.0 ml/min controlled at 103.4 kPa), hydrogen, and air. The hydrogen and air were held at 275.6 kPa.A mix standard ofFAMEs was used for detection and quantification of individual fatty acid. Chromatographic data were integrated and recorded using a Chemstation™ software (version 6.0). Identification of individual fatty acid in the blended oil samples was determined by comparing the retention time of the peak of FAME in the sample with those of the FAME standard.

2.5. Statistical analyses

All data were presented as mean ± standard deviation of three replicates. Analysis of variance (ANOVA) was used for comparison of free fatty acid values and fatty acid composition of the blended oils. The significant value was set at $P < 0.05$. Statistical Package for Social Science (SPSS™) version 17 was used for the statistical analyses.

3. Results and discussions

3.1. Free fatty acids content

Changes in FFA of the blended oils during potato frying at 0 time (unheated oil), 10 times, and 20 times, are shown in Figure 1. FFA values of the blended oils were compared with the values of PO. The FFA values of all samples were increased after 1-20 times of potato frying. Before frying potato, FFA level of the unheated PORBO was the highest (1.6%), followed by the unheatedPOCO (0.3%) and POSO (0.2%). These values were higher

than the FFA value of PO (0.18%). The FFA values obtained were within the acceptable range for the unheated oils, in which FFA of the oils should be less than 2% (Wrostald et al., 2000) or 2.5 mg/g (Osawa et al., 2007). An increased frequency of potato frying from 10 to 20 times altered FFA values of the blended oil samples, from 0.4 to 0.8% for POSO, 1.8 to 2.0% for PORBO, and 0.4 to 0.2% for POCO. However, no change in FFA value was found for PO.

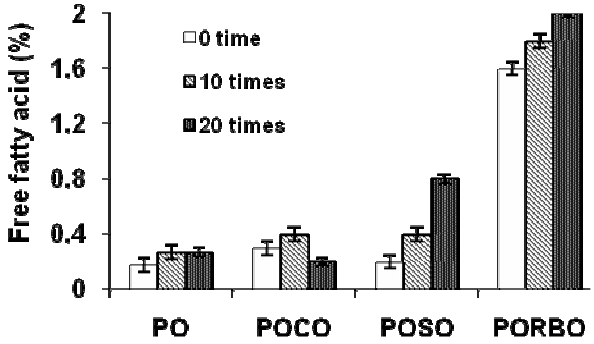

Figure 1. Free fatty acid values of the blended oil at 0 (unheated oil), 10, and 20 times of potato frying. PO: palm oil; POCO: blended palm oil-corn oil; POSO: blended palm oil-sesame oil; PORBO: blended palm oil-rice bran oil.

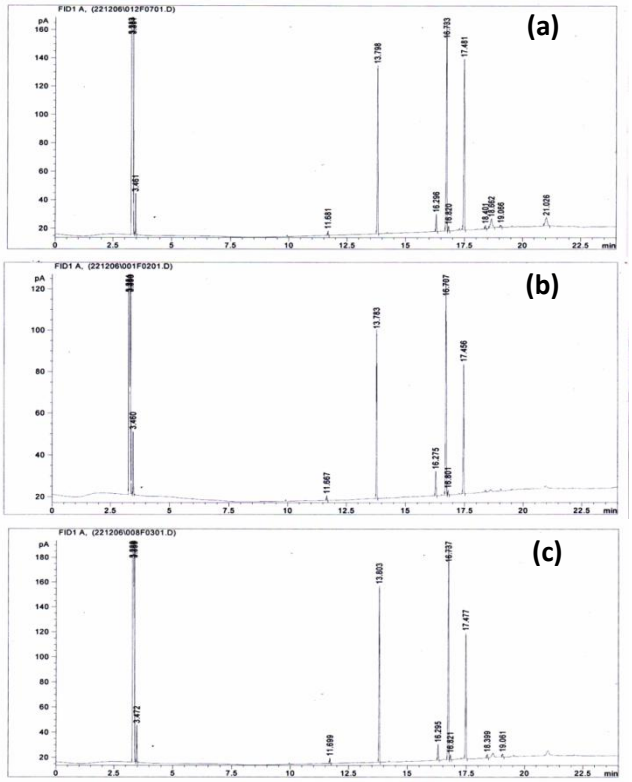

Figure 2. Representative chromatograms of blended oils, (a) POCO, (b) POSO and (c) PORBO.

The results indicate that POCO had the most reduction in FFA content compared with the other blended oils. Therefore, POCO is the most stable frying oil after mixing of PO with CO.

The high FFA value of the unheated oil can be explained by various factors prior to the purchase of oil, such as damaged packaging or old stocks of the oil. Besides, these oils were bought from the supermarkets with different storage conditions. Nevertheless, the FFA values were not significantly differed from our preliminary data (data not shown). Theoretically, acid value of an oil sample is dependent on the FFA value. A high FFA could also be attributed to a high acid value. However, FFA and acid values are differed in terms of the contribution of acid phosphates and amino acids in the determination of acid value (Wrostald et al., 2000). Moreover, the oil sample only contained FFA.

In this study, the oil samples were used for frying potato is 100% fat, but the sliced potato might have a high water content (Brown, 2011). Moisture and catalytic agents are known to cause hydrolytic rancidity of fat and increased FFA content in the frying oil (Berchmans and Hirata, 2008). As a result, formation of monoglycerides, diglycerides, and glycerol will speed up degradation of the remaining triacylglycerides by allowing a greater emulsification of water from food into the oil (Sangdehi, 2005). The increased in FFA values of these oils during 10 times of potato frying was due to the high water content in the potato slices as the water molecules escaped as steam that further accelerate hydrolysis of the oils. Besides, continuous heating of oil at high temperature (180°C) and exposure of fatty acid to air and light for extended duration promote chemical changes in the oil (Irwandiet al., 2005). Theoretically, FFA value of POCO should be increased that similar to PORBO and POSO. It is because heating process enhanced release of fatty acids from glycerol backbone. The reduction of FFA found for POCO after 20 times of potato frying might be due to the volatilization of the FFA since the oil was left overnight after each frying process.

3.2 Fatty acid composition

In this study, the types of fatty acid were identified based on the peaks obtained from GC chromatograms (Figure 2). Retention time of the identified fatty acid from oil sample was compared with retention time from FAME standard. Table 1 shows the fatty acid composition (FAC) of PO samples. FAC of the samples was expressed as percentage area of total fatty acids. In this study, PO had the highest level of SFA compared with the other blended oils. Before potato frying, total SFA of PO was 41.83%, and MUFA and PUFA were 45.12% and 13.05%, respectively. Palmitic acid (C16:0) was the major SFA in PO, 36.31% of total fatty acids. Other SFAs in PO were C13:0 (0.38%), C14:0 (0.06%), C15:0 (1.08%), C18:0 (3.83%), and C20:0 (0.15%). C18:1c was the major MUFA that contributed almost half of total fatty acids (44.70%), followed by C16:1 and C18:1t (0.23% and 0.20%). Besides that, the major PUFA was C18:2c (12.5% of total fatty acids), followed by C18:3n6 (0.26%) and C18:3n3 (0.29%). Further increased in the frequency of potato frying elevated the levels of SFA and MUFA, from 42.12% (10 times frying) to 44.54% (20 times frying), and from 47.38% (10 times frying) to 47.59% (20 times frying), respectively. On the contrary, the level of PUFA in PO reduced from 11.59% to 8.04%.

For the blended vegetable oil samples, the levels of PUFA, MUFA, and SFA ranged between 25% and 33%, between 37% and 43%, and between 28% and 33%, respectively (Tables 2-4). A highest percentage of PUFA was determined in POCO (33.39%), followed by POSO (27.93%) and PORBO (24.87%). Among the three blended oils, the major PUFAs were C18:2c (31.85% in POCO, 26.77% in POSO, and 23.34% in PORBO), followed by C18:3n3 (0.36% in POCO, 0.46% in POSO, and 0.41% in PORBO), C18:3n6 (0.53% in POCO, 0.41% in POSO, and 0.67%

in PORBO), and C18:2t (0.33% in POCO, 0.20% in POSO, and 0.17% in PORBO).Besides PUFA, the major MUFAs were C18:1c (37.73% in POCO, 42.66% in POSO, and 42.78% in PORBO) and C14:1 (0.17% in POCO and 0.08%in POSO only). In addition, C16:0 was the major SFA in the blended oils (24.74% in POCO, 23.32% in POSO, and 28.29% in PORBO). Other SFAs were C18:0 (2.87-4.93%), C15:0 (0.54-0.75%), C13:0 (0.19-0.20%), C20:0 (0.20-0.27%), and C14:0 (0.09-0.14%).

As shown in Table 2, POCO had the highest level of PUFA compared with the other blended oil samples. The oil mainly consisted of C18:2 (linoleic acid) and C18:3 (linolenic acid). These PUFAs are heat sensitive and easily expose to autoxidation (Frankel and Huang, 1994). The resistance of oils and fats to oxidation is depending onthe factors such as degree of unsaturation, presence of antioxidants or pro-oxidants, and prior abuse (Coppin and Pike, 2001). Antioxidants are proven to inhibit or delay oxidation of fats and lipids by slowing down the propagation of chain reaction of free radicals (Javanmardi et al., 2003). Thus, a high PUFA level in oil increases the rate of oil oxidation.

During intake of oil as source of energy (about 30% of total energy intake), the blended oils supply about 8.4-9.9% of total SFA, 11.1-12.9% of total MUFA, and 7.5-9.9% of total PUFA. With the increased frequency of frying, the composition of fatty acids in the three blended oil samples increased from 29-35% to 29-37% for total SFA and from 38-44% to 38-45% for total MUFA. However, after potato frying from 10 times to 20 times, total PUFA in these oil samples reduced from 22-33% to 19-32%. As observed, the total PUFA in PORBO reduced the most compared with POCO and POSO, whereas total SFA and total MUFA increased the most.

Total PUFA in PORBO reduced by 6.23% after 20 times of potato frying, while total SFA and total MUFA increased as much as 4.12% and 1.82%, respectively. It was followed by POSO, where its total PUFA was reduced by 1.75%, and its total SFA and total MUFA were increased by 0.65% and 1.00%, respectively. Besides, the reduction of total PUFA in POCO was determined as 1.67%, and increments of total SFA and total MUFA were 0.79% and 0.86%, respectively. Hence, after 20 times of potato frying, PO had a reduction in total PUFA of 5.01% and increased levels of total SFA and total MUFA by 2.71% and 2.47%, respectively. Therefore, we can conclude that among the three blended vegetable oils, POCO was the most resistant to oxidation, followed by PORBO and POSO.

In this study, blending of PO with other unsaturated vegetable oils (CO, SO, and RBO) resulted in a great change of FAC of the resulting oils. A previous study showed that blending of PO with the unsaturated vegetable oils reduced the level of SFA, increased MUFA and PUFA content (Noor Lida et al., 2002). The beneficial effect of PO appears to be due to the intrinsic quality from the blending with other PUFA-rich oil, which may contribute to the desirable effects onhuman plasma lipids. Although palmitic acid is a saturated fatty acid, its presence in PO does not increase blood cholesterol level (Edem, 2002). CO was selected since it is fairly stable towards oxidation. It contains a trace amount of C18:3 fatty acid that oxidize faster than C18:2 and 20 times faster than C18:1 (Ching, 2000). Even though SO contains a lower level of vitamin E than CO (Kamal-Eldin and Andersson, 1997), it has a good amount of other plant phenols that seem unique to SO (Shahidi et al., 1997). The plant phenols may act as antioxidants in maintaining oxidative stability of SO.

On the other hands, RBO was selected due to its potential advantage over the other edible oils with similar FAC. It contains high levels of tocopherols, tocotrienol, and γ-oryzanol, as well as its previously reported cholesterol lowering potential (Berger et al., 2003). Therefore, the results obtained from this study can promote the use of blended PO as a better frying oil.

Table 1. Fatty acid composition of palm oil at different frying times

Fatty acids	0 time (Unheated)	10 times	20 times
Tridecanoic acid (C13:0)	0.38 ± 0.05	0.37 ± 2.34	0.40 ± 15.22
Myristic acid (C14:0)	0.06 ± 12.55	0.52 ± 0.22	0.77 ± 6.85
Myristoleic acid (C14:1)	ND	ND	ND
Pentadecanoic acid (C15:0)	1.08 ± 22.33	0.99 ± 12.95	1.11 ± 12.22
Palmitic acid (C16:0)	36.31 ± 9.21	36.27 ± 10.22	38.33 ± 10.2
Palmitoleic acid (C16:1)	0.23 ± 13.2	0.14 ± 13.37	0.32 ± 13.0
Heptadecanoic acid (C17:0)	ND	ND	ND
Stearic acid (C18:0)	3.83 ± 12.0	3.73 ± 0.0	3.81 ± 12.38
Trans-elaidicacid (C18:1t)	0.2 ± 5.89	0.29 ± 6.21	0.25 ± 6,32
Oleic acid (C18:1c)	44.7 ± 3.33	46.95 ± 3.34	47.02 ± 4.0
Trans-linoelaidicacid (C18:2t)	ND	ND	ND
Linoleic acid (C18:2c)	12.5 ± 22.35	11.06 ± 20.00	7.60 ± 8.5
Gamma-linolenic acid (C18:3n6)	0.26 ± 24.2	0.24 ± 13.96	0.2 ± 13.97
Linolenic acid (C18:3n3)	0.29 ± 13.88	0.29 ± 13.34	0.24 ± 9.72
Arachidic acid (C20:0)	0.15 ± 0.0	0.24 ± 6.98	0.12 ± 7.0
Dihomo-gamma-linoleic acid (C20:3n6)	ND	ND	ND
Docosadienoic acid (C22:2)	ND	ND	ND
∑SFA	41.83 ± 9.99	42.12 ± 10.02	44.54 ± 10.3
∑MUFA	45.12 ± 22.31	47.38 ± 22.0	47.59 ± 22.58
∑PUFA	13.05 ± 19.2	11.59 ± 17.8	8.04 ± 19.45

Data are expressed as percentages of fatty acids (mean ± standard deviation). ND: not detected; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 2. Fatty acid composition of palm oil blended with corn oil (POCO) at different frying times

Fatty acids	0 time (Unheated)	10 times	20 times
Tridecanoic acid (C13:0)	0.19 ± 0.02	0.19 ± 0.17	0.19 ± 0.23
Myristic acid (C14:0)	0.14 ± 0.0	0.18 ± 0.0	0.2 ± 0.0
Myristoleic acid (C14:1)	0.17 ± 0.0	0.11 ± 0.0	0.09 ± 2.56
Pentadecanoic acid (C15:0)	0.55 ± 0.12	0.56 ± 0.37	0.55 ± 0.42
Palmitic acid (C16:0)	24.74 ± 3.32	25.15 ± 6.71	25.44 ± 4.58
Palmitoleic acid (C16:1)	ND	ND	ND
Heptadecanoic acid (C17:0)	ND	ND	ND
Stearic acid (C18:0)	2.87 ± 0.92	2.79 ± 4.11	2.82 ± 3.46
Trans-elaidicacid (C18:1t)	ND	ND	ND
Oleic acid (C18:1c)	37.73 ± 14.13	38.3 ± 7.03	38.67 ± 22.04
Trans-linoelaidicacid (C18:2t)	0.33 ± 1.02	0.35 ± 0.84	0.36 ± 1.07
Linoleic acid (C18:2c)	31.85 ± 3.95	31.22 ± 6.58	30.61 ± 7.5
Gamma-linolenic acid (C18:3n6)	0.53 ± 0.74	0.44 ± 1.97	0.4 ± 1.81
Linolenic acid (C18:3n3)	0.36 ± 1.19	0.3 ± 2.83	0.28 ± 2.82
Arachidic acid (C20:0)	0.23 ± 1.64	0.31 ± 0.36	0.3 ± 0.64
Dihomo-gamma-linoleic acid (C20:3n6)	ND	ND	ND
Docosadienoic acid (C22:2)	0.32 ± 0.0	0.10 ± 0.0	0.07 ± 0.0
∑SFA	28.71 ± 9.83	29.18 ± 9.99	29.50 ± 9.84
∑MUFA	37.90 ± 26.54	38.41 ± 26.99	38.76 ± 27.28
∑PUFA	33.39 ± 14.07	32.41 ± 13.83	31.72 ± 13.57

Data are expressed as percentages of fatty acids (mean ± standard deviation). ND: not detected.

Table 3. Fatty acid composition of palm oil blended with sesame oil (POSO) at different frying times

Fatty acids	0 time (Unheated)	10 times	20 times
Tridecanoic acid (C13:0)	0.2 ± 0.06	0.2 ± 0.35	0.2 ± 0.36
Myristic acid (C14:0)	0.09 ± 2.62	0.13 ± 0.0	0.11 ± 0.0
Myristoleic acid (C14:1)	0.08 ± 2.48	0.08 ± 2.96	0.1 ± 3.36
Pentadecanoic acid (C15:0)	0.54 ± 0.75	0.54 ± 0.6	0.55 ± 0.5
Palmitic acid (C16:0)	23.32 ± 8.41	23.47 ± 9.65	24.13 ± 9.68
Palmitoleic acid (C16:1)	ND	ND	ND
Heptadecanoic acid (C17:0)	0.17 ± 0.03	0.39 ± 5.2	0.47 ± 7.85
Stearic acid (C18:0)	4.93 ± 2.01	4.80 ± 7.25	4.37 ± 20.23
Trans-elaidicacid (C18:1t)	ND	ND	ND
Oleic acid (C18:1c)	42.66 ± 16.92	43.26 ± 16.88	43.64 ± 41.71
Trans-linoelaidicacid (C18:2t)	0.2 ± 0.9	0.42 ± 0.75	0.51 ± 1.27
Linoleic acid (C18:2c)	26.77 ± 11.66	25.79 ± 5.61	24.59 ± 10.04
Gamma-linolenic acid (C18:3n6)	0.41 ± 0.67	0.2 ± 1.75	0.18 ± 1.51
Linolenic acid (C18:3n3)	0.46 ± 0.89	0.33 ± 4.76	0.26 ± 2.74
Arachidic acid (C20:0)	0.2 ± 0.53	0.25 ± 0.0	0.27 ± 0.0
Dihomo-gamma-linoleic acid (C20:3n6)	0.09 ± 0.83	0.12 ± 0.0	0.64 ± 0.0
Docosadienoic acid (C22:2)	ND	ND	ND
∑SFA	29.45 ± 8.61	29.78 ± 8.64	30.10 ± 8.87
∑MUFA	42.74 ± 30.11	43.34 ± 30.53	43.74 ± 30.79
∑PUFA	27.93 ± 11.84	26.86 ± 12.72	26.18 ± 10.82

Data are expressed as percentages of fatty acids (mean ± standard deviation). ND: not detected.

Table 4. Fatty acid composition of palm oil blended with rice bran oil (PORBO) at different frying times

Fatty acids	0 time (Unheated)	10 times	20 times
Tridecanoic acid (C13:0)	0.2 ± 0.38	0.22 ± 25.12	0.22 ± 0.56
Myristic acid (C14:0)	ND	ND	ND
Myristoleic acid (C14:1)	ND	ND	ND
Pentadecanoic acid (C15:0)	0.75 ± 0.93	0.8 ± 0.15	0.85 ± 2.67
Palmitic acid (C16:0)	28.29 ± 46.3	29.8 ± 7.57	32.01 ± 38.88
Palmitoleic acid (C16:1)	ND	ND	ND
Heptadecanoic acid (C17:0)	ND	ND	ND
Stearic acid (C18:0)	2.85 ± 6.36	3.24 ± 1.23	3.11 ± 15.22
Trans-elaidicacid (C18:1t)	ND	ND	ND
Oleic acid (C18:1c)	42.78 ± 15.74	43.61 ± 25.12	44.60 ± 4.67
Trans-linoelaidicacid (C18:2t)	0.17 ± 1.0	0.18 ± 0.36	0.18 ± 1.31
Linoleic acid (C18:2c)	23.34 ± 29.29	20.38 ± 5.87	17.53 ± 39.64
Gamma-linolenic acid (C18:3n6)	0.67 ± 2.57	0.55 ± 0.4	0.32 ± 2.23
Linolenic acid (C18:3n3)	0.41 ± 3.4	0.64 ± 0.19	0.47 ± 3.4
Arachidic acid (C20:0)	0.27 ± 3.14	0.36 ± 0.27	0.29 ± 3.72
Dihomo-gamma-linoleic acid (C20:3n6)	0.08 ± 0.96	ND	0.09 ± 1.8
Docosadienoic acid (C22:2)	0.2 ± 2.97	ND	0.05 ± 0.03
∑SFA	32.36 ± 12.25	34.41 ± 12.87	36.48 ± 13.87
∑MUFA	42.78 ± 0.45	43.61 ± 25.12	44.6 ± 4.67
∑PUFA	24.87 ± 9.41	21.97 ± 8.19	18.64 ± 7.07

Data are expressed as percentages of fatty acids (mean ± standard deviation). ND: not detected.

Also, blending of PO with PUFA-rich oil gives a well-balanced FAC and a high amount of vitamin E in the blended oil (Cottrell, 1991; Ong, 1994). Besides that, blending of partially saturated oil such as PO with high-PUFA oil (CO, SO or RBO) is beneficial in terms of reduction in FFA content and increased resistance towards oxidation and hydrolysis.

4. Conclusions

Among the blended oils, POCO was the most stable form of frying oil, followed by PORBO and POSO. After potato frying for 20 times, SFA, PUFA, and MUFA contents in POCO did not change much in comparison to POSO and PORBO. Although PO is the most stable oil and can be reused after repeated heating, it has moderately high level of SFA. Blending of PO with PUFA-rich oils is an alternative choice for deep-frying, where the results obtained from this study showed the beneficial side of the blended oils. Due to the present study was performed based on a small-scale laboratory experiment, future studies need to consider kinetic stability of the blended oils in a larger setting.

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ANTIOXIDATIVE AND SENSORY PROPERTIES OF INSTANT COFFEE FORTIFIED WITH GALACTOSE-FISH SKIN GELATIN HYDROLYSATE MAILLARD REACTION PRODUCTS

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ABSTRACT

Antioxidative Maillard reaction product (MRP) prepared from the mixture of galactose-gelatin hydrolysate from unicorn leatherjacket skin was fortified in instant coffee brew at different levels (0, 0.5, 1.0 mg/mL) and the resulting brew was characterised. Addition of MRP up to 1.0 mg/mL had no effect on browning index, L*, a*, b*, ΔE* and ΔC* of coffee brew (P>0.05). The pH value of resulting instant coffee brew was decreased with increasing MRP level added (P<0.05). ABTS radical scavenging activity and ferric reducing antioxidant power of coffee brew increased as MRP levels increased (P<0.05). Based on sensory evaluation, there were no differences in likeness score between coffee brew fortified with MRP at all levels used and the control (without MRP). Much higher abundances of cyclohexanone and dimethydisulfide were observed in coffee brew added with MRP, compared with the control. Thus, MRP could serve as the rich source of antioxidant, which could be supplemented in coffee brew without negative effect.

1. Introduction

Maillard reaction products (MRPs) have the wide applications in several foods. MRPs showed antioxidative activity in biscuit (Goya, 2009), cereals (Rufiãn-Henares and Delgado-Andrade 2009), coffee (Goya et al., 2007) and nut (Açar et al., 2009). Antioxidative xylan-chitosan MRPs were able to retard lipid oxidation in the refrigerated pork meat (Li et al., 2013). Although MRPs have been shown as the potential antioxidants in a variety of foods, the practical use of MRP in food industry has been limited. This is due to the fact that MRPs have their own characteristic dark colour, bitter taste and peculiar flavour, which cause the negative impact on the finished products.

Recently, MRP derived from the mixture of galactose and gelatin hydrolysate of unicorn leatherjacket skin (2:1, w/w) has been reported to show higher ABTS radical scavenging activity and reducing power than mother gelatin hydrolysate (Karnjanaratum et al., 2015). Furthermore, the MRP also had *in vitro* cellular bioactivities including antioxidant activity, immunomodulatory potential and anticancer effects in different cell lines. MRP demonstrated *in vitro* suppression of oxidative stress and inflammation and was able to inhibit the proliferation of human colon cancer cells (Karnjanapratum et al., 2015b). Therefore, antioxidative MRP derived from fish skin gelatin hydrolysate could be used as an alternative antioxidant or a functional ingredient. Additionally, it can be supplemented or fortified in the foods or drinks with dark colour, e.g. coffee brew, etc. The

present study therefore aimed to investigate the effect of the fortification of antioxidative Maillard reaction product (MRP) derived from gelatin hydrolysates of unicorn leatherjacket skin on characteristics, antioxidative and sensory properties of fortified coffee brew.

2. Materials and methods

2.1. Chemicals

Instant coffee (Nestle, Quality coffee product Ltd., Chachoengsao, Thailand) was purchased from a local market in Hat Yai, Songkhla, Thailand. D-galactose and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were procured from Fluka (Buchs, Switzerland). 2,2′-azinobis (3- thylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

2.2.Preparation of Maillard reaction product from gelatin hydrolysate of unicorn leatherjacket skin

2.2.1. Preparation of fish skins

The skins of unicorn leatherjacket (*Aluterus monoceros*) were obtained from a dock, Songkhla, Thailand. Skins were pooled and used as a composite sample. The skins were washed with iced tap water (0-2°C) and cut into small pieces (0.5×0.5 cm^2).

The skins were subjected to pretreatment as per the method of Kaewruang et al. (2013). Fish skins (0.5×0.5 cm^2) were soaked in 0.05 M NaOH with a skin/alkaline solution ratio of 1:10 (w/v). The pretreatment solution was changed after 2 h and total pretreatment time was 4 h. Skins were then washed with tap water until neutral or faintly basic pH of wash water was obtained. Subsequently, pretreated skins were autolysed following the method of Karnjanapratum and Benjakul (2015). The resulting autolysed skins were used for preparation of gelatin hydrolysate.

2.2.2. Preparation of glycyl endopeptidase

Glycyl endopeptidase (GE) was fractionated from papaya latex using the method of Karnjanapratum and Benjakul (2014). Aqueous two phase system (ATPS) with 10% PEG 6000 and 10% ammonium sulphate $(NH_4)_2SO_4$ was used for fractionation of GE. The obtained GE was stored at -40 °C until use.

2.2.3. Preparation of gelatin hydrolysate

Autolysed skin suspension (3%, w/v) was mixed with GE (8%, based on solid matter) and incubated at 40 °C for 60 min (Karnjanapratum and Benjakul, 2015). After enzyme inactivation by heating at 90 °C for 15 min, the resulting gelatin hydrolysate was centrifuged at 9,000×g at 4 °C for 20 min using a refrigerated centrifuge, model Avanti J-E (Beckman Coulter, Inc., Palo Alto, CA, USA). The supernatant was collected and lyophilised using Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). The gelatin hydrolysate (GH) was collected and placed in polyethylene bag. GH was stored at -20 °C until used.

2.2.4. Preparation of Maillard reaction product (MRP)

Maillard reaction between GH and galactose was conducted following the method of Karnjanapratum et al. (2015a). The mixture of GH and galactose (2:1, w/w) was heated at 70 °C and 55% relative humidity for 36 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting galactose-gelatin hydrolysate MRP was collected, analysed and fortified into coffee brew.

2.3. Fortification of MRP from gelatin hydrolysate in coffee brew

Coffee brew was prepared by adding 2 g of instant coffee powder and MRP in hot water (75 °C) to obtain 100 mL of different final MRP concentrations (0, 0.5, 1 mg/mL). The resulting coffee brew samples were subjected to analyses.

2.4. Analyses

2.4.1 Browning index

Prior to measurement, the samples were appropriately diluted (50-fold) using distilled water and the absorbance was measured at 420 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). A_{420} was used as the index of browning intensity (Ajandouz et al., 2011).

2.4.2. Colour

Colour was measured by Hunter Lab (C04-1005-631 colorFlex, Reston, VA, USA). The value was expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/ blueness) values. Total difference in colour (ΔE*) and chroma (ΔC*) were calculated according to the following equation (1) and (2), respectively (Gennadios et al., 1996; Jangchud and Chinnan, 1999).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$\Delta C^* = \sqrt{\Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

where ΔL*, Δa*and Δb* are the differences between the corresponding colour parameter of the sample and that of black standard (L*= 0.63, a*= -0.39 and b*= -1.02).

2.4.3. pH

The pH value of coffee brew was measured using a pH meter (Sartorius PB-10, Göttingen, Germany).

2.4.4. Antioxidative activities

2.4.4.1. ABTS radical scavenging activity

ABTS radical scavenging activity was determined as described by Binsan et al. (2008). Sample with appropriate dilution (10-fold, 150 μL) was mixed with 2850 μL of ABTS solution and the mixture was left at room temperature for 2 h in the dark. The absorbance was then measured at 734 nm using a spectrophotometer (Binsan et al., 2008). A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was expressed as μmol Trolox equivalent (TE)/mL sample.

2.4.4.2. Ferric reducing antioxidant power (FRAP)

FRAP reagent was prepared by mixing acetate buffer (30 mM, pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM iron (III) chloride solution at a proportion of 10:1:1 (v/v/v) (Benzie and Strain, 1996). The sample solution (120-fold diluted, 100 μL) was mixed with 3 mL of working FRAP reagent and incubated in the dark at room temperature for 30 min. The absorbance of the reaction mixture was read at 593 nm using a spectrophotometer (Benzie and Strain, 1996). The standard curve was prepared using Trolox ranging from 0 to 500 μM. The activity was expressed as mmol TE/mL sample.

2.4.4.3. Ferrous chelating activity

Chelating activity of samples towards ferrous ion (Fe^{2+}) was measured by the method of Thiansilakul et al. (2007) with a slight modification. Sample (200 μL) was mixed with 800 μL of distilled water. Thereafter, 0.1 mL of 2.0 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine were added. The mixture was allowed to react for 20 min at room temperature. The absorbance was then read at 562 nm. The standard curve of EDTA (0-1.0 mM) was prepared. The control was prepared in the same manner except that distilled water was used instead of the sample. Ferrous chelating activity was expressed as μmol EDTA equivalents (EE)/mL sample.

2.4.4.4. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was assayed according to the method of Wettasinghe and Shahidi (2000) with a slight modification. Briefly, the sample (1 mL) was mixed with 83 μL of 100 mM hydrogen peroxide (prepared in 0.1 M phosphate buffer, pH 7.4). The mixture was allowed to react for 40 min at room temperature. The absorbance at 230 nm of the reaction mixture was read and the sample blank (devoid of hydrogen

peroxide) was used for background subtraction. Trolox (0-10 mM) was used as the standard. The hydrogen peroxide scavenging activity was expressed as mmol TE/mL sample.

2.4.5. Sensory evaluation

Fifty panelists were randomly recruited from Faculty of Agro-Industry, Prince of Songkla University. Panelists were selected for evaluation of samples when they consumed at least one cup of coffee per day. Panel included 38 % males and 62 % females. Fifty-five percent of the panelists were older than 25 years. Most panelists (97 %) preferred coffee with milk/cream and sugar.

The coffee brews were prepared freshly in a thermostatically controlled flask (75 °C) and evaluated within 15 min. Each panelist received one cup of coffee (100 mL containing 2 g instant coffee without and with MRP at various levels) separately. All samples were blind-coded with three-digit randomised numbers. Panelists were instructed to add milk/cream and sugar if preferred. A sufficient number of 3 g-cream sachets and 6 g-sugar sachets, were provided. The panelists expressed their liking of each sample using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (Meilgaard et al., 1999). Appearance, colour, aroma, flavour, taste and overall likeness were assessed.

2.5. Study on volatile compounds in coffee brews without and with MRP addition

The volatile compounds in coffee brew fortified with 1 mg/mL MRP were determined using a solid-phase microextraction gas chromatography mass spectrometry (SPME GC-MS) following the method of Iglesias and Medina (2008) in comparison with coffee brew without MRP addition as well as MRP.

2.5.1. Extraction of volatile compounds by SPME fibre

The samples were heated at 60 ºC in 20 headspace vial with equilibrium time of 10 h. The SPME fibre (50/30 μm DVB/Carboxen™/ PDMS StableFlex™) (Supelco, Bellefonte, PA, USA) was conditioned at 270 ºC for 15 min before use and then exposed to the headspace. The 20 mL-vial (Agilent Technologies, Palo Alto, CA, USA) containing the sample extract and the volatile compounds were allowed to absorb into the SPME fibre at 60 ºC for 1 h. The volatile compounds were then desorbed in the GC injector port for 15 min at 270 ºC.

2.5.2. GC–MS analysis

GC–MS analysis was performed in a Trace Ultra gas chromatography coupled with a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific Inc., San Jose, CA, USA) and equipped with a splitless injector. Compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m ± 0.25 mm ID, with film thickness of 0.25 μm). The GC oven temperature program was: 35 ºC for 3 min, followed by an increase of 3 ºC/min to 70 ºC, then an increase of 10 ºC/min to 200 ºC, and finally an increase of 10 ºC/min to a final temperature of 260 ºC and holding for 5 min. Helium was employed as a carrier gas with a constant flow of 1.5 mL/min. The injector was operated in the splitless mode and its temperature was set at 260 ºC. Transfer line temperature was maintained at 265 ºC. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and source temperature was set at 200 ºC. Initially, full-scan-mode data was acquired to determine appropriate masses for the later acquisition in scan mode under the following conditions: mass range: 10-200 amu and scan rate: 0.220 s/scan. All analyses were performed with ionisation energy of 70 eV, filament emission current at 150 μA, and the electron multiplier voltage at 500 V.

2.5.3. Analyses of volatile compounds

Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search

(Wiley 275.L). Quantification limits were calculated to a signal-to-noise (S/N) ratio of 10. The identified volatile compounds were presented in the term of abundance of each identified compound (peak area).

2.6. Statistic analysis

Experiments were run in triplicate using three lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussions

3.1. Browning index, colour, pH and antioxidative activities of coffee brew fortified with MRP

Browning index, colour and pH values of coffee brew added with MRP at different concentrations (0-1 mg/mL) are shown in Table 1. In general, there were no differences in A_{420}, L*, a*, b*, ΔE* and ΔC* values (P>0.05) between the coffee brew without and with MRP at various levels added. The result suggested that the fortification of MRP in coffee brew at the tested concentrations did not affect the colour of resulting coffee brew. Nevertheless, the decreases in pH of coffee brew added with MRP were found with increasing MRP level used (P<0.05). It was reported that galactose-gelatin hydrolysate MRP had the acidic pH due to the production of formic and acetic acids from the reducing sugar, which was partially degraded into these compounds via Maillard reaction (Karnjanapratum et al., 2015a; Rufiãn-Henares et al., 2006). Therefore, the addition of MRP more likely led to the decrease in pH of resulting coffee brew.

Antioxidative activities of coffee brew fortified with MRP at different concentrations were evaluated and expressed as ABTS radical scavenging activity, ferric reducing antioxidant power (FRAP), hydrogen peroxide scavenging activity and ferrous chelating activity as shown in Figure 1A, B, C and D, respectively. All antioxidative activities tested of coffee brews increased with increasing MRP levels (P<0.05). Karnjanapratum et al. (2015a) reported that the highest antioxidative activity was obtained when MRP was prepared from the mixture of unicorn leatherjacket skin gelatin hydrolysate and galactose (2:1) at 70 °C and 55% RH for 36 h. The resulting MRPs had the increases in ABTS radical scavenging activity and FRAP by 15 and 150-fold, respectively, compared with those of the mother GH (Karnjanapratum et al., 2015a). Antioxidative Maillard reaction products with radical scavenging, reducing and chelating abilities had been produced by heat treatment of several protein-sugar model systems such as silver carp protein hydrolysate-glucose (You et al., 2011) and hydrolyzed β-lactoglobulin-glucose (Dong et al., 2012) systems. The results revealed that MRP could serve as the rich source of antioxidants with radical and H_2O_2 scavenging activities. It was also capable of providing electron as well as chelating ferrous ion. From our previous study, the mild cytotoxic activity of MRP was demonstrated in human hepatoma (HepG2) and human histiocytic lymphoma (U937) cell lines with IC_{50} of 1.0 and 1.25 mg/mL, respectively. Moreover, MRP (0-1.50 mg/mL) had a very low cytotoxicity in mouse macrophages (RAW264.7) cells, in which an IC_{50} value for MRP could not be determined (Karnjanapratum et al., 2015b). Additionally, MRP did not cause DNA damage to U937 cell lines under tested concentrations (750-1500 μg/mL) (Karnjanapratum et al., 2015b). The fortification of MRP could therefore increase the antioxidative activity of coffee brew. Furthermore, MRP showed very weak cytotoxic effect without DNA damage against cell line.

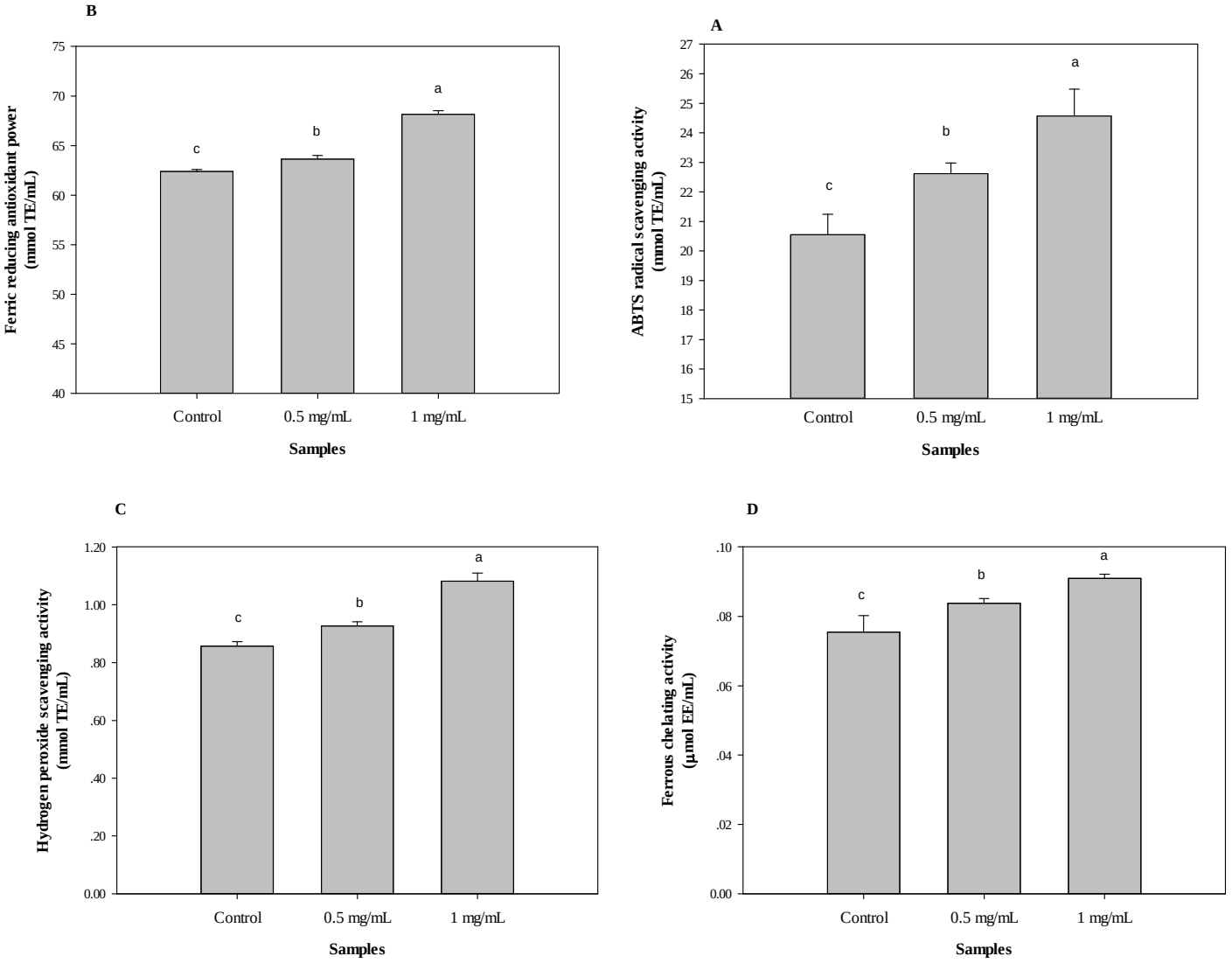

Figure 1. ABTS radical scavenging activity (A), ferric reducing antioxidant power (B), hydrogen peroxide scavenging activity (C) and ferrous chelating activity (D) of coffee brew added with galactose-fish gelatin hydrolysate MRP at different concentrations. Bars represent standard deviation (n=3). Different lowercase letters on the bars indicate the significant differences ($P < 0.05$).

3.2. Sensory property of instant coffee brew fortified with MRP

Likeness score of coffee brew added with MRP at various levels is shown in Table 2. There was no difference in likeness score for all attributes including appearance, colour, aroma, flavour, taste and overall amongst all samples ($P>0.05$). This result indicated that the addition of MRP up to 1 mg/mL had no effect on the likeness of coffee brew. All samples had likeness score of approximately 7 for all attributes, suggesting the moderate likeness of coffee brew, regardless of MRP fortification ($P>0.05$). Thus, the incorporation of MRP could enhance antioxidative activity of resulting coffee brew without the negative effect on sensory property. This was plausibly due to the strong odour or smell of coffee brew, which could mask the typical flavour or odour of MRP.

Table 1. Browning index, colour and pH of coffee brew added with galactose-fish skin gelatin hydrolysate MRP at different concentrations

Samples	Browning index (A_{420})^{ns}	Color					pH
		L^{*ns}	a^{*ns}	b^{*ns}	ΔE^{*ns}	ΔC^{*ns}	
Control	12.00±0.52	5.54±0.23	4.61±0.63	5.59±0.27	9.55±0.19	6.18±0.34	4.85±0.02^{a}
0.5 mg/mL	12.86±1.97	5.36±0.13	4.57±0.48	5.54±1.17	9.55±0.62	6.16±0.66	4.80±0.02^{b}
1 mg/mL	13.53±0.74	5.63±0.19	4.70±0.87	5.32±1.34	9.84±0.74	6.13±0.62	4.75±0.02^{c}

Values represent the mean ± SD (n=3). A_{420}: the absorbance at 420 nm.
^{ns} non-significant differences ($P < 0.05$) between values in the same column.
Different lowercase superscripts in the same column indicate significant differences ($P < 0.05$).

Table 2. Liking score of coffee brew added with galactose-fish skin gelatin hydrolysate MRP at different concentrations

Samples	Appearance^{ns}	Colour^{ns}	Aroma^{ns}	Flavour^{ns}	Taste^{ns}	Overall^{ns}
Control	7.62±0.82	7.65±0.92	6.68±1.43	6.97±1.29	7.0±1.30	7.0±1.26
0.5 mg/mL	7.74±0.75	7.65±0.81	6.88±1.47	6.94±1.28	6.79±1.53	6.85±1.41
1.0 mg/mL	7.41±0.92	7.47±0.90	6.91±1.44	7.12±1.59	7.0±1.65	7.06±1.30

Values represent the mean ± SD (n=3). ^{ns} non-significant differences ($P < 0.05$) between values in the same column.

Table 3. Volatile compounds in coffee brew without and with galactose-fish skin gelatin hydrolysate MRP

Compounds	Abundance×10^8		
	Coffee brew		MRP
	without MRP	with MRP*	
2-Methylbutanal	377.56	327.56	nd
1-Methylpyrrole	42.30	55.15	nd
Pyridine	212.46	144.88	nd
Furfuryl methyl ether	25.81	30.79	nd
p-Cymene	13.83	4.28	nd
Methylpyrazine	152.70	153.22	4.55
Cyclohexanone	1.68	95.25	135.41
2,5-Dimethylpyrazine	51.86	46.01	nd
Ethylpyrazine	135.90	69.53	nd
2,3-Dimethylpyrazine	24.22	17.75	nd
2-Ethyl-6-methylpyrazine	44.14	49.94	nd
2-Methyl-6-methylpyrazine	32.45	31.13	nd
2-Ethyl-3-methylpyrazine	35.24	36.40	nd
2-Ethyl-3,5-dimethylpyrazine	24.03	24.28	nd
Furfural	110.08	110.16	39.97
2-Furfurylmethylsulfide	37.14	45.47	nd
2-Acetylfuran	22.12	26.26	7.94
Benzaldehyde	30.09	28.67	15.60
Furfuryl acetate	44.28	60.55	nd
5-Methyl-2-furfural	56.09	58.18	5.79
2-Furfurylfuran	30.26	35.12	nd

2-Formyl-1-methylpyrrole	24.69	36.21	nd
Furfuryl alcohol	116.32	94.00	nd
5-Methyl-2-furfurylfuran	18.95	20.63	nd
1-(2-furfuryl)pyrrole	24.12	28.27	nd
2-Methoxyphenol	22.13	21.67	nd
2-Acetylpyrrole	7.33	7.03	2.91
2-Formylpyrrole	9.37	8.60	nd
p-Ethylguaiacol	7.76	8.20	nd
4-Vinyl-2-methoxy-phenol	5.69	5.75	nd
2,4-Ditert-butylphenol	3.29	5.05	12.79
Methyleugenol	nd	13.19	2.90
Pyrrole	nd	12.90	5.76
2-Ethyl hexanol	nd	14.95	11.98
Acetol acetate	nd	16.31	0.38
Diethyl-2,6-pyrazine	nd	14.34	1.50
2-Methyl-2-cyclopentenone	nd	5.96	5.53
3-Heptanone	nd	7.40	7.16
2-Methylfuran	nd	4.14	0.26
Dimethyldisulfide	nd	228.74	302.80
Dimethyltrisulfide	nd	nd	5.53
5-Nonanone	nd	nd	1.19
Nonanal	nd	nd	2.85
Cyclohexanol	nd	nd	5.91
Benzeneacetaldehyde	nd	nd	3.21
Benzeneacetonitrile	nd	nd	3.46

nd: not detected. MRP: Maillard reaction product. *MRP at 1 mg/mL was added.

Thus, MRP could be fortified to coffee brew up to 1 mg/mL, in which antioxidative activity was enhanced.

3.3. Volatile compounds of instant coffee brew fortified with MRP

Volatile compounds present in coffee brew without and with MRP addition in comparison with those of MRP are shown in Table 3. Thirty volatile compounds were identified and quantified in coffee brew (without MRP). Those included aldehydes, ketones, furans, pyrroles, pyrazines and others, which were commonly found in coffee brew (Petisca et al., 2013; Lopez-Galilea et al., 2006; Sanz et al., 2002). 2-Methylbutanal, pyridine, methylpyrazine, ethylpyrazine, furfural and furfuryl alcohol were found as the major volatile compounds in instant coffee. Lopez-Galilea et al. (2006) reported that 2-methylbutanal was responsible for chocolate-like and fruity note. Pyridine and pyrazines are well-known as Maillard reaction products and give characteristic roasted or toasted flavours to coffee (Moon and Shibamoto, 2009). On the other hand, furfural and furfuryl alcohol do not have a high odorant impact (Lopez-Galilea et al., 2006). Similar volatiles were found in instant coffee brew added with MRP. However, some compounds including cyclohexanone and dimethyldisulfide were much higher in abundance, compared with the control. It was noted that methyleugenol, pyrrole, 2-ethyl hexanol, acetol acetate, diethyl-2,6-pyrazine, 2-methyl-2-cyclopentenone, 3-heptanone, 2-methylfuran and dimethyldisulfide were found

only in coffee brew added with MRP. Moreover, other volatile compounds were found in both coffee brew (control) and MRP added sample at similar levels. When considering volatiles in MRP, cyclohexanone and dimethyldisulfide were found as the major constituents. Those compounds were detected as dominant volatiles in coffee brew when MRP was fortified. Volatile compounds from MRP were reported in coffee such as 2-ethyl hexanol, diethyl-2,6-pyrazine, 2-methyl-2-cyclopentenone, 2-methylfuran, methyldisulfide and cyclohexanal (Petisca et al., 2013; Lopez-Galilea et al., 2006; Sanz et al., 2002). In general, the Maillard reaction was responsible for the generation of roasted, toasted or caramel-like aromas as well as the development of brown colour in foods (Sanz et al., 2002). Nevertheless, sensory test revealed that the volatiles in MRP at levels tested had no impact on the likeness of coffee brew.

4. Conclusions

Fortification of galactose-fish skin gelatin hydrolysate MRP in coffee brew could increase ABTS radical scavenging activity, FRAP, hydrogen peroxide scavenging activity and ferrous chelating activity. The addition of MRP had no effect on colour and sensory property of resulting coffee brew, though MRP was rich in 2-methylbutanal and dimethyldisulfide. Therefore, MRP derived from fish skin hydrolysate could be used as an alternative antioxidant, which was used as supplement in foods or drinks, particularly coffee brew.

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EMULSION PROPERTIES OF BEEF AS AFFECTED BY pH AND IONIC STRENGTH OF PEA SOLUTION

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ABSTRACT

The effects of pH (3.5-8.5) and ionic strength (0.2-0.7 M NaCl) of pea solution (2%) on the emulsion properties of beef were studied using a model system. A central composite rotatable design was used to determine the response surface. pH and ionic strength had significant effects on the emulsion properties of beef. pH and ionic strength increased emulsion capacity (EC), emulsion stability (ES) and emulsion activity (EA). Emulsion density (ED) decreased with increasing pH and increased with ionic strength until a critical point, which was reached at an ionic strength of 0.348 M and a pH of 6.74. pH of pea solution had a quadratic effect on the studied emulsion characteristics which were minimum between pH of 5.5-6.5.

1. Introduction

In recent years pea products have been recognized as a valuable and low cost source of plant products. They have increasing potential to contribute to food manufactures as a more sustainable and cheaper alternative to animal proteins (Munialo et al., 2014). Pea products have presented as flour, concentrates and isolates. Pea is a good source of protein which consists of legumin (11S), vicillin (7S) and albumins (2S), where 11S and 7S are the most abundant ones (Söderberg, 2013, O'Kane et al., 2005). It contains around 25% proteins, 58% starch and 12% fibre (Söderberg, 2013, Polesi et al., 2011). They have a positive effect on health. Human nutritional interest in pea proteins has focused on carbohydrates in the form of fibres, and particularly high levels of lysine which can be used to balance the deficiencies of this essential amino acid in diets (Tian et al., 1999, Chel-Guerrero et al. 2007, Barac et al., 2015).

Pea products in formulations of food products are the most important factor for product quality, technological properties. The technological properties of pea proteins play a significant role in the emulsification (Franco et al. 2000, Gharsallaoui et al. 2009, Barać et al. 2015). Pea protein isolates contain soluble proteins with good gel formation, emulsification and water-binding properties that are needed for applications in processed meat system (Tarté, 2009). Protein solubility is strictly related to emulsion properties of meat products (Schut, 1976, Barać et al., 2015). It is affected by extrinsic factors like pH, ionic strength and temperature (Söderberg 2013, Bolontrade et al., 2013). Water holding and oil absorption capacities increase in proportion to protein contents of the flour, protein fraction,

and isolate of pea (Sosulski and McCurdy, 1987).

In emulsified meat products, the technological properties of proteins and their interaction with polysaccharide play an important role. Among the meat proteins, myofibrillar proteins with ionic strength have high solubility (Schut, 1976). Their increasing solubility and interactions with non-meat proteins and polysaccharides affect emulsifying properties of meat systems. Kinetically stable emulsions can be formed by adding emulsifiers and/or thickening agents, such as proteins and polysaccharides, to overcome the activation energy of the system (Sun et al., 2007). To make pea products more versatile as a food ingredient, an understanding of the relationship between the physico-chemical properties of these products and meat emulsions is important. The objective of this study was to determine the effects of modified physicochemical properties of ground pea on the emulsion properties of beef.

2. Materials and methods

The meat source used in this study was beef (M. Semimembranosus muscle). Meat and refined sunflower oil were obtained from local markets. Ground pea (protein 24%, fat 2%, carbohydrate 52.5%, fibre 12%) was obtained from Kimbiyotek (Istanbul, Turkey). Analytical grade chemicals were used.

2.1. Meat sample, homogenate and pea solution preparation

The muscles were ground using a grinder (Tefal, Le Hachoir 1500, France) with a 3 mm diameter hole plate. Each meat sample was divided into equal lots and packaged by using medium polyethylene and this was stored as –22 ºC until it was used.

Homogenate was prepared as; 100 ml of water and 25 g ground meat were placed into a blender (Waring 80011S, USA) jar and comminuted for 1 min at 18.000 rpm. Then it was filtered through a cheese cloth to remove rough pieces of connective tissue. Pea solution (2%) was prepared as; required amount of pea, NaCl and water placed into a blender jar (Waring 80011S, USA) and mixed for 1 min at 18.000 rpm. Then pH of pea solution was adjusted to required pH using either 0.5-1 N HCl or 0.5-1 N NaOH.

2.2. pH determination

The pH of the prepared pea solution and emulsion were measured by a pH meter (Hanna 2215, USA) equipped with temperature probe.

2.3. Emulsion capacity (EC)

EC is the maximum amount of oil emulsified by a unit of protein. It was determined by using a model system described by Ockerman (1985). The end point was determined as described by Webb et al. (1970). 12.5 ml homogenate, 37.5 ml of pea solution and 50 ml of oil were placed into the blender (Kenwood KM010, UK) jar at first. During emulsification at 6500 rpm, oil was added at a rate of 0.5-0.6 ml/sec until the emulsion broke. Water was circulated around the burette as to maintain the oil at a constant temperature of 20oC. The electrical conductivity of the emulsion was monitored with a microprocessor. At the breaking point of the emulsion, conductivity rapidly dropped and the addition of oil was stopped. The amount of oil added including the first 50 ml was recorded as the EC.

2.4. Emulsion preparation

25 ml of homogenate solution, 75 ml pea solution and 70 ml of oil were placed in the blender jar (Kenwood KM010, UK). During emulsification at 6,500 rpm, 50 ml of oil was added at a rate of 0.5-0.6 ml/s. After all the oil was added, the emulsion was mixed for an additional 5 s.

2.5. Emulsion stability

ES was determined using model systems, as described by Ockerman (1985) and Zorba et al. (1993). Raw emulsion (10 g) was weighed into

a centrifuge tube, which was capped and immediately heated at 80°C in a water bath for 30 min. The tubes were transferred in a cold water bath immediately and cooled to approximately 25°C. Then, the emulsion was held for temperature standardisation for 1 h at ambient conditions and then centrifuged at 350 x g for 40 min. The water and oil separations were measured, and emulsion stability (ES) was calculated using the following equations:

ES(%)=100-(SW+SO) (1)
SW(%)=ml of water seperated x 10 (2)
SO (%)=ml of oil separated x d x 10 (3)

where: d is specific gravity.

2.6. Emulsion density

ED measurement is one of the simplest methods of determining emulsion properties. It can be required inexpensive equipment that is available in many laboratories (Mcclements, 1999). Emulsion of 20 ml was pipetted with enlarged mouth side of the pipette and weighed. ED was determined as the weight of 20 ml of emulsion.

2.7. Emulsifying Activity

Emulsifying activity was determined using the method of Neto et al. (2001). The emulsion was immediately transferred into the tubes. After the centrifugation at 350 x g for 40 min, the height of emulsified layer and that of the total contents in the tube was measured.

The emulsifying activity (EA) was calculated as:

$$EA = \frac{\text{(Height of emulsified layer in the tube) x 100}}{\text{Height of the total contents in the tube}} \quad (4)$$

2.8. Experimental design and statistical analysis

The experimental design and statistical analysis were performed using JMP Software (SAS Institute Inc.). The experiments were based on a central composite rotatable design. A quadratic model was employed to study the combined effect of two independent variables (pH and ionic strength). A total of 12 combinations including four replicates of the center point were carried out in random order. The codified and actual levels are given in Table 1.

Table 1. Central composite rotatable design of two independent variables

Run order	Codified levels		Actual levels	
	Ionic Strength (X_1)	pH (X_2)	Ionic Strength (M NaCl)	pH
1	0	-1.67	0.45	3.50
2	-1	-1	0.30	4.50
3	1	-1	0.60	4.50
4	-1.67	0	0.20	6.00
5	0	0	0.45	6.00
6	0	0	0.45	6.00
7	0	0	0.45	6.00
8	0	0	0.45	6.00
9	1.67	0	0.70	6.00
10	-1	1	0.30	7.50
11	1	1	0.60	7.50
12	0	1.67	0.45	8.50

The variables were coded according to the following equation:

$$X_i = (x_i - \overline{x}_i) / \Delta x_i \quad (5)$$

where X_i is the coded value of an independent variable, x_i is the real value of an independent variable, $\overline{x}_i$ is the real value of an independent variable at the centre point, Δx_i is the step change.

The variance for each factor assessed was partitioned into linear, quadratic and interactive components and were represented using a second order polynomial equation. The equation is

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2 + \sum_{\substack{i=1 \\ i<j}}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j \quad (6)$$

where Y is the estimated response, β_0, β_i, β_{ii}, β_{ij} are constant coefficients, k is the number of factor variables. X_i, X_j, which are defined as the

independent variables. The analysis was performed using uncoded units.

3. Results and discussions

The results of analysis of variance indicating the pH-e (pH of the emulsion) are summarised in Table 2. The linear and quadratic effects of pH on pH-e of beef were found to be significant. As shown in Figure 1 and 2, pH-e increased with increasing pH of the pea solution. This increasing rate slowed down at high pH levels (Figure 1).

This change in the pH may be associated with buffering properties of muscle proteins. pH plays an important role during emulsification and is related to the physicochemical and functional properties of an emulsion (Schut 1976).

The effects of ionic strength and pH on EC of beef were found to be significant ($p<0.01$, Table 2). As shown in Figure 3, ionic strength increased EC values of beef emulsion as linearly. However, EC decreased with increasing pH until a critical value and then increased with increasing pH (Figure 1). Hsu et al. (1982) reported that emulsion capacity of pea protein isolate was poor at pH 6.5 but was improved at pH 7.5. This was probably related to isoelectric range of the pea proteins. Moreover, hydrophilic and lipophilic characteristics of proteins and the balance between them might play an important role in EC values. Hydrophilic and lipophilic characteristics of proteins and polysaccharides reduced interfacial tension between oil and water, allowing much-reduced energy input (Elizalde et al., 1988 Krause, 2002). Barać et al. (2015) reported that the isoelectric point (pI) of major pea proteins was 4.5. The solubility of proteins increases when pH shifts from the isoelectric point (Schut, 1976). The highest solubility of pea protein concentrate was observed at pH ranging from 1 to 3 and 7 to 10 (Boye at al., 2010). Barać et al., (2015) reported that solubility of pea protein isolates was poor at pH 5.0 but was improved at pH 7.0 and 8.0. Also, they reported that pea contains a considerable amount of starch that affects the emulsifying and foaming properties and water- and oil-binding capacities. Aluko et al., (2009) reported that emulsifying capacity of pea protein isolate was higher than soy protein isolate when emulsions were prepared at the pH 5.0 and 7.0. Tömösközi et al., (2001) stated that emulsifying capacity of pea protein isolate was better than emulsion stability when compared to soy protein isolate. Moreover, Söderberg (2013) reported that ionic strength (NaCl) improved emulsion capacity of pea and soy proteins. The linear effect of ionic strength on ES were found to be significant ($p<0.01$, Table 2). ES increased with increasing ionic strength of pea solution (Figure 1 and 4). However, the quadratic effect of pH on ES were found to be significant ($p<0.05$, Table 2). ES decreased with increasing pH levels until a critical value and then increased with increasing pH (Figure 1). These findings about ES might be related to the protein and polysaccharide content of pea. pH can affect the interactions occurring between polysaccharides and proteins at the interface (Khalloufi et al., 2009). When pH moves to isoelectric point, a decrease in droplet surface net charge leads to emulsion destabilisation (Laplante et al., 2006).

Major pea proteins had minimum emulsifying stability in the range of pI (Barać et al., 2015). Moreover, the increasing ionic strength might be reduced the electrostatic repulsion, so that the droplets may come close together, and are prone to coalescence (McClements, 1999, Srinivasan et al., 2000). Varying pH, ionic strength and ionic species were created different network structures from pea proteins (Munialo et al. 2014). Also, heat treatment affects protein properties allowing protein-protein interactions.

A strong network structure of proteins can hold oil droplets in that structure leading to increased emulsion stability (Smith, 1988).

The linear and quadratic effects of pH on ED were found to be significant (Table 2). However, the linear effects of ionic strength on

ED values were found to be significant ($p<0.05$).

Table 2. Analysis of variance of the linear, quadratic and interaction effects of pea solution with pH and ionic strength on the emulsion characteristics of beef

Source of variation	pH-e		EC		ES		ED		EA	
	$R^2 = 0.98$		$R^2 = 0.88$		$R^2 = 0.83$		$R^2 = 0.85$		$R^2 = 0.89$	
	F-value		F-value		F-value		F-value		F-value	
Model	53,543	**	9,170	**	5,977	*	7,0180	*	9,8185	**
X_1 (µ)	0,035		23,933	**	18,677	**	6,5442	*	8,7024	*
X_2 (pH)	256,698	**	0,002		2,232		17,2373	**	1,2181	
X_1*X_2	0,428		0,114		0,015		1,3613		2,1617	
X_1*X_1	0,260		1,261		1,381		1,6887		0,2271	
X_2*X_2	10,307	*	21,702	**	8,882	*	9,8172	*	35,0570	**
Lack of fit	8,886		5,721		0,146		2,0515		2,7469	

*: $p<0.05$ significance level, **: $p<0.01$ significance level, µ: ionic strength, pH-e; Emulsion pH, EC: Emulsion capacity, ES: Emulsion stability, ED: Emulsion density, EA: Emulsion activity

Table 3. Predicted model equations for the effects of pea solution with ionic strength (X_1) and pH (X_2) on the emulsion characteristics of beef

$Y_{pH} = 5.763 + 0.006X_1 + 0.475X_2 + 0.030X_1X_2 - 0.0143X_1^2 – 0.090X_2^2$
$Y_{EC} = 64,758 + 2,111X_1 + 0,017X_2 + 0,225X_1X_2 + 0,457X_1^2 + 1,897X_2^2$
$Y_{ES} = 52,488+ 4,282X_1 + 1,480X_2 + 0,188X_1X_2 + 1,099X_1^2 + 2,788X_2^2$
$Y_{ED} = 0,9374 + 0,0021X_1 - 0,0035X_2 - 0,0015X_1X_2 + 0,0010X_1^2 + 0,0025X_2^2$
$Y_{EA} = 14,452 + 5,050X_1 - 1,889X_2 + 3,890X_1X_2 + 0,770X_1^2 + 9,568X_2^2$

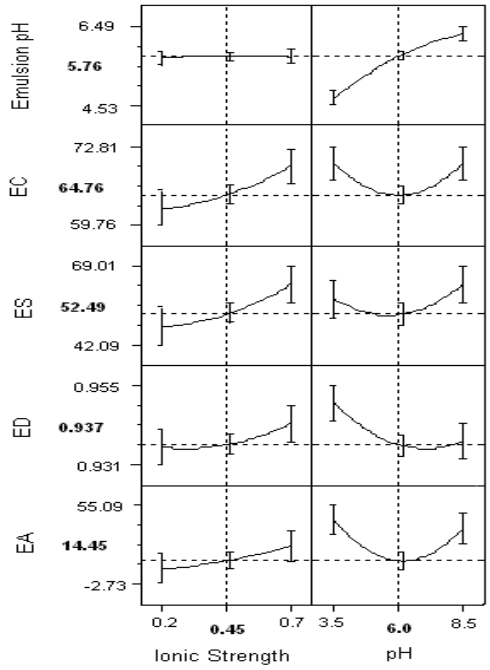

Figure 1. Prediction profilers of the effects of pea solution with pH and ionic strength (M NaCl) on the emulsion properties of beef.

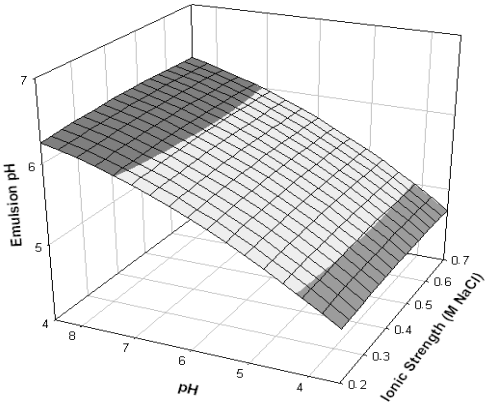

Figure 2. Effects of pea solution with pH and ionic strength on the pH of beef emulsion.

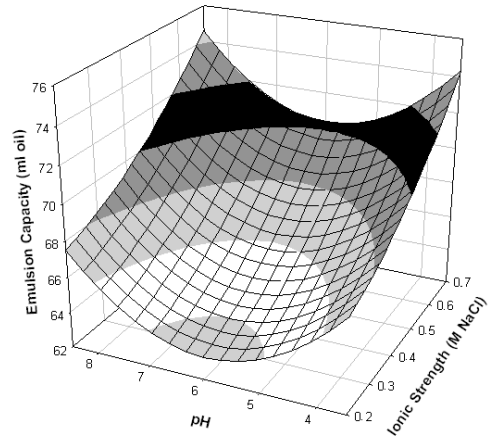

Figure 3. Effects of pea solution with pH and ionic strength on EC of beef.

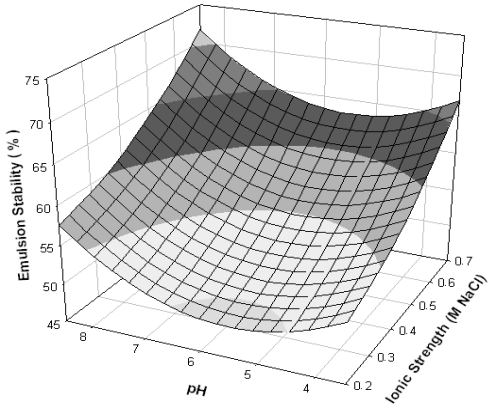

Figure 4. Effects of pea solution with pH and ionic strength on ES of beef

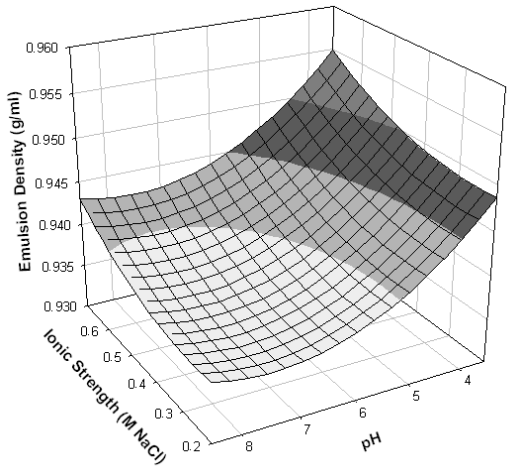

Figure 5. Effects of pea solution with pH and ionic strength on ED of beef.

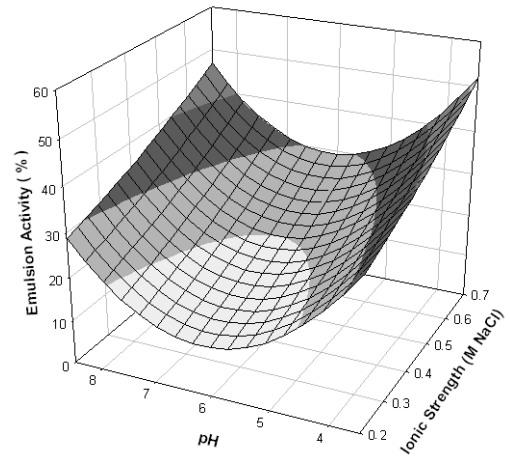

Figure 6. Effects of pea solution with pH and ionic strength on EA of beef.

The ED decreased with decreasing ionic strength and increasing pH until a critical point, which was reached at an ionic strength of 0.348M and a pH of 6.74.

As shown in Figure 1 and 5, the predicted value at solution was a minimum. These changes in emulsion density might be related to the size of oil droplet and air inclusion due to the lower density of oil and air. This might be related to the behaviour of adsorbed proteins due to the effects of pH and ionic strength.

The results of analysis of variance indicating the EA are summarised in Table 2. The linear effects of ionic strength on EA were found to be significant ($p<0.05$), and also the quadratic effects of pH on EA were found to be significant ($p<0.01$). Increasing ionic strength increased EA values (Figure 1, 6). EA decreased with increasing pH levels until a critical value and then increased with increasing pH. A significant relation had between EA and pH values (Neto et al., 2001), which affect protein and polysaccharide solubility leading to differences in the net electrical charge. Barać et al. (2015) reported that minimum emulsifying activity the major pea proteins showed in the range of isoelectric points. Also, they reported that when pH moves away from pI, emulsifying properties improve due to intensive dissociation, which is more pronounced in the case of legumin (Neto et al., 2001). Moreover, pea contains a considerable amount of fibres, which might improve emulsion volume due to the hydration and swelling properties of fibres (Rubilar et al., 2010). Khalloufi et al. (2009) reported that interaction between charged polysaccharides and proteins can be controlled to increase the thickness of the surface layer surrounding the droplets and to create multilayer surfaces.

The effects of pH and ionic strength of pea solution on emulsion characteristics of beef were also expressed mathematically in Table 3. These predicted model equations are useful for understanding levels of factors and the interactions between studied factors.

4. Conclusions

Increasing ionic strength of the pea solution improved emulsion properties of beef. However, the increasing levels of pH firstly decreased then started to increase the values of emulsion properties. In particular, minimum values of emulsion properties observed in the pH range of 5.5-6.5. Ground pea can be used as a functional ingredient in emulsified meat products to improve emulsion properties.

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COMPARATIVE STUDY ON POLYPHENOLS CONTENT, CAPSAICIN AND ANTIOXIDANT ACTIVITY OF DIFFERENT HOT PEPPERS VARIETIES (*CAPSICUM ANNUUM* L.) UNDER ENVIRONMENTAL CONDITIONS OF THESSALY REGION, GREECE

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ABSTRACT
Seven red hot peppers varieties grown in Central Greece, have been studied for evaluating and comparing their polyphenols content, the capsaicin content and antioxidant properties. It has been established that TP and the capsaicin content of the red hot varieties move in broad ranges depending on the variety and, to a lesser extent with the ecological conditions and soil chemical properties. The total phenols were expressed as gallic acid equivalent (GAE). The highest content of total phenol (TP) has been found in the *Uganda* variety with a value 1700 μg (GAE) g^{-1} FW, and the lowest in the Rainbow variety with a value 907.4μg (GAE) g^{-1} FW.The highest content of flavonoid phenols has been found in the *Uganda* variety with a value 1156 μg (GAE) g^{-1} FW, and the lowest in the Rainbow variety with a value 571.7 μg (GAE) g^{-1} FW.The highest content of capsaicin has been found in the *Cayenne* variety with a value 1775μg g^{-1} FW, and the lowest in the *Tomatini* variety with a value 539.2 μg g^{-1} FW.The antioxidant activity FRAP for the pepper fruits is varying from 13.2 to 24.9 μmol(equivalent ascorbic acid)g^{-1}FW. The FRAP value was highly correlated with total phenol content (r^2 = 0.8607).While, there is no linear correlation between the amount of capsaicin and of the antioxidant capacity.

1. Introduction

The free radicals are strong oxidisers, which damage the basic biological molecules (proteins, lipids), causing the occurrence of many diseases in the human organism (Halliwelland Gutteridge, 1989; Halliwell, 1991). Epidemiological investigations demonstrate that the oxidative destruction of biomolecules can be protected by using endogenous and exogenous antioxidants which are contained in fruits, vegetables etc. (Ou et al., 2002; Pellegrini et al., 2003; Pellegrini et al., 2006; Wu et al., 2004; Yanishlieva - Maslarova et al., 2001).

Phenolic compounds are secondary metabolites, which are synthesized by plants as a result of plant adaptation in conditions

stress, and contained in a high proportion in the plants, fruits and vegetables (Gougoulias, 2012a; 2012b; Gougoulias et al., 2012).Some contribute to shaping of the taste and color of fruits and plants, while others have protective role against some diseases (Gambacorta et al., 2009; Shahidi, 1997; Shahidi, 2000).The intake of polyphenols with food reduces the risk of cardiovascular, carcinogens and other diseases, because of the commitment of the oxygen free radicals (ROS), which damage the cells of the human body (Bravo, 1998; Dinis et al., 1994; Manach et al., 2004; Scalbertand Williamson, 2000).

Peppers fruits (*Capsicum annuum L.*) it is natural reservoir of nutrients and of natural antioxidants (de Jesús Ornelas-Paz et al., 2010; Gougoulias et al., 2016). Hot peppers cultivars are rich in capsaicin (capsaicinoids alkaloids), with medicinal propertiesand a high antioxidant activity (Basith et al., 2016; Díaz-Laviada and Rodríguez-Henche, 2014; Materska and Perucka, 2005; Reyes-Escogido et al., 2011). The techniques and cultivation systems, fertilization, irrigation and variety, affect the levels of the antioxidant activity in the peppers (Ayodele et al., 2015; Bae et al., 2014; Guinoza et al., 2015; Nunez-Ramirez et al., 2011).

The aim of the present study is to determine of the content capsaicin, phenolic compounds, their major fractions and the antioxidant activity, of different red hot peppers varieties, grown in central Greece.

2. Materials and methods

2.1. Experimental

The experiment was conducted at the Technological Education InstituteFarm of Thessaly (latitude 39°37′25″ N, longitude 22°22′48″ E, 80 m altitude). The region of Larissa is characterized by a continental climate with cold winter, hot summer and low precipitation, in spring and summer. The soil of the experiment was Sandy Loam (SL), with the physicochemical characteristics are presented in Table 1. Ground cover fabric for weed control was applied, 30 kg /ha fungicide BORD 20WPwere applied, and 30 tons manure for fertilizing per hectare, corresponding to 240 kg Nitrogen, 165 kg Potassium, 30 kg Phosphorus and 9 tons Organic matter. Peppers was cultivated in the Farm on an area of 700 m^2(100m^2 per cultivar), between April 2016 and October 2016.The planting took place with 0.4m distance, plant from plant and 0.8 m distance, line from line. Seven varieties red hot peppers were cultivated. The *Cayenne*, *Tomatini*, *Pellegrino*, *Uganda*, *Bolivian Rainbow*, *Chili* and *Picante rocho*. The fruits harvested in the ripeness. There were four replicates per treatment combination.

2.2. Methods of analyses

Soil was analyzed using the following methods which are referred by Page (1982).

Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.

Inorganic nitrogen was extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy. Exchangeable form of potassium was extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK). pH and (EC), Electrical conductivity measured in the extract (1 part soil or manure : 5 parts H_2O).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1} + triethanolamine 0.1 mol L^{-1}) and measured by atomic absorption. The samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture (Varian, 1989).

Preparation of the methanol extracts. Ten g of the peppers samples were two rounds treated by 20 ml of 80% aqueous methanol. Samples were incubated for 24 h in the extractant at stirring; the supernatant

material was removed. The pellet was re-treated with aqueous methanol for 2 h at stirring at ambient temperature. The extract was gathered after centrifugation/filtration and the volume was made up to 50 ml with aqueous methanol and used for further chemical analysis (Kanner et al., 1994).

Determination of total polyphenolics (TP). Total polyphenolic content was determined with the Folin-Ciocalteu (F-C) reagent according to the method by (Singleton and Rossi, 1965) using the microvariant proposed by (Badenschneider et al., 2015) and the results were expressed as gallic acid equivalent (GAE) in μg g^{-1} fresh weight.

Nonflavonoid phenols (NFP). The content of NFP was determined with the F.-C. reagent after removing the flavonoid phenols (FP) with formaldehyde according to the method by (Kramling and Singleton, 1969) and was expressed as gallic acid equivalent (GAE) in μg g^{-1} fresh weight.

Flavonoid phenols (FP). Flavanoid phenols were determined as a difference between the content of total phenols (TP) and nonflavonoid phenols (NFP). Their amount was evaluated as gallic acid equivalent in μg g^{-1} fresh weight.

Determination of ferric reducing antioxidant power (FRAP). The antioxidant activity of the methanol extracts was determined on the basis of the method by (Benzie and Strain, 1999) and was expressed as ascorbic acid equivalent (AA) in μM g^{-1} fresh weight.

Determination of capsaicin. Capsaicin content was determined after drying at 55°C for 24 h. 0.5 g of dry fruits was extracted with 25 ml Ethyl acetate and capsaicin was estimated colorimetrically in 430nm according to the method by Bajaj(1980).

2.3. Statistical analysis

Data were analyzed using the MINITAB (Ryan et al., 2005) statistical package. The experiment had four replicats. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

3. Results and discussions

The single extraction of the fruits with 80% methanol results in the extraction of phenol compounds which vary between 907.4 and 1700 μg TP (GAE) g^{-1} FW (Figure 1). Among the peppers fruits studied, the *Uganda* variety characterised by the highest TP content, with 1700 μg (GAE) g^{-1} FW, followed by of the *Picante rocho* variety with 1403μg(GAE)g^{-1} FW, and the lowest by those of the *Cayenne*, *Pellegrino* and *Rainbow* varieties with 963.0, 912.2 and 907.4 μg(GAE)g^{-1}FW, respectively.

Our results are in agreement with the data obtained by other authors (de Jesús Ornelas-Paz et al., 2010). Studies of other authors show, that hot red varieties are characterized by higher total phenols content compared to hot green varieties (Materska and Perucka, 2005). Furthermore, according to some authors, many sweet peppers have shown higher content of total phenols compared to hot peppers (Blanko-Rios et al., 2013; Gorinstein et al., 2009; Zhuang et al., 2012).

Flavonoid phenols content (equivalent to gallic acid) in the red hot peppers varieties ranges from 571.7 to 1156.0μg (GAE) g^{-1} FW (Table 2). Of the seven peppers varieties analyzed, peppers of the hot variety *Uganda* characterized by higher contents of flavonoid phenols (1156.0 μgGAEg^{-1} FW). The comparison of the content of FP with respect to the total phenols in the fresh matter of the hot peppers shows that they represent about (60.1 –77.5) % of TP. The flavonoid phenols in the hot peppers variety, *Tomatini* and *Picante rocho* constitute 77.5 % and 75% respectively of the TP amount, and for *Red chili*, *Uganda*, *Bolivian Rainbow*, *Pellegrino* and *Cayenne*, reached 69%, 68%, 63%, 62.8 % and 60.1 %, respectively. Some authors (Lee et al., 1995) reported that the FP fraction consists mainly of quercetin and luteolin in conjugate forms. These compounds affect the

antioxidant properties in peppers. Luteolin had highest antioxidant activity followed by capsaicin and quercetin.

Non-flavonoid phenols content (as equivalent to gallic acid) in the red hot peppers of ranges from 235.8 to 544.0 μg (GAE)g^{-1}FW (Table 2). Peppers of the variety *Uganda* characterized by higher contents of nonflavonoid phenols (544.0 μg GAE g^{-1} FW). The comparison of the content of NFP with respect to the total phenols in the fresh matter of the red hot peppers shows that they represent about 22.5 - 39.9 % of TP. The nonflavonoid phenols in the *Cayenne* peppers variety constitute 39.9 % of the TP amount, while in the *Tomatini* variety constitute 22.5% of the TP amount. Some authors (Materska et al., 2003) reported that the NFP fraction consists mainly of ferulic, cinnamic and other acids, and their derivatives.

Table 1. Chemical properties of the soil cultivated peppers, before fertilizing

Property	Soil depth (0-30) cm	Soil depth (30-60)cm
Texture	Sandy Loam (SL)	Sandy Clay Loam (SCL)
pH, extract (1:5)	7.83±0.05	7.96±0.07
EC, extract (1:5), dS m^{-1}	0.15±0.01	0.15±0.01
$CaCO_3$ (%)	1.32±0.03	5.89±0.20
Organic matter (%)	1.82±0.05	1.04±0.03
N-inorganic (mg kg^{-1})	32.6±5.12	25.2±3.82
K- Exchangeable (mg kg^{-1})	227.9±7.02	165.7±3.62
P -Olsen (mg kg^{-1})	52.3±3.22	22.3±2.30
Na-Exchangeable (mg kg^{-1})	66.7± 3.12	89.7±1.20
*CEC (cmol kg^{-1})	19.10±0.40	16.02±0.30
Cu- DTPA (mg kg^{-1})	5.07±0.14	3.42±0.07
Zn- DTPA (mg kg^{-1})	2.15±0.05	0.90±0.02
Mn- DTPA (mg kg^{-1})	13.0±0.90	8.67±0.70
Fe- DTPA (mg kg^{-1})	3.73±0.18	4.07±0.20

*CEC: Cation exchange capacity; Data represent average means and SE deviation. (n) = 4.

Table 2. Flavonoid phenols content and Nonflavonoid phenols in the peppers under study

Hot peppers varieties	Flavonoid phenols (FP) (μg GAE g^{-1}FW)	Nonflavonoid phenols (NFP) (μg GAE g^{-1}FW)
Cayenne	578.8^{d}	384.2^{b}
Tomatini	812.2^{c}	235.8^{c}
Pellegrino	572.9^{d}	339.3^{b}
Uganda	1156.0^{a}	544.0^{a}
Bolivian Rainbow	571.7^{d}	335.7^{b}
Red chili	747.9^{c}	336.1^{b}
Picante rocho	1052.3^{b}	350.7^{b}

Rows in each characteristic of each column with the same letter do not differ significantly according to the Tukey's test (P=0.05).

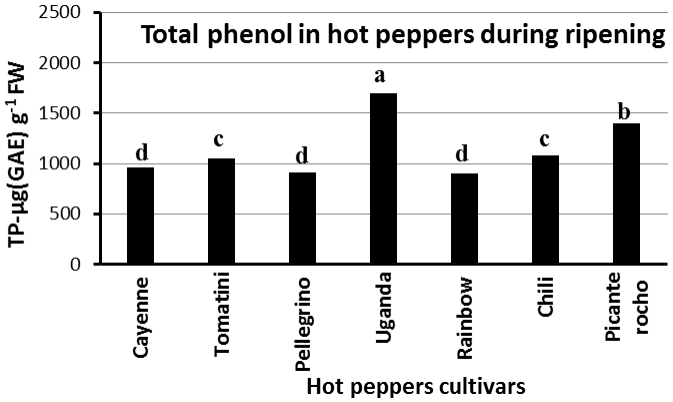

Figure 1. Total phenol content of different hot peppers varieties. Columns in each characteristic of each graph with the same letter do not differ significantly according to the Tukey's test (P=0.05).

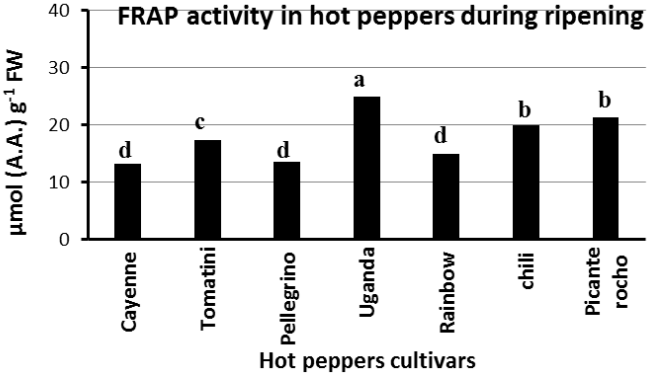

Figure 2. Antioxidant activity FRAP of different hot peppers varieties. Columns in each characteristic of each graph with the same letter do not differ significantly according to the Tukey's test (P=0.05).

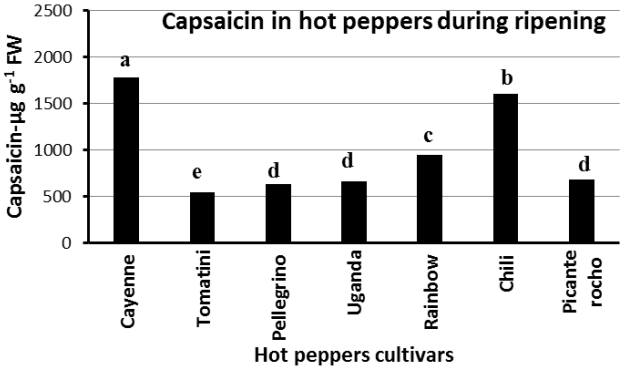

Figure 3. Capsaicin content of different hot peppers varieties. Columns in each characteristic of each graph with the same letter do not differ significantly according to the Tukey' s test (P=0.05).

The antioxidant activity of the hot peppers assayed by the FRAP reagent depends also on the peppers variety (Figure 2) and varies from 13.2 to 24.9 µmol (A.A) g^{-1} FW. The results of the seven varieties reveal that the *Uganda* variety exerts the highest antioxidant activity with 24.9 µmol (A.A) g-1 FW (Figure 2) and those of the *Cayenne, Pellegrino* and *Rainbow* varieties the lowest with 13.2, 13.6, and 15.0 µmol (A.A.) g^{-1}FW, respectively. The correlation between the antioxidant FRAP activity of the

peppers and the contents of TP was high, with correlation coefficient (r^2) equal to: 0.8607.

Studies of other authors also show, that the antioxidant activity of the hot peppers depends on the variety, the climatic and culture conditions (Bae et al., 2014; Guinoza et al., 2015). According to some authors, methanolic extract of red hot pepper showed greater antioxidant activity than the green hot peppers (Conforti et al., 2007).

The hot peppers varieties analyzed, content capsaicin ranges from 539.2 to 1775 $\mu g g^{-1}$ FW (Figure 3). The *Cayenne* variety characterised by the highest capsaicin content, with 1775$\mu g\ g^{-1}$ FW, followed by the Chili variety with 1604.4$\mu g\ g^{-1}$ FW, and this of the *Tomatini* variety the lowest with 539.2 $\mu g\ g^{-1}$ FW. In our study there is no linear correlation between the amount of capsaicin and antioxidant capacity of red hot peppers.

Our results are in agreement with the data obtained by other authors (Ziino et al., 2009). Studies of other authors show, that hot red varieties are characterized by higher capsaicin content compared to hot green varieties (Materska and Perucka, 2005).

4. Conclusions

Hot peppers varieties (*Uganda, Picante rocho*, *Chili* and *Tomatini*), grown under specific climatic and soil conditions, in a sandy loam soil in the region Thessaly, are rich in phenol compounds and exert strong antioxidative activity compared with other fruits and vegetables.

Among hot peppers varieties, grown under the same climatic and soil conditions of the region Thessaly, the *Cayenne* variety and the Chili variety are characterized by higher capsaicin content.

Fruits of hot peppers varieties which are cultivated in region Thessaly are a source of bioactive components that could be included in functional foods composition.

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EFFECT OF THE ADDITION OF CHITOSAN PREPARED FROM GREEN SHRIMP (*Penaeus semisulcatus*) ON THE SENSORY CHARACTERISTICS OF CUPCAKES

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ABSTRACT

The present study was conducted to investigate the the effect of chitosan addition at different concentrations (0%, 0.5%, 1%, 1.5%, 2% and 2.5%) on the sensory properties and the shelf-life of cupcake. Sensory analysis of stored cupcakes prepared with different levels of shrimp shells chitosan concentrations (0.5%, 1%, 1.5%, 2%, and 2.5%) as well as that stored at room temperature 18°C ±3 and relative humidity (RH) 70% ±5 for 35 days. The results showed that the cupcake with 1.5% chitosan had the highest overall acceptability storage compared with other concentrations at a delay of oxidative processes in the fat of cupcakes. A cupcake without added chitosan has the lowest organoleptic quality rating. Cupcakes supplemented with 2% and 2.5% chitosan have showed poor organoleptic acceptability, but lipid hydrolysis and oxidation in that cupcake's sample were less intense. The addition of 1.5% chitosan to cupcake improved their sensory properties and prolong the shelf-life. All of that leads to the conclusion that, chitosan can be considered as a potential natural antioxidant to stabilize the lipids contained in the confectionery products to prolong their shelf life.

1.Introduction

A major source for the production of a wide range of chitin and chitosan is crustacean processing waste (shrimp, krill, and crab shells). Physicochemical and structural properties of chitin and chitosan depend on both sources and production conditions. Shrimp is usually peeled in standard seafood processing factories to obtain shrimp meat for export, and the leftover shells and heads, approximately represent 35– 45% of the total weight, are considered to be waste. Consequently, shrimp processing leads to huge amounts of shrimp biowaste estimated to be more than 200,000 metric tons (*wet weight*) per year (Hirano, 1996). To date, the shrimp waste has been used primarily for the preparation of chitin and chitosan. In addition to potential applications of chitin and the chitosan relies on their biological activities and physicochemical properties. However, information got from green shrimp waste relying on the physicochemical properties and the biological activities of chitin and chitosan which has been prepared from shrimp waste are limited. Chitin, a linera polymer of β (1→4) linked 2-acetamido-2-deoxy-D- glucopyranose, it is one of the most abundant natural polymers found in almost all crustaceans. Chitin, especially its deacetylated form and Chitosan are well-known marine biopolymers, having many applications

in the food industry, agriculture, biotechnology, cosmetics, medicine, and also wastes treatment (Trung *et al.,* 2003). The reactive functional groups of chitosan include an amino group and both primary and secondary hydroxyl groups at C-2, C-3, and C-6 positions, respectively. Contents of amino chitosan are the main factors influencing their physicochemical properties and structures and are associated with their chelation and biological functions (Rinaudo, 2006). Chitosan has attracted considerable attention, not only because of being a resource that is underutilized, but also due to its biological behaviours, namely, antioxidant,antimicrobial, hypocholesterolemic, impunity enhancing, antitumor activity, drug delivery, its capacity accelerate calcium, ferrum absorption, and so forth (Hirano, 1996; Kumar, 2000). The antioxidant properties of chitosan have been studied in vitro and in vivo. Antioxidant effects have been shown from chitosan on the oxidation of lard and rapeseed oil by (Abdou *et al.,* 2008; Cahú *et al.,* 2012) who reported that chitosan could significantly reduce malondialdehyde concentrations and serum free fatty acids, elevated superoxide dismutase's, and display catalase and glutathione peroxidase activities, the latter being the principal antioxidant enzymes in the body. This suggests that chitosan organizes enzyme, antioxidant activities, and reduced lipid peroxidation. The biological activities of chitosan and its derivatives correlated with their physicochemical properties and structures. Research has been increased on alternative natural products for synthetic food additives replacement because of the need to satisfy safety standards. Natural food additives from many biological materials have attracted much interest has increased because of their supposed safety. Potential sources of natural food additive compounds have been found in several types of natural material. One of these is chitosan. Chitosan is biocompatible, not antigenic, not nontoxic and bio-functional (Kumar, 2000; Xia *et al.,* 2011). It has received considerable attention as a novel excipient and/or functional a material of great potential in the pharmaceutical and food applications. Chitosan, determined from shrimp has been recognized as a GRAS (Generally Recognised as Safe) for general use in foods, including poultry and meat, and for various technique effects by the US food and Drug Administration (GRAS, 2010). In Japan and Korea, chitosan has been allowed as a food additives since 1983 and 1995, respectively (Sachindra *et al.,* 2006). The present research, therefore, was conducted to investigate the physical and chemical properties of chitosan and sensory evaluation of chitosan prepared from green shrimp waste in a high-fat product.

2. Materials and methods

2.1. Materials

Green shrimp waste (*Penaeus semisulcatus*) was collected from a seafood processing factory. The cold shrimp waste was transported at 0 °C temperature to a laboratory. The waste was washed in running water and ground using knife mill to obtain pieces of 0.2 to 0.4 cm. Portions of 1000 g were packed into plastic bags and frozen at −20°C until processing. Glacial acetic acid, hydrochloric acid, sodium chloride, sodium hydroxide, methyl orange was all purchased from Sigma-Aldrich Inc., USA. Aniline –blue were purchased from ROTH Bestellen sie zum (Nulltarif, Germany). All other reagents and solvents were used according to analytical grade.

2.2. Purification of chitosan

Chitin and chitosan were prepared from green shrimp waste by chemical treatment (Trung *et al.,* 2006). Chitin has been isolated by treatment with a dilute HCl solution for demineralization by 3M HCL at 45 °C for 2 h, and dilute NaOH for deproteinization by 2M NaOH at 75 °C for 3 h for removal protein from shrimp shells then washed to a neutral pH and dried to obtain chitosan from chitin. The resulting solids were washed until till reaching the neutral point. Chitosan was prepared from

chitin by a chemical method (deacetylation), 10-15 % (v/v) NaOH at 90 º C for 2 h, and then washed to a neutral pH and dried to obtain chitosan.

2.3.Molecular weight of chitosan

The Definition of the molecular weight of chitosan samples using the Mark-Houwink-Sakurada (MHS) equation (Ghosh, 2000). $[\eta]=K(M_v)^{\alpha}$ [η] and Mv are the intrinsic viscosity [[η], (dl/g)] and viscosity molecular weight [Mw x 10^5 (g/Moll)] while K and α are constants for given solute-solvent system and temperature. The ubbelohde-form capillary viscometer was used to estimate the flow time of the solutions through the capillary in a constant temperature bath at 20 °C. Three measurements were made on each sample. The running times of the solution and dissolvent were recorded as seconds (s) and used to calculate intrinsic viscosity [η]. The reported values of K and α are 1.81×10-5 and 0.93, sequentially (Kasaai, 2007).

2.4.Deacetylation of chitosan (D.D%)

The acid–base titration method was used to determine the D.D from the amino group content in chitosan. Chitosan (0.3 g) was dissolved in 30 ml of HCl standard solution (0.1mol/l). Methyl orange and aniline blue mixing indicators were added (Stevens, 2001)**.** A standard solution of 0.1M NaOH was used for titration until the solution became blue green. The following formulas were used to calculate the D.D:

$$(-NH_2)\,\% = \frac{0.016\,(C_1V_1 - C_2V_2)}{W} \times 100$$

$$DD\% = \frac{203(-NH_2\%)}{16+42\,(-NH_2\%)} \times 100$$

Where C1, V1, C2, and V2 are the concentrations and volumes of the HCl standard solution and NaOH standard solution, respectively, and W is the weight of the sample.

2.5.Physicochemical and functional properties methods

Fat binding capacity (FBC) and Water binding capacity (WBC) of chitosan were determined by using the method of (No *et al.*, 2000). Briefly, the procedure was carried out by weighting a centrifuge tube containing 1.0 g sample, adding 10-15 ml of water or corn oil, and mixing via vortex mixer for 1 min to disperse sample. The contents of chitosan shells were left at ambient temperature for 30 min with shaking for 10s every 15 min and centrifuged at 5000 RPM for 25 min. The supernatant was decanted and the tube was weighted again. Fat binding capacity (FBC) and Water binding capacity (WBC) of chitosan were calculated using next formula:

WBC (%)= [water bound (g) /sample weight (g)] ×100

FBC (%)= [fat bound (g)/sample weight(g)]× 100

Chitosan (0.1 g) was placed into a centrifuge tube (known weight) then dissolved in 10 ml of 1% acetic acid for 30 min using an incubator shaker operating at 240 RPM and 25ºC. The solution was then immersed in a boiling water bath for 15 minutes, cooled to room temperature (25 °C) and centrifuged at 9000 RPM for 15 min. The supernatant was decanted. The residue particles were washed with distilled water (25ml) then centrifuged at 9000 RPM. The supernatant was removed and the residue dried at 60 °C for 24hr. Finally, the dried residue weighted and the percentage of solubility was calculated as follows:

$$\%\ \text{Solubility of Chitosan} = \frac{(\text{The initial weight of tube + chitosan}) - (\text{final weight of tube + chitosan}) \times 100}{(\text{Initial weight of tube + chitosan}) - (\text{Initial weight of tube})}$$

2.6. Preparation of cupcakes

Cupcakes were prepared according to the progress of the study (Sudha *et al.*, 2007b). The formula included 31.01% egg, 25.84% wheat flour, 6.46% shortening, 25.84% sugar, 10.34%

refined corn oil, 0.13% baking powder and 0.39% salt. The wheat flour (as well as the flour substituted with 0.5, 1, 1.5, 2.0 and 2.5 % chitosan) salt, shortening and baking powder were creamed together to prepare a fluffy cream. Cupcake batter was prepared in a Hobart mixer (N-50) using a flour batter method. Eggs and sugar were whipped together until semi-firm foam formed. The sugar-egg foam was associated with the creamed flour and oil, after which the vegetarian oil was added in little portions. For each cupcake variation, twelve 50-g portions of batter were weighed and put in a baking cup in an aluminum muffin pan. The cupcakes were baked in a 160 °C oven for 45min. Cupcakes were cooled to room temperature before wrapping in polyethylene film and stored on the shelf at 18°C ±3 and relative humidity (RH) 70% ±5 for 35 days. Samples were analyzed at 5 day intervals.

2.6.1. Sensory evaluation of cupcake

Sensory evaluations of the cupcake were carried out using a simple triangle test on the next day after the preparation and after the end day of storage (35 days). Ten staff members of the Department of Technology of Goods and Merchandising, Astrakhan State Technical University., Astrakhan- Russia. The panelists were asked to evaluate the appearance, odor, texture, flavor and taste (Sudha *et al.,* 2007a).

2.7. Statistical analysis

A completely randomized 5 or 6 (Chitosan replacing levels) x 7 or 6 (storage periods) x 3 (replication) factorial design was used Data were analyzed by analysis of variance SAS (1990). Statistical analysis was done using analysis of variance (ANOVA), and least significant difference (LSD) was obtained to compare the means of treatments, using Costat-version 6.311. Duncan's multiple range tests (http://cran.r-project.org/bin/windows/base/) was used to compare between the treatments means of portability of 5% level.

3. Results and discussions

3.1. Physicochemical and functional properties

Physicochemical properties of chitosan recovered from green shrimp *Penaeus semisulcatus.* The present study shows that the degree of deacetylation (D.D%) 84.56 %, water binding capacity (518.746 %) and fat binding capacity (425.47 %). The solubility of the produced chitosan reached to 58.93 %. The values obtaining for the produced shrimp shells chitosan are too close to those stated by several investigators (Figure 1). The molecular weight of alkali treated samples was determined and is presented in Fig 1. From the chart, higher the duration of alkali treatment, a higher was the molecular weight of chitosan sample 10.05 kDa ($M_W = 1{,}05 \times 10^4$). With the alkali treatment, the number of amino groups (-NH2) increased while the number of acrylamide (-NHCOCH3) group decreased (Khan *et al.,* 2002). The higher the duration of alkali treatment, the higher was the formation of amine groups. Earlier studies have demonstrated that the physicochemical characteristics of chitosan affect its functional properties (Khan *et al.*, 2002). The values obtained for our produced shrimp shells chitosan is too close to those stated by several investigators. Moradi *et al.* (2010) stated that the D.D is an important parameter affecting chemical reactivity, solubility and biodegradability. Depending on the source and preparation procedure, D.D may range from 30% to 90%. Among the many characteristics, the degree of deacetylation is one of the most important chemical characteristics, which influences the performance of Chitosan in many of its applications. Emi-Reynolds *et al.,* (2007) reported DD value of 89.7%. Fat binding capacity signifies how the chitosan can easily bend or absorb fat, especially when used in the manufacturing of dietary supplement and food additive. The trend recorded for WBC was similarly observed for FBC. Values for un-irradiated and irradiated shrimp chitosan were 560.55% and 431%, respectively, while that of

the commercial chitosan sample was 490.10% and 529.05%, respectively. The average of fat binding capacities of crawfish chitosan and commercial crab chitosan for soybean oil was 706 % and 587%, respectively. Water and fat binding capacity of commercial chitosan are lower than the extracted chitosan. WBC and FBC of different commercial chitosan were reported as 458–805% and 314–535%, respectively. WBC and FBC of six commercial chitosan products observed by Singh, (2001) were in the range of 355–611% and 217–477%, respectively. The WBC (492.67%) and FBC (383.04%) of commercial chitosan in the present study were compatible with those reported by (Emi-Reynolds *et al.*, 2007; Thebaudin *et al.*, 1997).

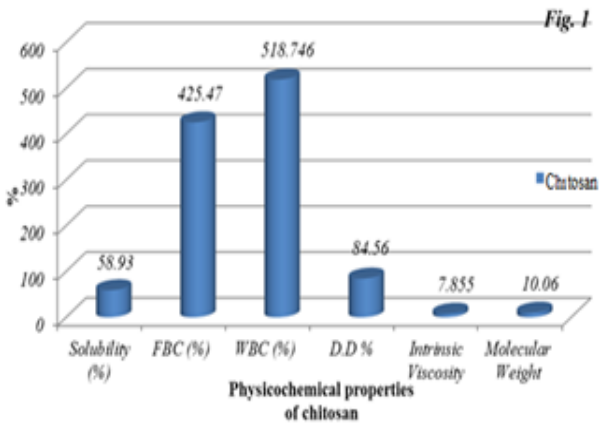

Figure 1. Physicochemical properties of Chitosan.

3.2. Sensory evaluation of cupcakes

Results of sensory analysis of stored cupcakes prepared with different levels of chitosan concentrations (0.5%, 1%, 1.5%, 2%, and 2.5%) as well as that stored at room temperature 18°C ±3 and relative humidity (RH) 70% ±5 for 35 days are presented in Fig 2 and Fig 3. When storing cupcake samples for a period of 1 - 5 days from the beginning of manufacturing till storage, No significant ($p \leq 0.05$) decrease was observed in general appearance, odor, texture and taste of stored cupcake prepared with 0.5%, 1%, 1.5%, 2% and 2.5% shrimp shells chitosan compared with control after 5 days of storage as illustrated in Figure 2 and Figure 3.

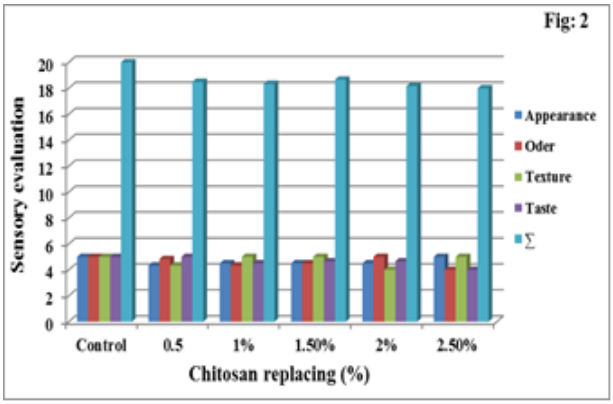

Figure 2. Sensory attributes of unstored (fresh preparation) cupcake prepared with different levels of shrimp chitosan (First day).

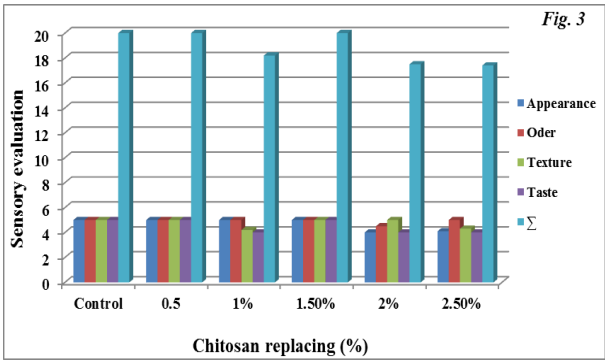

Figure 2. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan(After 5 days).

Cupcake prepared with 2%, 2.5% chitosan had significantly ($p \leq 0.05$) lower general appearance, texture and taste compared with control, 0.5 %, 1%, 1.5% chitosan. While cupcake prepared with up to 0,5 % and 1 % had no significant ($p > 0.05$) differences were observed in general appearance, taste, texture and taste between control and cupcakes prepared with 0.5 and 1% shrimp shells (After 10 -15 days of storage) were present in Figure 4 and Figure 5. Stored cupcake prepared with 1,5% shrimp shells chitosan had the highest overall acceptability compared with that prepared with other concentrations, but it still higher than control. Meanwhile, cupcake prepared with up to 2% and 2,5% after 20 days

(Figure 6) had significant (p>0.05) difference compared with control, 0.5%, 1%, and 1.5 % chitosan after 25 days of storage till the end of storage period to cupcake prepared with 2% and 2,5% after 25 days of storage) were present in Figure 7.

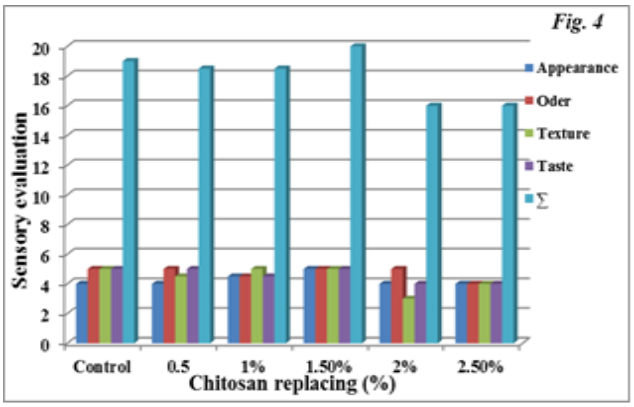

Figure 4. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan (After 10 days).

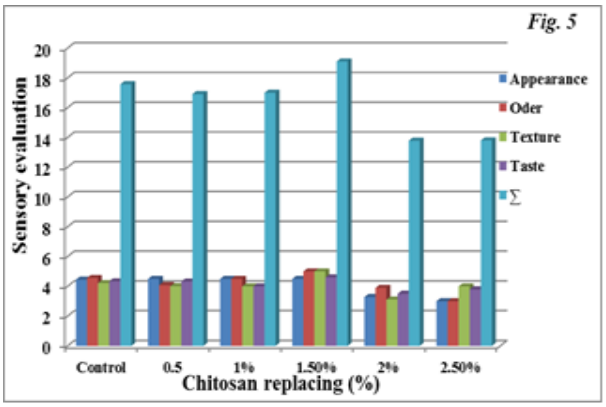

Figure 5. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan (After 15 days).

But when increasing the emphasis of chitosan led to a decrease in the ability to form the emulsion and a rapid drying of the product. Resulting in an appearance of final product's defects and undesirable as well for the consumer (Sudha *et al.*, 2007b; Sudha *et al.*, 2007a). Adverse effects of flour replacement by dietary fibers such as chitosan 2.5% in disruption of the starch, gluten matrix and bread making include weakened gluten network and in dough, reduction of the initial starch granule swelling, reduction in starch availability for gelatinization, decreased loaf volume, lowered gas retention and unsuitable taste (Sudha *et al.*, 2007b). On the other side (Fig 8), no significant (p>0.05) differences were observed among general appearance, taste, texture, and taste between 0,5% chitosan and cupcakes prepared with 1% shrimp shells chitosan, but it's still higher than control (after 30 days of storage). But stored cupcake prepared with 1,5% shrimp shells chitosan had the highest overall acceptability compared with that prepared with other concentrations as illustrated in Figure 9. Meanwhile, cupcake control without chitosan had significant (p>0.05) difference compared with 0.5%, 1%, and 1.5 % chitosan after 25 -30 days of storage.

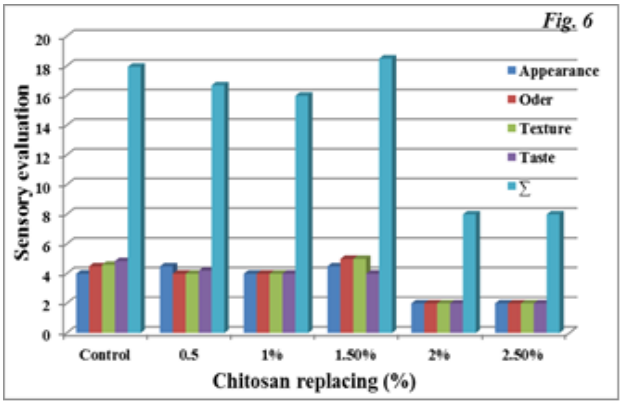

Figure 6. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan (After 20 days).

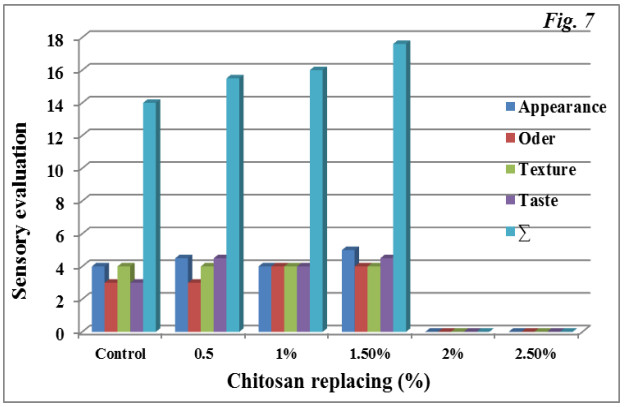

Figure 7. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan (After 25 days).

But stored cupcake prepared with 1,5% shrimp shells chitosan had the highest overall acceptability compared with that prepared with other concentrations as illustrated in Fig 9.

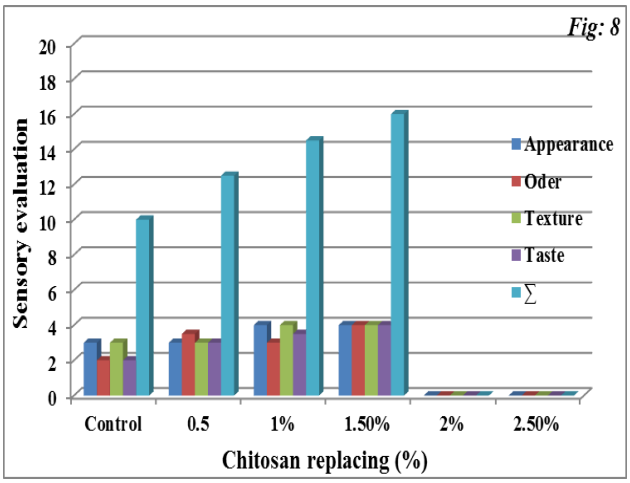

Figure 8. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan (After 30 days).

Meanwhile, cupcake control without chitosan had significant ($p>0.05$) difference compared with 0.5%, 1%, and 1.5 % chitosan after 25 -30 days of storage. The end of storage period to cupcake control without Chitosan after 30 days of storage were presented in Fig, 8.

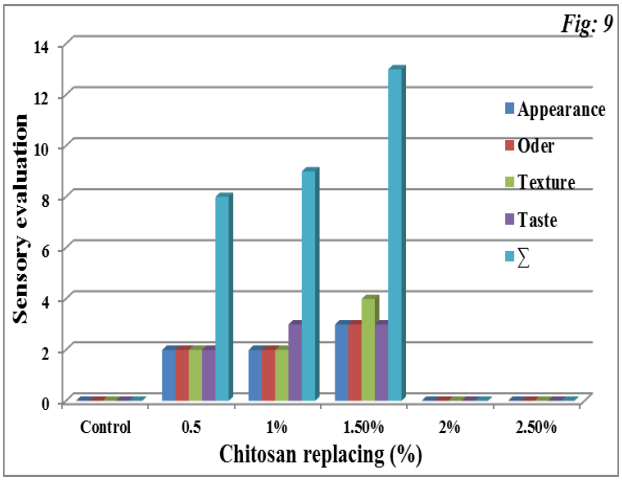

Figure 9. Sensory attributes of stored cupcake prepared with different levels of shrimp chitosan (After 35 days).

Chitosan increases the rate of bread staling, increases water migration rate from crumb crust, prevents amylose-lipid complications, and increases dehydration rate of both starch and gluten (Hirano, 1996). Our results revealed that improvements in shelf-life of cupcake prepared with 1.5% shrimp shells chitosan can be achieved to cupcake. It is concluded that cupcake prepared with 1.5% shrimp shells chitosan achieved the best antioxidant protection against cupcake deterioration during 35 days of storage. The results also demonstrated that stored cupcake prepared with 1,5% shrimp shells chitosan had the highest overall acceptability compared with that prepared with other concentrations.

4. Conclusions

Chitosan was prepared from chitin by a chemical method. The degree of deacetylation (DD %) of chitosan samples determined by different methods was found in a good agreement with each other. The present study shows that the DD 84.56 %, WBC (518.746 %), and FBC (425.47 %). The solubility of the produced chitosan reached to (58.93 %). This study aimed to prepare chitosan and using in a high fat product such as cupcakes. Sensory analysis of stored cupcakes prepared with different levels of shrimp shells chitosan concentrations (0.5%, 1%, 1.5%, 2%, and 2.5%) as well as that stored at room temperature 18°C ±3 and relative humidity (RH) 70% ±5 for 35 days. The present study, therefore, was conducted to investigate the physicochemical properties and Sensory analysis of cupcake of extracted chitosan confirms that chitin and chitosan, had a good quality and having characteristics compatible with a broad range of applications in the food industry.

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CHEMICAL PROPERTIES OF WATERMELON SEED AND THE UTILIZATION OF DEHULLED SEED IN COOKIES PRODUCTION

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ABSTRACT

The physicochemical properties of whole seed, dehulled, and hull of watermelon seed was investigated. In addition, the ability of the dehulled seed flourto serve as margarine substitute in cookies was examined. The protein and fat contents of the dehulled seed (25.50%; 48.06%) was observed to be the highest, followed by the whole seed (21.62%; 31.27%) and the hull (5.78%; 6.24%). The functional properties of the flours showed that water and oil absorption capacities were 0.50 g/g-3.43 g/g and 1.34 g/g-1.85 g/g respectively, while foaming capacity (28.61%) was highest in the dehulled seed. All the three samples contained considerable amounts of calcium (79.3 mg/100g-707.3 mg/100g), sodium (939.0 mg/100g-2256.7 mg/100g) and potassium (137.0 mg/100g-918.0 mg/100g). Cookies were produced with varying amount of watermelon seed. Cookies with no watermelon seed flour which served as the control (NWC), cookies with 10g dehulled watermelon seed flour + 20 g margarine (WC1), cookies with 20 g dehulled watermelon seed flour +10 g margarine (WC2) and WC3-cookies with 30 g dehulled watermelon seed flour and no margarine. The analysis of the formulated cookies revealed that increase in the levels of dehulled watermelon seed flour resulted in increase in the protein content (22.65% to 33.25%) and weight (4.23 g-5.29 g), with reduction in fat (3.00%-7.50%) and carbohydrate content (57.55%-58.98%). It was observed that cookies with 20 g watermelon seed +10 g margarine produced acceptable cookies, which were not significantly different from the control.

1. Introduction

One possible means of achieving nutrition security in developing countries,where the supply of animal protein is inadequate and not affordable by all, is through exploitation and utilization of locally sourced food materials. Also, consumer's demand for healthier foods from natural products have necessitated research efforts towards the study of potential utilization of protein from locally grown food crops (Enujiugha, 2000). The consumption of cereal based foods which include biscuits and bread have become very popular especially among children.

Margarine is considered as a major contributor of total *trans* fatty acids in diets through baked products and fast foods (Ascherio et al., 1999). Concerns have been raised for several decades that consumption of *trans* fatty acids might have contributed to coronary heart disease (Booyens et al., 1988).

According to the American Heart Association, saturated fat is the main alimentary cause of increased plasmatic cholesterol levels (AHA, 2000). There is need to source for a substitute of fat or food ingredients that can replace totally or partially the lipids in baked foods without altering the organoleptic properties.

Watermelon is grown in the warmer parts of the world. The juice or pulp from the fruit is used for human consumption, while the rind and seeds are major solid wastes (Lewinsohn et al., 2005). Watermelon seed contain about 35% protein, 50% oil, 5% dietary fiber, vitamins, antioxidants, minerals and phytochemicals (El-Adawy and Taha, 2001; Hayashi et al., 2005). Interestingly, the seed of some fruits have higher vitamins, fibres, minerals and other essential nutrients than the pulp fractions (Jayaprakasha,2001). However, the nutritional values of many seeds have not been given much attention such that most times this part of fruits is discarded even with their hidden nutrients. It is therefore necessary to evaluate the chemical and functional properties of water melon seed and the ability of dehulled watermelon seed to serve as substitute for margarine in the production of cookies

2.Materials and methods

2.1.Sample Collection

Watermelon seeds, wheat flour, sugar, salt, margarine, milk, sodium bicarbonate and vanilla essence used in this study were purchased from alocal market in Akure, Ondo State, Nigeria. The chemicals used were of analytical grade and purchased from Pascal Scientific Laboratory, Akure, Nigeria.

2.2.Sample Preparation

The fruits were sliced open and the seeds were manually separated from the pulp. The seeds were washed with portable water to remove adhering pulp and dried at 45 ^{o}C in an oven for 1 h. The seeds were divided into two portions; one part was left unbroken (whole seed), while the other half was broken with hands to obtain dehulled seed and the hull. They were separately milled into flour using a kitchen blender, packaged in airtight containers and labeled. Watermelon seed flours were stored in the refrigerator for further analyses.

2.3. Determination of proximate composition of watermelon seed flour and the formulated cookies

Crude protein, lipid, crude fibre, moisture and ash content were determined in accordance with the standard methods of AOAC (2005). Carbohydrate was determined by difference.

2.4.Determination of Functional Properties of watermelon seed flour

The method of Onwuka (2005) was used for the determination of functional properties. The functional properties determined include water and oil absorption capacities, bulk density swelling index and foaming properties.

2.5.Determination of mineral content of watermelon seed flour

Two grams of sample was placed in a crucible, ashed in a muffle furnace at 550 ^{o}C for 5 h and transferred into the dessicator to cool. The ashed sample was dissolved with 1ml nitric acid and 1ml HCl and made up to 100 ml. This was used to analyze for Mg, Na, Ca, K, Mnand Zn. The atomic absorption spectrophotometer was used to determine these elements (AOAC, 2005).

2.6. Determination of Amino acid composition

The amino acid composition of the whole and dehulled seed was determined using an amino acid analyser after hydrolysing the samples with 6 M HCl at 100 ^{o}C for 22 h. The contents of different amino acids recovered are presented in g/100 g (Bassler and Buchholz, 1993).

2.7.Production of Cookies

Four samples of cookies were formulated as presented in Table 1, where margarine was substituted with dehulled watermelon seed flour. This include; the control, cookies with 30 g margarine and no watermelon seed flour (NWC), cookies with 20 g margarine + 10 g watermelon seed flour (WC1), cookies made with 10 g of margarine +20 g of watermelon

seed flour (WC2), and cookies prepared from 30 g watermelon seed flour with no margarine (WC3). Cookies were prepared as described by Ayo *et al*. (2007) using wheat flour, sugar, fat (margarine and watermelon flour), salt, sodium bicarbonate, water, milk and vanilla essence. All the ingredients were weighed and were mixed thoroughly (except flour) in a Kenwood mixer (a 3-speed hand mixer) appropriately and two stage creaming up method was used. Flour and sodium bicarbonate were later added with continuous mixing for 15 min until smooth dough was obtained. A piece of this dough was cut, placed on a clean platform then rolled out using rolling pin until the desired uniform texture and thickness was obtained. Cookies cutter was used to cut the sheet of the dough into required shapes and sizes. These were transferred onto a greased baking tray. The baking was done for 15 min -20 min. After baking, the hot cookies were removed from the pan and placed on a clean tray to cool down. The cookies were then packed in polyethylene sachets, sealed and kept at room temperature for further analyses.

Table 1. Formulation of the cookies

Ingredients	NWC	WC1	WC2	WC3
Wheat flour (g)	100	100	100	100
Sugar(g)	10	10	10	10
Margarine (g)	30	20	10	0
Dehulled watermelon seed flour (g)	0	10	20	30
Sodium bicarbonate (g)	1	1	1	1
Milk (g)	10	10	10	10
Water (ml)	50	50	50	50
Vanilla essence (ml)	2	2	2	2

NWC-cookies with 30 g margarine and no watermelon seed flour (control), WC1-cookies with 10g dehulled watermelon seed flour + 20 g margarine, WC2-cookies with 20 g dehulled watermelon seed flour +10 g margarine, WC3-cookies with 30 g dehulled watermelon seed flour and no margarine.

2.7.1.Physical analysis of the formulated cookies

The weight of the biscuits was measured on a weighing balance. The diameter and spread ratio were determined as described by Ayo *et al*. (2007). Three rows of the five well-formed biscuits were made and the height measured as well as arranging the same cookies horizontally edge and the sum of the diameter measured.

2.7.2. Sensory analysis of the cookies

Twenty-two untrained tasters evaluated the cookies characteristics such as crispiness, aroma, taste, color and overall acceptability using a nine point Hedonic scale where 1 represents "extremely dislike" and 9 "extremely like" respectively (Solomakos et al., 2008).

2.8. Statistical Analysis

Results were expressed as the means of three separate determinations. The data were subjected to analysis of variance (ANOVA) using the statistical package for social statistics (SPSS version 12). Means were separated using the Duncan's new multiple range test

3. Results and discussions

3.1.Proximate composition of whole, dehulled and hull of watermelon seed flour

Proximate compositions of whole, dehulled and hull of watermelon seed flour are presented in Table 2. The results revealed that the moisture content of the dehulled seed flour (7.17%) was significantly higher than that of whole seed flour (3.66%) and seed hull flour (3.45%). Echendu (2004) reported that low moisture content enhances keeping quality of flours. The protein and fat contents of the seed

flours was observed to be highest in the dehulled seed (25.50%; 48.06%), followed by whole seed (21.62%; 31.27%) and seed hull (5.78%; 6.24%). The crude protein value of the dehulled seed competes favourably with those of germinated pumpkin and sesame seeds (Fagbemi et al., 2006; Olagunju and Ifesan, 2012). The fat content (48.06%) falls within the range of oil seed (43% to 51%) as reported by Ige et al. (1984). However, the whole seed (21.17%) contains highest content of crude fibre compared to the dehulled seed (12.57%) and seed hull (7.54%). This may suggest that the watermelon whole seed should be considered when the purpose of application in food formulation is the fibre content.

Table 2. Proximate composition (%) of whole, dehulled and hull from seeds of watermelon

Component	Seed Hull	Whole seed	Dehulled seed
Moisture	3.45±0.02^{c}	3.66 ±0.12^{b}	7.17±0.07^{a}
Crude Protein	5.78±0.40^{c}	21.62±0.09^{b}	25.50±0.05^{a}
Fat	6.24±0.05^{c}	31.27±0.03^{b}	48.06±0.06^{a}
Ash	1.30±0.03^{c}	2.79±0.01^{a}	2.08±0.03^{b}
Crude fibre	7.54±0.04^{c}	21.17±0.36^{a}	12.57±0.04^{b}
Carbohydrate	75.69±0.38^{a}	19.49±0.21^{b}	4.62±0.19^{c}

All values are means of triplicate determinations ± standard deviation.
Means within rows with different letters are significantly different (P ≤0.05).

3.2.Functional properties of watermelon seed flours

The functional properties of watermelon seed flours (whole, dehulled and seed hull) are shown in Table 3. The bulk density of the whole seed (0.94 g/ml) and dehulled seed flour (0.91 g/ml) were higher than that of the seed hull flour (0.79 g/ml). The bulk densities of dehulled and whole seed were higher than those reported for cowpea varieties (0.71 g/cm^3) and pigeon pea (0.68 g/cm^3) (Butt and Batool 2010). High bulk density is desirable for greater ease of dispersibility of flours. In contrast, however, low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999). Since seed hull flour had the least bulk density it could be the most suitable for the production of complementary foods. Functional properties are the intrinsic physicochemical characteristics, which may affect the behavior of food systems during processing and storage. Seed hull flour had significantly higher water absorption capacity (3.43 g/g) than whole (1.02 g/g) and dehulled seed flour (0.50 g/g). The low WAC of dehulled seed flour might due to its high protein and fat content which cover the water binding sites on the side chain groups of protein (Compaoré et al., 2011). According to Butt and Batool (2010) protein has both hydrophilic and hydrophobic properties, and so can interact with water in foods. The observed highwater absorption in the seed hull flour may be due toits high carbohydrate content which have been reported to influencewater absorption capacity of foods (Adejuyitan et al., 2009). The oil absorption capacity of the flours ranged from 1.34 g/g to 1.85 g/g. The highest oil absorption capacity was noticed in the seed hull flour (1.85 g/g), followed by the dehulled seed flour (1.67 g/g) while the whole seed flour (1.34 g/g) had the lowest. The ability of the proteins to bind oil makes them useful in food systems where oil imbibition is desired. The flours could, therefore, have functional uses in foods such as sausage production.The high oil absorption capacity also makes the flours suitable in facilitating enhancement in flavor and mouth feel when used in food preparations. The foaming capacity of the three flours varied significantly with highest foaming capacity from dehulled seed flour (28.61%), 14.47%

from whole seed flour and seed hull flour (0.09%). Oil seed proteins have recently found application as aerating agents in whipped toppings, frozen desserts, angel food and sponge cakes. Watermelon whole and dehulled seed flour may be utilized for these food products in view of their good foaming properties.

Table 3. Functional properties of watermelonseed flours

Sample	Seed hull	Whole seed	Dehulled seed
Bulk density (g/ml)	0.79 ± 0.01^{b}	0.94 ± 0.01^{a}	0.91 ± 0.03^{a}
Water absorption capacity (g/g)	3.43 ± 00.03^{a}	1.02 ± 0.05^{b}	0.50 ± 0.01^{c}
Oil absorption capacity (g/g)	1.85 ± 0.01^{a}	1.34 ± 0.05^{c}	1.67 ± 0.01^{b}
Foaming capacity(%)	0.09 ± 0.12^{c}	14.47 ± 0.02^{b}	28.61 ± 0.48^{a}
Swelling power(%)	62.50 ± 0.50^{a}	43.69 ± 0.69^{b}	6.43 ± 0.27^{c}

Results are expressed as mean ± standard deviation of three replicates. Means followed by different lowercase letters in the same row are significantly different at 0.05 significance level.

3.3.Mineral composition of watermelon seed flours

Mineral contents of the whole, dehulled and hull of watermelon seed flour are presented in Table 4. Dehulled seed flour had significantly higher magnesium (707.3 mg/100 g), potassium (918.3 mg/100 g), zinc (291.0 mg/100 g) and manganese (338.3 mg/ 100g) content than whole seed and seed hull flours. Calcium (2145.7 mg/100 g-5654.0 mg/100 g) was the predominant mineral followed by sodium, potassium and magnesium.Mineral content of agricultural produce is strongly influenced by cultivation conditions; soil structure condition, time of planting and harvesting and fertilization. The mineral content of watermelon seed flours may be an indication that they can be employed in food fortification to improve their nutritional properties.

Table 4. Mineral composition (mg/100 g) of watermelon seed flours

Element	Seed hull	Whole seed	Dehulled seed
Magnesium	79.3 ± 0.06^{c}	267.0 ± 0.17^{b}	707.3 ± 15.12^{a}
Sodium	2256.7 ± 2.25^{a}	1610.7 ± 0.70^{b}	939.0 ± 0.66^{c}
Potassium	137.0 ± 4.27^{c}	492.7 ± 4.07^{b}	918.3 ± 7.52^{a}
Zinc	ND	32.0 ± 0.30^{b}	291.0 ± 1.04^{a}
Calcium	5654.0 ± 3.92^{a}	2145.7 ± 2.72^{c}	2409.7 ± 4.55^{b}
Manganese	49.3 ± 0.06^{c}	193.0 ± 0.10^{b}	338.3 ± 0.12^{a}

Results are expressed as mean ± standard deviation of three replicates. Means in the same row with different letters significantly are different ($p \leq 0.05$). ND (not detected)

3.4.Amino acid composition of watermelon seed flours

Amino acid composition of whole and dehulled seed of watermelon seed are shown in Table 5. The amino acid profiles of the dehulled seed flour and whole seed flour were slightly different with higher concentration in the dehulled seed. This may be because protein is majorly concentrated in the endosperm. Dehulled watermelon seed flour was richer in total essential amino acids (36.19 g/100 g), isoleucine (4.67 g/100 g), leucine (7.37 g/100 g), total sulphur amino acid (3.02 g/100 g), total aromatic amino acids (6.32 g/100 g) and

threonine (5.06 g/100 g) as compared with the FAO/WHO (1991) reference pattern. The results were similar to previous reportthat aspartic acid and glutamic acid were the most abundant amino acids in legumes and nuts (Olagunju and Ifesan, 2013). Dehulled watermelon seed could be therefore used to complement protein source that are low in essential amino acid. The proportions of arginine, and other essential amino acids that are present in the dehulled watermelon seed indicated that the seeds may be useful in formulating weaning food.

Table 5. Amino acid composition (g/100 g) of whole and dehulled watermelon seed flours

Amino acid	Whole seed	Dehulled seed	FAO/WHO (1990)
Isoleucine	3.98	4.67	2.8
Leucine	6.96	7.37	6.6
Lysine	3.05	3.15	5.8
Cystine	1.12	1.29	
Methionine	1.77	1.93	
Total sulfur amino acids	2.89	3.02	2.5
Tyrosine	1.80	1.77	
Phenylalanine	4.05	4.55	
Total aromatic amino acid	5.85	6.32	6.3
Threonine	4.28	5.06	3.4
Valine	4.20	4.53	3.5
Total essential amino acids	35.04	36.19	36.0
Histidine	3.20	3.37	1.9
Arginine	8.96	10.02	
Aspartic acid	9.41	9.74	
Glutamic acid	15.65	15.97	
Serine	6.29	6.04	
Proline	3.72	3.82	
Glycine	6.28	6.13	
Alanine	4.34	5.42	

3.5.Proximate composition of the formulated cookies

The proximate composition of the formulated cookies is presented in Table 6. The moisture content of the formulated cookies ranged from 3.00% to 7.57% and sample WC3 (3.00%) had the lowest moisture content which might be due to its low fat content. However all samples presented a low moisture content required for stability of food product. An increase in the proportion of watermelon seed flour substitution resulted in increase in the protein content progressively. Cookies WC3 possessed significantly higher protein content (33.25%) than WC2 (22.75%) and WC1 (22.65%), while NWC (control) contained the least protein content (21.00%). Addition of watermelon seed flour improved the quantity of protein content of the product, thereby has the great potential in combating protein energy malnutrition. The protein content of wheat-watermelon cookies was found to be higher than that reported for wheat-sesame cookies (Olagunju and Ifesan, 2013). Regarding the fat content of the samples, a significant reduction in the fat content of the formulations was observed as percentage of watermelon seed flour increased (3.00% to 9.50%). Cookies with no margarine but 30 g watermelon seed flour (WC3) presented the lowest value (3.00%) when compared to (NWC) cookies with 30 g

margarine and no watermelon seed (9.50%). It was observed that the crude fibre values of all the samples were not different significantly, which could be due to low percentage of dehulled watermelon seed in the total formulation. There was also a downward drift in the carbohydrate content of the formulated cookies as the proportion of watermelon seed flour increased. Formulation WC3 possessed the lowest carbohydrate content (57.55%) when compared with the control sample (60.65%), while the carbohydrate content of WC1 (58.98%) and WC2 (58.48%) were not significantly different($P \leq 0.05$) but higher than that of WC3. It was observed that the fat and carbohydrate content of cookies followed the same trend.

Table 6. Proximate composition (%) of the cookies

Sample	NWC	WC1	WC2	WC3
Moisture	5.79±0.01^{b}	7.17±0.15^{a}	7.57±0.07^{a}	3.00±0.50^{c}
Protein	21.00±0.50^{c}	22.65±0.05^{b}	22.75±0.05^{b}	33.25±0.15^{a}
Fat	9.50±0.15^{a}	7.50±0.70^{b}	6.50±0.40^{c}	3.00±0.50^{d}
Ash	3.00±0.05^{c}	3.50±0.02^{b}	4.50±0.04^{a}	3.00±0.02^{c}
Crude fibre	0.15±0.03^{a}	0.20±0.03^{a}	0.20±0.04^{a}	0.20±0.06^{a}
Carbohydrate	60.65±0.01^{a}	58.98±0.05^{b}	58.48±0.03^{b}	57.55±0.03^{c}

Means followed by different lowercase letters in the same row are significantly different. NWC-cookies with no watermelon seed flour (control), WC1-cookies with 10g dehulled watermelon seed flour + 20 g margarine, WC2-cookies with 20 g dehulled watermelon seed flour +10 g margarine, WC3-cookies with 30 g dehulled watermelon seed flour and no margarine.

Table 7. Physical properties of the cookies

Sample	NWC	WC1	WC2	WC3
Weight (g)	3.91±0.08^{c}	4.23±0.02^{b}	4.28±0.03^{b}	5.29±0.08^{a}
Height (cm)	0.68±0.08^{c}	0.93±0.12^{b}	1.27±0.04^{a}	1.37±0.08^{a}
Diameter (cm)	2.67±0.15^{a}	2.65±0.05^{a}	2.63±0.06^{a}	2.73±0.08^{a}
Spread ratio	1.10±0.04^{b}	1.21±0.10^{a}	1.04±0.01^{b}	0.81±0.01^{c}
Breakability (%)	40.00±0.00^{b}	40.00±0.32^{b}	40.00±0.32^{b}	60.00±0.00^{a}

Means followed by different lowercase letters in the same row are significantly. NWC-cookies with no watermelon seed flour (control), WC1-cookies with 10g dehulled watermelon seed flour + 20 g margarine, WC2-cookies with 20 g dehulled watermelon seed flour +10 g margarine, WC3-cookies with 30 g dehulled watermelon seed flour and no margarine.

Table 8. Sensory analysis of cookies substituted with watermelon seed flour

Sample	NWC	WC1	WC2	WC3
Crispiness	7.20±0.85^{b}	6.00±1.49^{c}	8.40±0.70^{a}	8.40±0.70^{a}
Colour	6.20±01.48^{c}	6.80±0.63^{bc}	7.30±0.82^{b}	8.30±0.95^{a}
Aroma	6.60±0.84^{a}	5.90±1.52^{a}	6.60±1.35^{a}	6.00±1.15^{a}
Taste	7.50±1.08^{a}	5.30±1.70^{b}	6.90±1.60^{a}	4.80±1.62^{b}
Overall acceptability	7.10±0.57^{a}	5.80±1.62^{b}	7.20±1.14^{a}	5.50±0.71^{b}

Means followed by different lowercase letters in the same row are significantly different. NWC-cookies with no watermelon seed flour (control), WC1-cookies with 10g dehulled watermelon seed flour + 20 g margarine, WC2-cookies with 20 g dehulled watermelon seed flour +10 g margarine, WC3-cookies with 30 g dehulled watermelon seed flour and no margarine.

3.6.Physical characteristics of the cookies

The physical properties of the formulated cookies are presented on Table 7. The results revealed that significant difference ($P \leq 0.05$) in weight, height, spread ratio and breakability were found among the different cookies formulated. However, substitution of margarine with watermelon seed flour showed no significant difference in the diameter of the cookies but higher effect in breakability from 40.0%-60.0% which could be attributed to the fat content of the samples.The value recorded for breakability for cookies NWC, WC1 and WC2 is the same with that reported for wheat-sesame cookies (Olagunju and Ifesan, 2013). The mean weight of the cookies increased from 3.91 g in cookies without watermelon seed flour (NWC) to 5.29 g in cookies with 30 g watermelon seed flour (WC3) while the mean weight of cookies WC1 and WC2 were not significantly different.The spread ratio of formulated cookies decreased from 0.81 - 1.21 with increase in watermelon seed content.
Generally, the cookies spread ratio decreased with increase in protein content of the cookies. It was reported that rapid partitioning of free water to hydrophilic sites during mixing increased dough viscosity and limited cookies spread ratio during baking (McWatters, 1978).

3.7. Sensory analysis of the cookies

Sensory rating of the cookies on Table 8 showed crispness which is a desirable quality of cookies were significantly ($p \leq 0.05$) superior in cookies with no margarine (WC3) and (WC2) watermelon seed flour than in other samples. It is possible that the high fat content of the control and cookies with 10 g watermelon seed flour could be responsible for their lower score in crispness. Colour is an important sensory attribute for any food because of its influence on acceptability (Ifesan et al., 2009). The mean score of colourincreased significantly as substitution of margarine with watermelon seed increased. Cookies with no margarine (8.30) was scored higher and was superior to all other cookies (6.20-7.30). The brown colour resulting from Maillard reaction is always associated with baked goods. With increasing level of watermelon seed, the colour of the cookies turned from dark brown to light brown, leading to higher acceptance. Mean score for taste revealed that the control cookies (7.50) and sample WC2 (6.90) had highest score followed by sample WC1 (5.30) and WC3 (4.80). The response of panelist to taste attribute also pointed out the difference between sample WC3 and others, indicating that total replacement of margarine with watermelon seed flour affected the taste, resulting in scores lower compared to samples with margarine and watermelon seed. This may be explained due to the difference in the taste of margarine and watermelon seed flour. For overall acceptabilityit was observed that there was no significant difference between the control and cookies WC2 while the other two samples were not scored below average.

4.Conclusions

The findings from this study show that watermelon seed is rich in fat, protein and essential amino acids. This may be an indication that dehulled watermelon seed flours could be used to fortify conventional flours which are low in protein and would be an important step towards alleviating protein malnutrition. The good functional properties exhibited by watermelon seed flours may suggest their application in food systems. Furthermore, cookies with 20 g dehulled watermelon seed flour +10 g margarine could compete very well with cookies with 30 g margarine. Therefore, the dehulled watermelon seed flour may be considered as a source of fat in baking industry.

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THE EFFECTS OF CONTINUOUS WAVE LASER RADIATION IN THE VISIBLE REGION AGAINST *E.COLI O157: H7* IN PROBIOTIC *DOOGH*

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ABSTRACT

This study is an attempt to explore the possibility of carrying live microorganisms and active probiotic *doogh* as a functional food with acceptable organoleptic properties as well as assessing the survival of pathogenic *E. coli* bacteria in probiotic products affected by the continuous wave laser (EW). In this study, probiotic microorganisms (*Lactobacillus casei*) was inoculated with the amount of 10^9-10^8 CFU/mL and pathogenic *E. coli* bacteria was inoculated with the amount of 10^3 CFU/mL into the *doogh*. The *doogh* samples were radiated by the continuous laser with the wavelength of 437 nm for 10, 20, 30, and 60 minutes. During the cold storage period (days 1, 3, 5 and 7), microbial evaluation (bacterial pathogen destruction, survival of probiotic bacteria) and changes in pH were investigated. Based on the findings of this study survival of the pathogenic bacteria was statistically significant during different days of storage time ($p<0.05$). The lowest count of all treatments was observed on the 7^{th} day of storage period. Laser treatment and probiotics had a synergistic effect on reducing the pathogenic bacteria ($p< 0.05$). However, the decrease in the survival of *E. coli* in the probiotic *doogh* and laser radiation treatment was much more than these treatments individually. According to the results of the probiotic bacterial count, the highest survival was observed after the 60-minute radiation at the end of the storage time. Laser treatment has no significant effect on the pH reduction in compared with the control sample.

1.Introduction

Food contamination draws a lot of attention as one of the major challenges in the food industry, whereas many diseases are caused by food contamination. Different researches have been done on the different technologies of reducing or eliminating microbial dangers of foods. Methods used for reducing these risks are heat, drying, freezing and additives. Despite the positive impact of this approach in improving food safety, these methods cause undesirable changes in food quality and nutritional compounds and ultimately reduce the nutritional value. To eliminate or reduce the adverse effects of these methods, numerous efforts have been made in the field of non-thermal technologies for production of healthy food with the least adverse impact on the qualitative, organoleptic, and nutritional

characteristics (Alikhani et al., 2011). The non-thermal treatments that can be cited are (PEF) Pulsed Electric Field, (PLT) Pulsed Light and ultraviolet and infrared radiation (Mahmoudi et al., 2012).

Strong optical radiation has been used as a protective technique to treat the contamination levels of food through the destruction of microorganisms in the wavelength of 200 to 1000 nm (Dunn, 1995). The use of radiation in the food industry reduces or eliminates the chemical preservatives and this fact is of importance in keeping and promoting the health of consumers is important and in this way it reduces health problems and economic disadvantages. Other mentionable advantage is absence of residues in the food samples. Optical radiation has impressive performance in reducing the microbial population, vegetative cells and spores in the food surface. However, no specific information is available on the effect of optical radiation on food and food industry, which is a very important fact (Rowan et al., 1999). Non-thermal technology is widely used in food processing as an alternative to thermal treatments (Barbosa-Cánovas et al., 1995). Traditionally, most food products are processed by using thermal treatments of 60°C for a few minutes or 100°C for a few seconds. During this time, large amount of energy is transferred to the food and so can cause the production of undesirable secondary compounds or undesirable changes. On the other hand, non-thermal treatments the temperature of the food products is much lower than the thermal treatments. So, the vitamins and the aromatic compounds are hardly altered or don't get changed at all. Food consumers ask for high quality processed food with minimal changes in nutritional and sensory factors, therefore non-thermal treatments are more efficient in keeping the foods' quality than the thermal technology (Gemma et al.,2010). UV radiation is one of the physical and optical methods for eradicating food contamination. This treatment is widely used for surface and drinking water sterilization (MurpH., 2008).

Pulsed-light treatment on milk proteins using electrophoresis (SDS-PAGE) showed the formation of mostly dimers in the BLG proteins after a 5-pulse treatment, while no noticeable changes happened in the composition of the proteins' amino acids and the fat oxidations (Funtenberger et al., 1997; Guerrero et al., 2004). Variations in the amount of polarity of some of the amino acids and sedimentation of some proteins were other changes in this study. Popo et al. (2004) and Liu et al. (2007) reported the sedimentation of soy proteins after a treatment of high-power pulsed electric field (Liu et al., 2007, Puppo et al., 2004). Fantinburger et al. (1997) considered the formation of di-sulfide bonds as a reason to BLG sedimentation (Funtenberger et al., 1997; Guerrero-Beltrán et al., 2004). Liu et al. (2007) considered the presence of BLG dimers following HHPT as a marker of low presence of non-reducible dimers following HHPT and PLT (Liu et al., 2007). Many more studies on the proteins and other food components are required in order to fully understand the radiation treatments in food systems, so that ideal circumstances are used for applying these treatments for various food models.

2.Material and Methods

2.1. Preparation of probiotic and spiked out *doogh* samples

First of all, the homogenized milk with 1.5% fat was pasteurized in 90°C. It was cooled down to 43°C. Then, Streptococcus thermophiles, *Lactobacillus* delbrueckii sub. bulgaricus, and 2% of salt were added. It was incubated with the starter solution in 42°C and the fermentation was continued until the pH reached over 4.6. In this study, the produced *doogh* was divided into 4 different samples. *Doogh* samples were inoculated with (probiotic culture) initial number of 8 CFU/mL, 48 hours

Incubation time and 30 °C temperature. The *doogh* samples were dried up to 9.5% of dry matter.

The samples, taken in days 1, 3, 5 and 7, were kept in 4°C until the end of the research. Doogh samples were spiked out with *E. Coli* by 3 CFU/mL initial cell count.

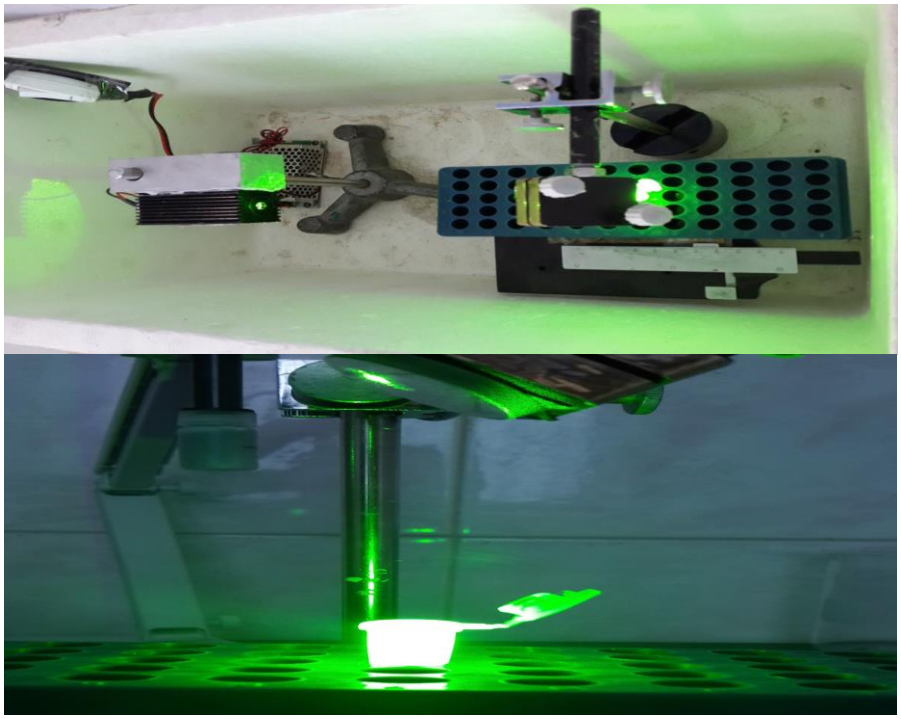

Figure 1. Laser device.

2.2.Laser radiation treatment

A box, which was stabilized on the table, was used in this study. Then, the laser-producing source in one end and the adjustable base was set at the other side, in order to ensure the precise position of laser reflex after meeting the mirror in the center of the sinks. The sinks were 5 millimeters apart.

Laser with constant wavelength produced by a diode laser with a wavelength of 437 nm and intensity of 50 w/cm2 was radiated to the *doogh* samples (with *Lactobacillus casei*, *E. coli* and control) for specific periods of time (10-20-30-60 minutes), and at last the counting was done on the days 1, 3, 5, and 7 while keeping them refrigerated.

2.3.Microbiological analysis

Enumeration of *E. coli* and probiotic bacteria (*Lactobacillus casei*) were implemented based on culturing method by EMB agar at 37 °C for 48 hours and MRS agar at 30 °C for 48 hours respectively.

2.4.Statistical methods

Statistical analysis was done by SPSS17 and variance analysis (ANOVA). The meaningful results were considered by $P< 0.05$.

3. Results and discussions

The results of this study demonstrated that the survival of the pathogen bacteria during different days of storage leads to meaningful statistical differences ($p<0.05$), in which the lowest enumeration was found on the 7^{th} day in all samples (Figure 1). Laser treatment in

different times had significant statistical differences in pathogen bacteria decrease in compared to the control group ($p<0.05$) (Figure 1).

Also, the amount of decrease in the probiotic *doogh* and laser treated samples had more effect in comparison with any of simple treatments ($p<0.05$) (Figure 2).

The results of pathogen bacteria survival rate in probiotic *doogh* during different periods of radiation implemented significant statistical differences in comparison with the control group ($p<0.05$), as the lowest enumeration in all the samples was seen on the 7th day of storage period (Figure 2).

The bacterial count in the probiotic *doogh* treated with 20 minutes radiation was 1.0 log CFU/mL on the first day and 2.66 log CFU/mL on the 7th day (Figure 2).

Also, bacterial counts in *doogh* samples with 30 minutes of radiation was 2.95 log CFU/mL on the first day and 2.64 log CFU/mL.

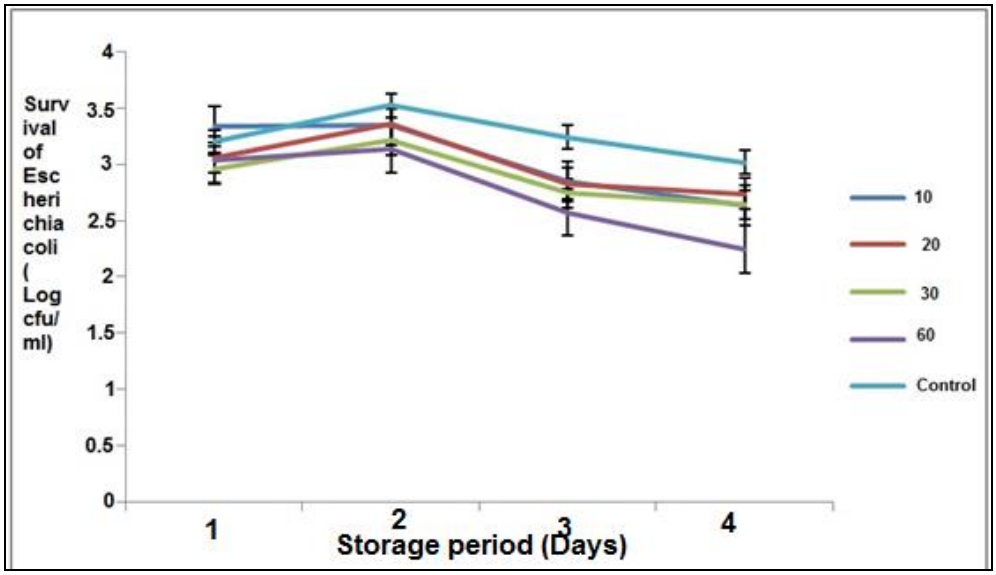

Figure 2. Laser radiation affected the survival of Escherichia coli in doogh with the continuous wave (EW) during the shelf life of 7 days.

The comparison of the results of decreased *E. coli* survival in the probiotic *doogh* with 20-minute-radiation and 30-minute-radiation showed that the 10-minute-decrease in laser radiation treatment caused similar results in the probiotic *doogh*, which demonstrated the synergistic effect of probiotic bacteria and laser radiation treatment on *E. Coli* enumeration.

This study showed that the value of pH decreased during the 7 days of storage time. Even though, the laser radiation treatment had no significant correlation with the inclination compared to the control group samples (Figure3).

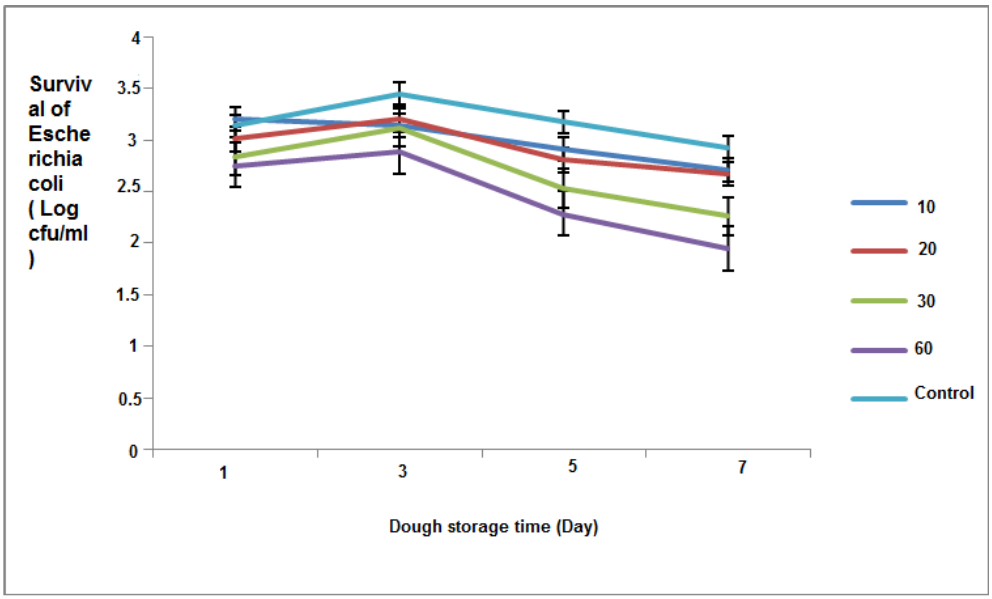

Figure 3. Survival of Escherichia coli bacteria in probiotic doogh continuous wavelength laser radiation treated (EW) during the shelf life of 7 days.

The results of pH value changes evaluation demonstrated decrease during the 7-day storage (Figure 4).

According to the results of the probiotic bacterial count, the highest survival was seen in the 60-minute radiation (8.41 log CFU/mL) and the lowest survival was observed in the control samples (7.63 log CFU/mL) (Figure 5).

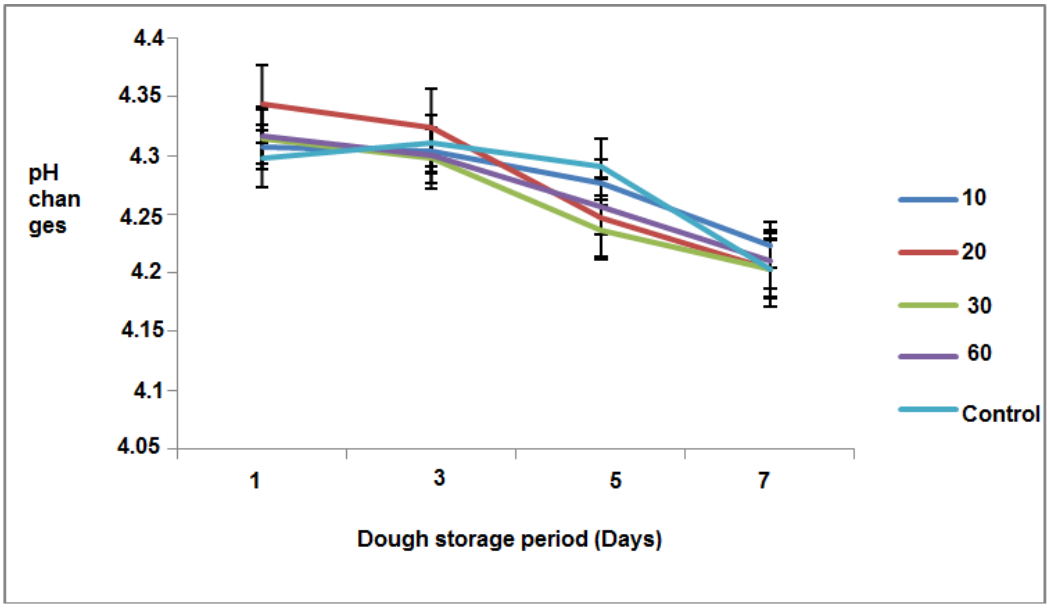

Figure 4.pH changes in the doogh under the treatment of laser radiation with continuous wavelengths (EW) During the storage period of 7 days.

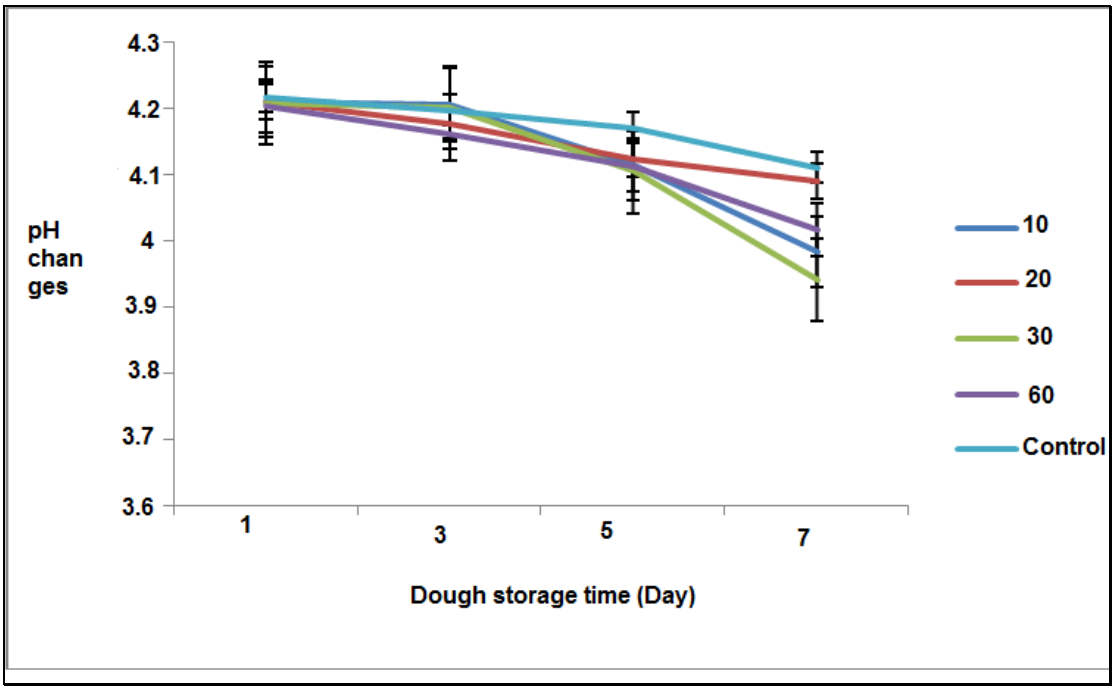

Figure 5. pH changes in probiotic doogh by laser radiation with continuous wavelengths (EW) during the shelf life of 7 days.

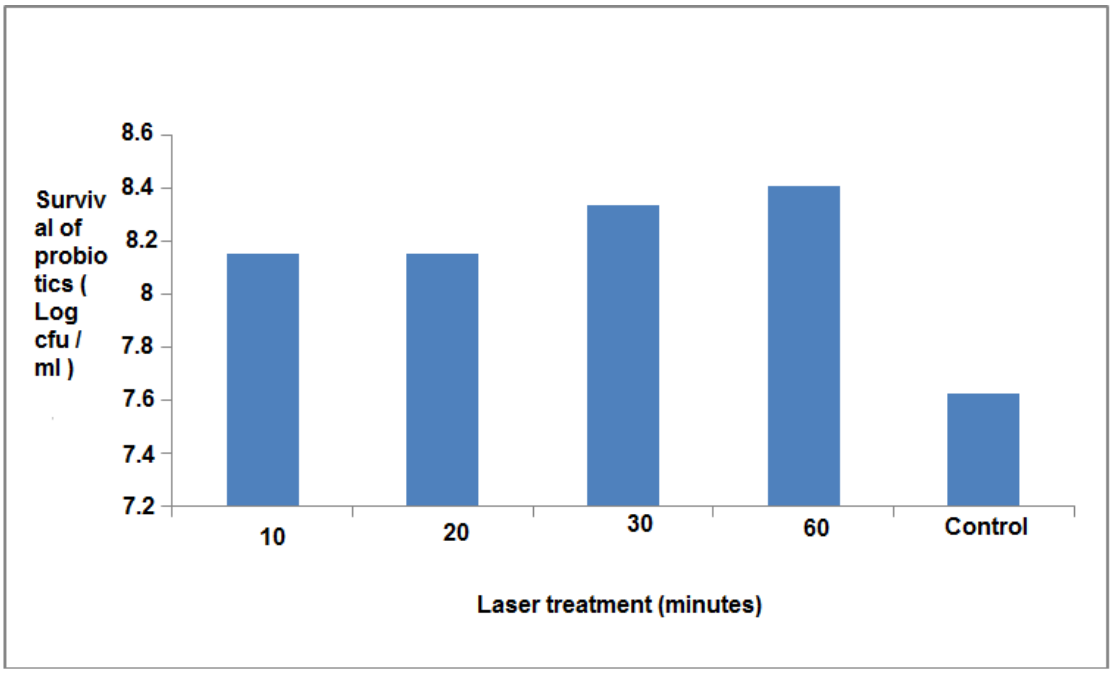

Figure 6. Shelf life of probiotics in different treated *doogh* samples.

In this study, we investigated the antagonistic effects of 3 types of probiotics (*Lactobacillus*) on the urinary tract infection-causing *E. coli*. The results showed that *Lactobacillus Casei* can be used as a probable candidate for a probiotic against urinary tract infection-causing *E. coli* (Dunn, 1995).

The performance of ultraviolet radiation in disinfecting the aquatic environments from *E. coli* showed that the ultraviolet process can be

used as an efficient method for disinfecting aquatic environments from *E. coli* (Alikhani et al., 2011).

Also in present study it is demonstrated that the radiation of constant-wave laser retains antibacterial effects against *E. coli*. In addition, the decrease in the survival of *E. Coli* in the probiotic *doogh* and laser radiation treatment was much more than these treatments separately. According to other researchers (Mahmoudi et al., 2012), the effect of the essential oil of mint on the physicochemical characters of probiotic *doogh* during different treatments showed that the simultaneous usage of mint essential oil extract and probiotic bacteria didn't cause any significant statistical difference in the pH variance.

Hermavan and et al. investigated the effects of PEF (Pulsed Electric Fields) treatment in 55°C on the sensory and physical characters of liquid egg, and they showed that the desired parameters (viscosity, color, and pH) didn't make any significant changes.

According to effects of constant-wave laser radiation treatment in the visible range on the physicochemical characters of the albumin proteins, it is demonstrated that the use of constant-wave green laser radiation had no any significant effect on the evaluated organoleptic characteristics of the albumin (smell and color), and the physical properties (pH) in compared with blank samples. In addition, the slight changes in pH gradually over the time in the treated samples and control group were in normal mode, and were due to CO2 outlet from the albumin (Hermawan et al., 2004; Sampedro et al., 2006; Anderson et al., 2000).

According to attained results in this study it can be investigated that the *doogh* pH values decreased during the 7 days of storage time. Although, the laser radiation treatment had no significant correlation with this inclination compared to the control group samples, while the changes in pH values in the probiotic *doogh* samples which were laser-radiation treated were significantly different ($p<0.05$).

4. Conclusions

According to results obtained in this study it can be inferred that constant wave laser treatment had no undesirable significant effects on the physicochemical characteristics of the *doogh* and finally this type of radiation has antibacterial effect on *E. Coli*. Also, the decrease in *E. Coli* survival in the probiotic *doogh* treated with laser radiation was higher compared to any of the individual treatments. The synergistic effects of constant wave laser and probiotic on the *E. Coli* was seen during the 7-day storage time of *doogh*. The pH values of probiotic *doogh* had significant inclination compared to the control samples, which can be due to production of lactic acid by the probiotic bacterial culture. Based on this study, *doogh* can be used as a appropriate probiotic bacteria-holding food product. However, many studies are required in order to achieve the best circumstances of using these treatments for different food models.

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COMPARATIVE STUDY ON POLYPHENOLS CONTENT AND ANTIOXIDANT EFFECT OF OLIVE CULTIVARS FROM THE ISLAND PAROS, GREECE

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Phenolic fractions;
Total phenols.

ABSTRACT

Four different varieties olive fruits (Koroneiki, Throubolia, Kalamon and Dafnolia) from the island Paros were studied for total polyphenol content, phenolic fractions and for antioxidant properties. It has been established that total phenols content, move in broad ranges depending on the variety, chemical soil properties, and with the ecological conditions. The content of TP in the olives varieties varied from 12.76 to 20.03 mg (GAE) g^{-1} FW, (NFP) from 2.72 to 4.659 mg (GAE) g^{-1} FW and (FP) from 8.422 to 15.827 mg (GAE) g^{-1} FW. The highest content of TP has been found in the Dafnolia variety with 20.03 mg (GAE) g^{-1} FW, and the lowest in the Throubolia variety with 12.76 mg (GAE) g^{-1}FW. Antioxidant activity (FRAP) and antiradical activity (DPPH•) is in descending order:Kalamon > Dafnolia,Koroneiki > Throubolia. The olives from the island of the Paros offer a high intake of antioxidants for the prevention of many diseases.

1. Introduction

The reactive oxygen and nitrogen containing free radicals (ROS and RNS), which are formed in the human cells as strong oxidants can cause oxidative damage in the biological molecules. These changes of the biological molecules are the basis for many diseases (Halliwell, 1991).

The epidemiological and laboratory investigations demonstrate that the oxidative destruction of biomolecules can be reduced by using endogenous and exogenous antioxidants such as polyphenols, etc.(Pellegrini et al., 2003; Pellegrini et al., 2006). Their inclusion in a diet reduces the risk of cardiovascular and some cancer diseases (Manach et al., 2004; Owen et al., 2004). Phenolic compounds contained an high proportion in the plants, fruits and vegetables (Gougoulias, 2009; Gougoulias et al., 2012; Gougoulias, 2014).

Olives fruits (*Olea Europaea* L.) are traditional Greek product and one important component of the Mediterranean diet, while it is natural reservoir of phenolic compounds and of natural antioxidants (Boskou and Visioli, 2003; Silva et al., 2006; Zamora et al., 2001). Phenolic compounds of the olives fruit affect the sensory properties of fruits (Brenes et al., 1995). Particular, secoiridoids they have inhibiting activity in the autoxidation of olive oil (Botia et al., 2001). Olive fruits are rich in flavonoids, secoiridoids, phenolic acids and phenolic alcohols who exercise high antioxidant activity (Bianco et al.,

1999;Bouaziz et al., 2005; Dağdelen et al., 2013).

The content and qualitative composition of phenolic compounds in the fruit depends on the genotype, cultivation techniques and the climatic conditions (Tovar et al., 2002; Vinha et al., 2005). Furthermore, changes in the phenolic composition occur during ripening of the olives (Briante et al., 2002; Romani et al., 1999).

The aim of the present study is to determine the content of phenolic compounds, their major fractions and the antioxidant activity, of olive fruits from different varieties, grown on the island Paros, Greece.

2. Materials and methods

2.1. Experimental

Four varieties olive fruits of farms Samaltanis from the island of Paros, Greece, were studied (latitude 37°03′00″ N, longitude 25°11′00″ E, 50 m altitude). The climate in the area is dry and temperate, with mild winters (11-13)^{0}C, hot summers (25-30)^{0}C and average annual precipitation 296 mm.

The Koroneiki variety used for olive oil production, the Kalamon variety used for table olives, and Throubolia and Dafnolia varieties for dual-use. In the olive orchard (A); The Koroneiki variety is grown, 2000 the planting took place with 5m distance tree from tree and 5 m distance line from line, 30 kg manure corresponding to 0.70 kg Nitrogen, 0.64 kg Potassium, 0.24 kg Phosphorus and 8.3 kg Organic matter per tree added, and 2500 m^3 irrigation water per hectare added. In the non-irrigated olive orchard (B); The Throubolia variety is grown,1940 the planting took place with 5m distance tree from tree and 5 m distance line from line, 30 kg manure corresponding to 0.70 kg Nitrogen, 0.64 kg Potassium, 0.24 kg Phosphorus and 8.3 kg Organic matter per tree added. In the non-irrigated olive orchard (C); The Kalamon and Dafnolia varieties is grown, 1900 the planting took place with 5m distance tree from tree and 5 m distance line from line, 30 kg manure corresponding to 0.70 kg Nitrogen, 0.64 kg Potassium, 0.24 kg Phosphorus and 8.3 kg Organic matter per tree added. The collection of fruits took place at November of 2015, at the stage of complete maturation (black color).Two trees by each olive grove were chosen, and were sampled of olive fruits one kilogram with four replicates from each tree, from all the orientations and without type of disease.

Furthermore, leaves were collected from all varieties at the end of their active photosynthesis period. These samples were stored at -18^{o}C and were further subjected to analyses.

2.2. Preparation of the methanol extracts:

Olive fruits. The flesh was separated from the kernel and 100 g of flesh for each treatment were subjected to freeze drying for further extraction and determination of humidity. The dry mass was crushed and stored in clean bottles in refrigeration (Boskou et al., 2006). Two g of dry sample was extracted two times with 50 mL methanol for 24 h at 150 rpm, the methanolic extracts were combined and washed two times with 25 mL n-hexane in order to eliminate the oil of the methanolic extract (Rigane et al., 2011). The separation of the phases was performed with separating funnels. Subsequently the methanolic extract was evaporated under nitrogen, and the residue was dissolved in 50 mL methanol, stored in clean bottles in refrigeration in the dark until its use.

Leaves olive Tree (***Olea europaea*****).** The extracts of the studied plants were obtained after twofold treatment of two g sample with 20ml methanol after one hour storage at dark and room temperature. After centrifugation the combined extracts were brought to 50 ml with methanol and used for further chemical analysis (Yi et al., 1997).

2.3. Methods of analyses

Soil was analyzed using the following methods which are referred by Page (1982).

Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.

Inorganic nitrogen was extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in

the presence of MgO and Devarda's alloy, respectively.

Available P forms (Olsen P) was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy. Exchangeable form of potassium was extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK). pH and (EC), Electrical conductivity measured in the extract (1 part soil : 5 parts H_2O).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1} + triethanolamine 0.1 mol L^{-1}) and measured by atomic absorption. The samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture (Varian, 1989).

Determination of total polyphenolics (TP). Total polyphenolic content was determined with the Folin-Ciocalteu (F.-C.) reagent according to the method by (Singleton and Rossi, 1965) using the microvariant proposed by (Badenschneider et al., 2015) and the results were expressed as gallic acid equivalent (GAE) in mg/g fresh weight.

Nonflavonoid phenols (NFP). The content of NFP was determined with the F.-C. reagent after removing the flavonoid phenols (FP) with formaldehyde according to the method by (Kramling and Singleton, 1969) and was expressed as gallic acid equivalent (GAE) in mg g^{-1} fresh weight.

Flavonoid phenols (FP). Flavanoid phenols were determined as a difference between the content of total phenols (TP) and nonflavonoid phenols (NFP). Their amount was evaluated as gallic acid equivalent in mg g^{-1} fresh weight.

Determination of ferric reducing antioxidant power (FRAP). The antioxidant activity of the methanol extracts was determined on the basis of the method by (Benzie and Strain, 1999) and was expressed as ascorbic acid equivalent (AAE) in μmol g^{-1} fresh weight.

Determination of antiradical activity (DPPH•). The antiradical activity of the methanol extracts was determined according to the method by (Brand-Williams et al., 1995) using the stable free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH•). The activity was evaluated as Trolox equivalent (TEAC) in μmol g^{-1} fresh weight.

2.4. Statistical analysis

Data were analyzed using the MINITAB (Ryan et al., 2005) statistical package. The experiment had four replications. Analysis of variance was used to assess treatment effects. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

3. Results and discussions

Soil samples collected from olive orchards in Paros were analyzed for inorganic nutrient element (N, P, K, Mn, Zn and Cu) and organic matter content. The soils was Sandy Loam (SL) and Loamy Sand (LS) with low content in organic matter and salinity (Table 1).

The single extraction of the fruits with 50 ml methanol results in the extraction of phenol compounds which vary between 12.76 and 20.03 mg (GAE) g^{-1} FW (Figure 1). Among the studied fruits, the Dafnolia variety characterised by the highest TP content, with 20.03 mg (GAE) g^{-1}FW, followed by those of the Koroneiki and Kalamon with 17.24 and 17.00 mg (GAE) g^{-1}FW respectively, and the lowest by the Throubolia variety with 12.76 mg (GAE) g^{-1} FW.

These values are higher than those reported by other authors for some Tunisian and Turkey varieties (Bouaziz et al., 2010; Gurel et al., 2014). Our results are in agreement with the data obtained by other authors who have established differences in phenols content in the different olive fruits varieties (Boskou et al., 2006). The established trend in the variation of phenols within one and the same variety under the action of climatic factors is observed also by other authors (Petridis et al., 2012).

The leaves for all varieties analyzed from the island of Paros, contain phenol compounds (as equivalent to gallic acid) ranged from 23.18 to 31.2 mg (GAE) g^{-1} FW (Figure 1). The

Koroneiki characterised by the highest TP content, with 31.2 mg (GAE) g^{-1} FW, followed by those of the Throubolia and Dafnolia with 27.34 and 25.22 mg (GAE) g^{-1} FW, respectively, and the lowest by the Kalamon with 23.18 mg (GAE) g^{-1} FW. These data show for all cultivars, that leaves had higher TP concentration than fruits. Furthermore, the leaves of olive tree compared with the leaves from other species fruit, they have higher concentration total phenols for some species fruit (peach), and lowest concentration of TP for other species fruit (plum, cherry) (Gougoulias, 2015).

Table 1. Chemical properties of soils cultivated olive fruits

	Soil depth (0-30) cm		
Soil properties	Olive orchard (A) - Koroneiki	Olive orchard (B) - Throubolia	Olive orchard (C) - Kalamon+Dafnolia
Texture	Loamy Sand	Sandy Loam	Sandy Loam
pH (1:5)	7.57 ± 0.25	7.83 ± 0.27	7.66 ± 0.25
EC (1:5), dS m^{-1}	0.16 ± 0.01	0.18 ± 0.01	0.36 ± 0.02
$CaCO_3$ (%)	2.96 ± 0.15	8.87 ± 0.47	9.09 ± 0.84
Organic matter (%)	0.94 ± 0.07	1.54 ± 0.07	2.01 ± 0.1
N-inorganic (mg kg^{-1})	98 ± 6.53	217 ± 15.5	91 ± 7.2
K-exchangeable (mg kg^{-1})	94.8 ± 4.51	199.1 ± 11.7	237.0 ± 12.2
P -Olsen (mg kg^{-1})	13.2 ± 1.02	28.3 ± 1.7	25.8 ± 1.92
Cu-DTPA (mg kg^{-1})	4.20 ± 0.20	4.80 ± 0.22	6.00 ± 0.38
Zn-DTPA (mg kg^{-1})	15.20 ± 0.60	15.30 ± 1.02	18.70 ± 0.78
Mn-DTPA(mg kg^{-1})	2.80 ± 0.30	4.50 ± 0.68	3.20 ± 0.42
	Soil depth (30-60) cm		
Texture	Loamy Sand	Sandy Loam	Loamy Sand
pH (1:5)	7.96 ± 0.30	8.43 ± 0.42	7.77 ± 0.34
EC (1:5), dS m^{-1}	0.12 ± 0.01	0.16 ± 0.02	0.36 ± 0.04
$CaCO_3$ (%)	3.38 ± 0.20	13.52 ± 0.68	10.42 ± 0.57
Organic matter (%)	0.80 ± 0.04	1.11 ± 0.06	1.51 ± 0.08
N-inorganic (mg kg^{-1})	91 ± 4.60	105 ± 6.40	70 ± 5.10
K-exchangeable (mg kg^{-1})	66.4 ± 3.201	104.3 ± 4.20	170.7 ± 6.40
P -Olsen (mg kg^{-1})	13.2 ± 1.12	11.7 ± 0.82	21.0 ± 1.22
Cu-DTPA (mg kg^{-1})	2.92 ± 0.22	3.62 ± 0.28	4.40 ± 0.32
Zn-DTPA (mg kg^{-1})	8.80 ± 0.40	11.2 ± 1.60	14.5 ± 0.52
Mn-DTPA(mg kg^{-1})	2.36 ± 0.32	3.80 ± 0.42	2.52 ± 0.28

Data represent average and SE deviation, (n) = 4.

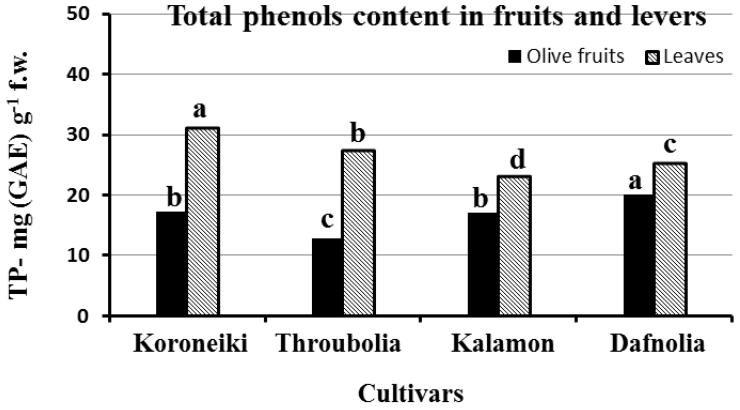

Figure 1. Total phenols content in fruits and leaves of different varieties. Columns in each characteristic of each graph with the same letter do not differ significantly according to the Tukey's test (P=0.05).

Table 2. Flavonoid phenols content and Nonflavonoid phenols in the olives under study

Olive fruits cultivars	Flavonoid phenols (FP) (mg GAE g^{-1} FW)	Nonflavonoid phenols (NFP) (mg GAE g^{-1} FW)
Koroneiki	12.581^{c}	4.659^{a}
Throubolia	8.422^{d}	4.339^{ab}
Kalamon	14.282^{b}	2.720^{c}
Dafnolia	15.827^{a}	4.207^{b}

Rows in each characteristic of each column with the same letter do not differ significantly according to the Tukey's test (P=0.05).

In our studies we have applied a systematic approach through separation of the flavonoid phenols with formaldehyde and thus the nonflavonoid (NFP), flavonoid phenols were determined and presented as gallic acid equivalent (GAE). The content of flavonoid phenols ranges from 8.422 to 15.827 mg (GAE) g^{-1} FW (Table 2). The highest content of FP was determined in the Dafnolia variety with 15.827 mg (GAE) g^{-1} FW, and the lowest in the Throubolia variety with 8.422 mg (GAE) g^{-1} FW. The FP content in the fruits Kalamon and Dafnolia constitute 84.0% and 79% of TP amount, whereas for fruits Koroneiki and Throubolia it is lower and reached 73.0 % and 66.0%, respectively.

The results on the content of nonflavonoid phenols (NFP) in the studied fruits are given in Table 2. The Koroneiki variety are distinguished by the highest amount of NFP, with 4.659 mg (GAE) g^{-1} FW, and that of the Kalamon – by the lowest, with 2.72 mg (GAE) g^{-1} FW. The nonflavonoid phenols in the fruits Throubolia and Koroneiki constitute 34 % and 27% of the TP amount, whereas for fruits Dafnolia and Kalamon, reached 21.0 % and 16.0 %, respectively.

The results of the FRAP antioxidative assay, of the four varieties of fresh fruits reveal that the Kalamon variety exert the highest activity (as ascorbic acid equivalent) with 92.2 μmol (AAE) g^{-1} FW (Figure 2), followed by those of the Dafnolia and Koroneiki with 78.44 and 75.12 μmol (AAE) g^{-1} FW respectively, and those of the Throubolia variety the lowest with 59.14 μmol (AAE) g^{-1} FW. Differences in antioxidant activity FRAP reported and by other authors for the different olive fruits varieties (Bouaziz et al., 2004). Furthermore, these values are higher than those reported by other authors for the same varieties, but from different regions of Greece (Petridis et al., 2012). The correlation between the antioxidant activity (FRAP) of fruits and the contents of TP was low, with correlation coefficient (r^2) equal to:0.2974.

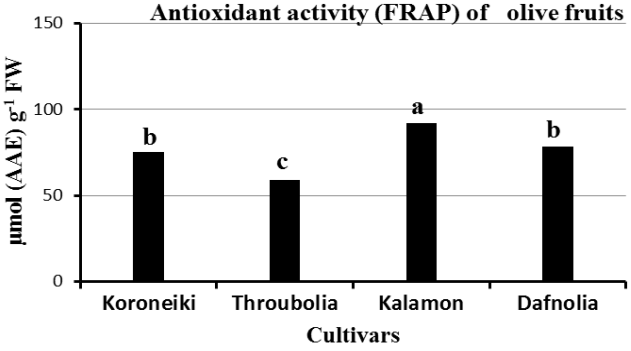

Figure 2. Antioxidant activity FRAP of different fruit varieties. Columns in each characteristic of each graph with the same letter do not differ significantly according to the Tukey's test (P=0.05).

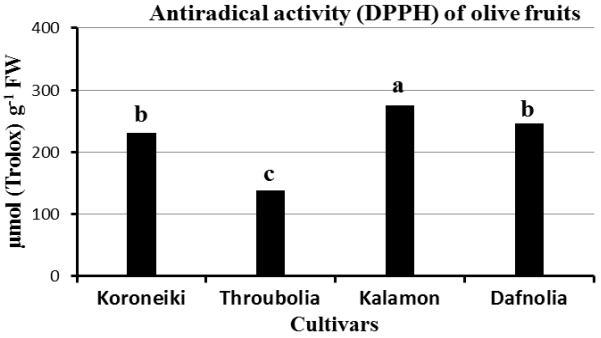

Figure 3. Antiradical activity (DPPH•) of different fruit varieties. Columns in each characteristic of each graph with the same letter do not differ significantly according to the Tukey' s test (P=0.05).

The antiradical activity (DPPH•) of fruits from the olive fruits varieties studied ranges from 138.14 to 274.72 μmol (Trolox) g^{-1} FW (Figure 3). The Kalamon variety exert the highest activity with 274.72 μmol (Trolox) g^{-1} FW, followed by those of the Dafnolia and Koroneiki with 246.44 and 231.04 μmol (Trolox) g^{-1} FW respectively, and those of the Throubolia variety the lowest with 138.14 μmol (Trolox) g^{-1} FW. These values are higher than those reported by other authors for some Turkey varieties (Gurel et al., 2014). Also, our results are in agreement with the data obtained by other authors where in the fresh olive fruit of the Kalamon variety manifested highest antiradical activity compared with Throubolia variety (Boskou et al., 2006). The correlation between the antiradical activity (DPPH•) of fruits and the contents of TP was low, with correlation coefficient (r^2) equal to: 0.232.

4. Conclusions

The results obtained showed differences in total phenols, flavonoid phenols, and nonflavonoid phenols composition.

Phenolic compounds, antioxidant and antiradical activity of olive fruit depends strongly on the fruit variety and by the climatic conditions of each region.

Olive fruits which are cultivated in the island Paros are a source of bioactive components that could be included in functional foods composition.

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