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CONTENT

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RHEOLOGY AND THERMAL PROPERTY OF WHEAT FLOUR DOUGH CONTAINING XYLOGLUCAN AND MALTODEXTRIN

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ABSTRACT

Rheological and thermal property of wheat flour added with xyloglucan (XG) and maltodextrin (MD) was studied to analyse the effect of added hydrocolloid on dough.The combined effect of both the hydrocolloid increased the arrival time, water absorption, elasticity, mixing tolerance index and peak energy as studied with Brabender farinograph. The water absorption ranged from 61.3-66.4% while mixing tolerance index value varied from 61.9-133.1 FU. Dough dynamic rheological parameters storage modulus, loss modulus and damping factor were investigated as function of frequency and temperature using rheometer. Dynamic rheological test performed showed that power law exponent 'n' values were close to 0 indicating 3D network in the mixture. The values of storage and loss modulus at a constant frequency of 0.935 rad^{-1} ranged from 22.1-33.9 kPa and 6.9-15.4 kPa respectively. Temperature sweep test showed that loss tangent values decreased as the temperature increased from 30-90 ^{0}C again indicating that dough became more elastic. The addition of both the hydrocolloid increased the storage modulus. Thermal property studied through differential scanning calorimeter (DSC) revealed two peaks in the temperature range of 30-150 ^{0}C. XG and MD addition lowered the on_set and peak gelatinization temperature at all levels.

1. Introduction

Food rheology deals with how the materials respond to the applied forces and deformation. During processing, the manufacturing and consumption of food are subjected to large deformation which decides the final texture attributes of the product (Pons and Fiszman, 1996).Knowledge of the rheological behavior of liquid foods/semi liquid is essential forprocess and equipment design such as mixers, extruders, homogenizers, heat exchangers. The rheological parameters are used inproduct development, shelf life testing, evaluation of textural property and its correlation to the sensory property of food.The Farinograph test measures the resistance of dough to mixing. The results are used as parameters in formulation to estimate the amount of water required to make a dough, to evaluate the effects of ingredients on mixing properties, to evaluate flour blending requirements, and to check flour uniformity. Farinograph results are also useful for predicting finished product texture characteristics.

Dynamic rheological testing is deployed to characterize and quantify the presence, rigidity and integrity of a material's internal structure

resulting from, for example, flocculation and interaction of dispersed particles or droplets, or cross-linking and entanglement of dissolved polymers. Dynamic rheological test has been extensively used to study viscoelasticity of polysaccharide solutions (Dublier et al., 1992). The measurement of dynamic rheological and microstructural characteristics can provide insights into the behaviour of polysaccharide systems (Canovas et al., 1996). Dynamic rheological test in the viscoelastic region gives storage modulus G', loss modulus G'', loss tangent tanδ = (G''/G'). Storage and loss modulus represents the elastic and viscous behaviour of the sample respectively. G' is measure of energy stored in the sample when shear stress is applied while G'' is a measure of energy dissipated as heat per cycle of deformation per unit volume. Loss tangent (or damping factor) is the ratio of viscous to the elastic portion of the deformation behaviour (Mezger, 2002).

Tamarind xyloglucan (XG) is a food grade polysaccharide obtained from tamarind seed used as valuable thicker and stabilizer. It is a soluble hemicellulose with a back bone composed of β (1, 4)-linked glucose residues similar to cellulose. The glucan backbone is is partially substituted at position 6 of the glucopyranosyl units mainly by single α-D-xylopyranosyl residues aswell as by disaccharide side chains composed of β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl residues (Gidley et al., 1991; Nishinari et al., 2000). XG has found to have high viscosity, broad pH tolerance and mucoadhesivity (Nishinari et al., 2000), non-carcinogenicity (Sano et al., 1996) biocompatibility (Burgalassi et al., 1996). Japanese food industry has been using XG as thicker due to its high viscosity and stability against heat, pH, and shear (Nishinari et al., 2000). The flow behaviour of the solution of xyloglucan is very close to Newtonian, and very stable against heat, pH and shear. It is expected to find new food applications, serving as thickening sauce, ice cream dressing, in processed vegetables, stabilizer, gelling agent, ice crystal stabilizer and starch modifier.

Maltodextrin has been used in every food category from infant formula to coffee creamers to enhance the texture and tenderness. It is purified, concentrated aqueous solution of nutritive saccharides obtained from edible starch or the dried product derived from said solution and having a DE of less than 20. Functional properties of maltodextrin include fat replacer, bulking, crystallization prevention, promotion of dispersibility, freezing control, and binding of flavourings, pigments, and fats. (Lucca et al., 1994, Garzon et al., 2003). Maltodextrin has been used in variety of food products including french fries, fried snacks and other foods, frozen foods, cooked rice products, noodle-like preparations, milky paste foods, jelly compositions, food glazes and films (Schmidt et al., 1993, Sobczynska and Setser, 1991).

Hydrocolloids control water absorption and consequently dough rheology (Mandala et al., 2007). The effect of different hydrocolloids on baked products has been studied by Barcenas and Rosell (2005), Rosell et al., (2001), Armero and Collar (1998). Hydrocolloid induces structural changes in the crystalline structure of starch present in wheat flour (Appelqvist and Debet, 1997). Xyloglucan has shown synergistic interaction with gellan gum (Ikeda et al., 2001), topoica starch (Pongsawatmanit et al., 2006). Xanthan gum (Nishinari et al., 2006). Similarly the effect of addition of maltodextrin alone or in conjugation with other polysaccharide has been studied. No work has been done to study the effect of blends of xyloglucan and maltodextrin on wheat flour dough. Therefore this work was carried out with the following objectives a. To investigate the effect of blends of XG and MD on the rheological including dynamic rheological properties of wheat flour dough. b. To study the effect of XG and MD on the thermal property of wheat flour dough.

2. Materials and methods

2.1. Flour and chemicals

The flour used was "Hathi", commercial brand white wheat flour purchased from local market of Sonipat, Haryana, India. The moisture content of the flour was 12.82% (w.b.). The tamarind seed powderused in the experiment was procured from Akshar Exim Company Private Limited, Kolkotta, India. Maltodextrin was obtained from Sigma-Aldrich®, (India).

2.2. Isolation of Tamarind Seed xyloglucan (TSX)

The isolation of Tamarind Seed xyloglucan was performed by Rao et al. (1973) method. 20 g of tamarind kernel powder was added to 200 mL of cold distilled water to prepare slurry. The slurry was poured into 800 mL of boiling distilled water. The solution was boiled for 20 min with continuous stirring. The resulting solution was kept overnight and the supernatant centrifuged at 5000 rpm for 20 min. The supernatant liquid was separated and poured into twice the volume of absolute alcohol with continuous stirring. The precipitate obtained was washed with absolute ethanol and dried at 50°C for 8h. The dried polymer was milled, passed through sieve no.60 and stored in a desiccator until further use.

2.3. Preparation of sample

Dough was prepared using wheat flour, distilled water with the addition of xyloglucan (XG) and maltodextrin (MD) in five different mixing ratios. The range of percentage addition of XG and MD were chosen based on application of these hydrocolloid in literature. XG and MD wereadded in the ratio of 3:1, 3:2, 2:1.5, 1:1 and 1:2 in the wheat flour sample. The same five ratios were used for all the study presented here. The control sample had no XG or MD added into it.

2.4. Rheological characteristics

Rheological characteristics of flour samples were determined with Brabender farinograph using the method adopted by Collaret. al (1999). The 300 grams flour samples having five different combination of XG and MD on a 14% moisture basis is weighed and placed into the corresponding farinograph mixing bowl. Water from a burette was added to the flour and mixed to form dough. As the dough was mixed, the farinograph recorded a curve on graph paper (AOCC method No.54-21). Dough characteristics such as water absorption, dough stability, dough development time, Mixing tolerance index (MTI) and softening of dough were interpreted from farinogram.

2.5. Dynamic Rheological Measurements

Dynamic rheological measurements were performed on controlled shear/stress rheometer (Anton Paar MCR 301, Germany) using the method adopted by Upadhyay et al (2012). Parallel plate geometry (25 mm diameter, 2 mm gap) was used for dynamic test. Batter was placed between plates of rheometer and excess batter was trimmed off carefully. The edge was coated with mineral oil to prevent drying. Dough samples were allowed to rest for 5 min after loading on plate to allow dough relaxation. The linear viscoelastic range was first determined using strain sweep test keeping the frequency constant (5 rad s^{-1}). Dough showed linear viscoelastic region below 0.1% stain. All the further test were carried out in linear viscoelastic region. The frequency sweep test were carried out at 0.1-200 rad s^{-1} at 30 ^{0}C.The experimental set up allowed the measurement of storage modulus (G'), loss modulus (G''), complex modulus and damping factor as a function of frequency.

2.6. Differential scanning calorimetry (DSC)

The thermal property of dough was studied with the method adopted from Collar et. al (1999). A differential scanning calorimeter (DSC, Pyris-1, Perkin Elmer, Norwalk, CT, USA) was used to determine thermal properties of wheat flour, XG and MD mixture. The heating rate was 10 ^{0}Cmin^{-1} from 30 to 150 ^{0}C with an empty pan as the reference. The DSC analyzer was calibrated using indium (T_m=156.6 ^{0}C, ΔH=28.5 J g^{-1}), mercury (T_m= -38.9 ^{0}C, ΔH=11.4 J g^{-1}), tin (T_m=231.9 ^{0}C, ΔH=60.6 J g^{-1}), and zinc (T_m=419.5 ^{0}C, ΔH=108.0 J g^{-1}).

Samples were weighed (~12 mg) into aluminium pans and hermetically sealed prior to analysis. Thermal transitions of dough samples were defined as T_o (onset temperature), T_p (peak temperature) and T_e (end point temperature).

2.7. Statistical analysis. The experimental data were analysed by the analysis of variance (ANOVA) and Duncan's multiple range test with significance defined at $P < 0.05$. All statistical analyses were carried out using the software program SPSS 16.0 (StatSoft, Inc., USA).

3. Results and discussions

3.1. Effect of XG and MD on dough rheological property

Arrival time increased with the addition of XG and MD. All the samples except 5 exhibited greater arrival time than the control. Maximum arrival time of 4.5 min was observed for sample having 1% XG and MD each. Arrival Time is the time when the top of the curve touches the 500-BU line. This indicates the rate of flour hydration. Collar et. al (1999) reported increase in arrival time with the addition of genu freeze, genu-freeze pectin type big, xanthan and hydroxylmethyl -propylcellulose in wheat dough. The effect of XG and MD addition on wheat flour farinogram is summarized in Table 1 and their comparative value is presented in Fig 1. The Farinograph test results are used as parameters in formulation to estimate the amount of water required to make dough as well as to evaluate the effects of ingredients on mixing properties. The flour blending requirements and its uniformity can also be evaluated by farinograph. Departure Time is the time when the top of the curve leaves the 500-BU line. This indicates the time when the dough is beginning to break down and is an indication of dough consistency during processing. All the sample showed less departure time as compared to control sample. Minimum departure time was observed for sample having 3% XG and 1% MD. Stability time indicates the time the dough maintains maximum consistency and is a good indication of dough strength. It is the difference in time between arrival time and departure time. All the sample tested had less stability as compared to control sample. Ghanbari and Farmai (2013) reported decrese in stability with the addition of k-Carrageenan and HPMC. Tavakolipour and Kalbasi, (2006) found a decrease in stability time by addition of CMC or HPMC to the dough formulation. Water absorption is the amount of water required to center the Farinograph curve on the 500-Brabender Unit (BU) line. This relates to the amount of water needed for a flour to be optimally processed into end products. The addition of XG and MD had increased water absorption of wheat flour dough except for the sample 5. Maximum water absorption was observed for the sample having 1% XG and MD each. Increase in water absorption with the addition of hydrocolloid is due the presence of hydroxyl group in the hydrocolloid which allows more water interaction due to hydrogen bonding (Guarda et al., 2004). Hydrocolloids absorb water in their interrelated network and interact with starch granules. Higher water absorption capacity of dough represents consistency which is desirable in bread making. The results agree with the earlier work reported by Ghanbari and Farmai (2013), Rodge et al. (2013), Smitha et al. (2008) and Guarda et al. (2004). Development time is the time required for the dough development or time required to reach the maximum consistency. Development time was found to be lesser as compared to control for all the sample except sample having 1% XG and MD each which had development time of 7.1 min. Mixing Tolerance Index (MTI) is the difference in BU value at the top of the curve at peak time and the value at the top of the curve 5 minutes after the peak. Mixing tolerance index (MTI) indicates resistivity of wheat flour to the mixing as well as the degree of softening during mixing. Stronger flour has higher the mixing tolerance. All the sample showed higher MTI as compared to control. Sample having concentration of XG and MD in the ratio 3:1 had highest MTI of 114.4 FU. This sample also showed highest elasticity or softening of 114.4

FU. Peak energy value also increased by the addition of XG and MD.

3.2.Effect of XG and MD on dough dynamic rheological property

In frequency sweep test the sample is exposed to small-deformation oscillations covering a range of frequencies to assess the structural response to deformations of longer or shorter timescales. It enables the viscoelastic properties of a sample to be determined as a function of timescale. Frequency sweep test results are depicted in Figure 2. Storage modulus (G') and loss modulus (G'') were found to be function of frequency for all the samples. G' was larger than G'' indicating that all samples had a firm, elastic-like behaviour.

Table 1. Rheological characteristics of dough prepared with different incorporation level of XG and MD.

Sample	Arrival Time (min)	Departure Time (min)	Stability time (min)	Water Absorption (%)	Development time (min)	Softening (FU)	MTI (FU)	Peak Energy (Wh/kg)
Control	2.4±0.02^{d}	12±0.12^{a}	9.6±0.22^{a}	60.2±0.37^{d}	6.9±0.56^{b}	24.6±0.58^{f}	33.7±0.47^{e}	10.2±0.54^{b}
1 (3XG:1MD)	3.2±0.07^{c}	6.9±0.54^{e}	3.6±0.13^{e}	61.4±0.87^{c}	4.8±0.75^{e}	114.4±0.51^{a}	133.1±1.04^{a}	11.4±0.29^{a}
2 (3XG:2MD)	3.2±0.05^{c}	7.7±0.19^{d}	4.5±0.09^{d}	61.3±0.88^{c}	5.0±0.48^{d}	84.1±0.49^{b}	103.5±1.07^{b}	11.6±0.75^{a}
3 (2XG:1.5MD)	3.5±0.04^{b}	8.6±0.43^{c}	5.1±0.06^{c}	64.4±0.44^{b}	6.1±0.56^{c}	71.8±0.95^{c}	86.9±0.92^{c}	11.6±0.61^{a}
4 (1XG:1MD)	4.5±0.03^{a}	10.2±0.14^{b}	5.8±0.28^{b}	66.4±0.67^{a}	7.1±0.30^{a}	50.6±0.31^{e}	61.9±0.66^{d}	11.1±0.22^{a}
5 (1XG:2MD)	2.0±0.01^{e}	7.0±0.32^{e}	5.1±0.09^{c}	61.4±0.39^{c}	4.8±0.24^{e}	67.2±0.89^{d}	85.8±0.46^{c}	9.2±0.38^{c}

Values in bracket in sample column indicates the percentage of XG and MD added in the flour for dough preparation
Values are means ± SD of triplicate. Means with different subscript within the same column are significantly different ($p < 0.05$), FU: Farinograph Unit

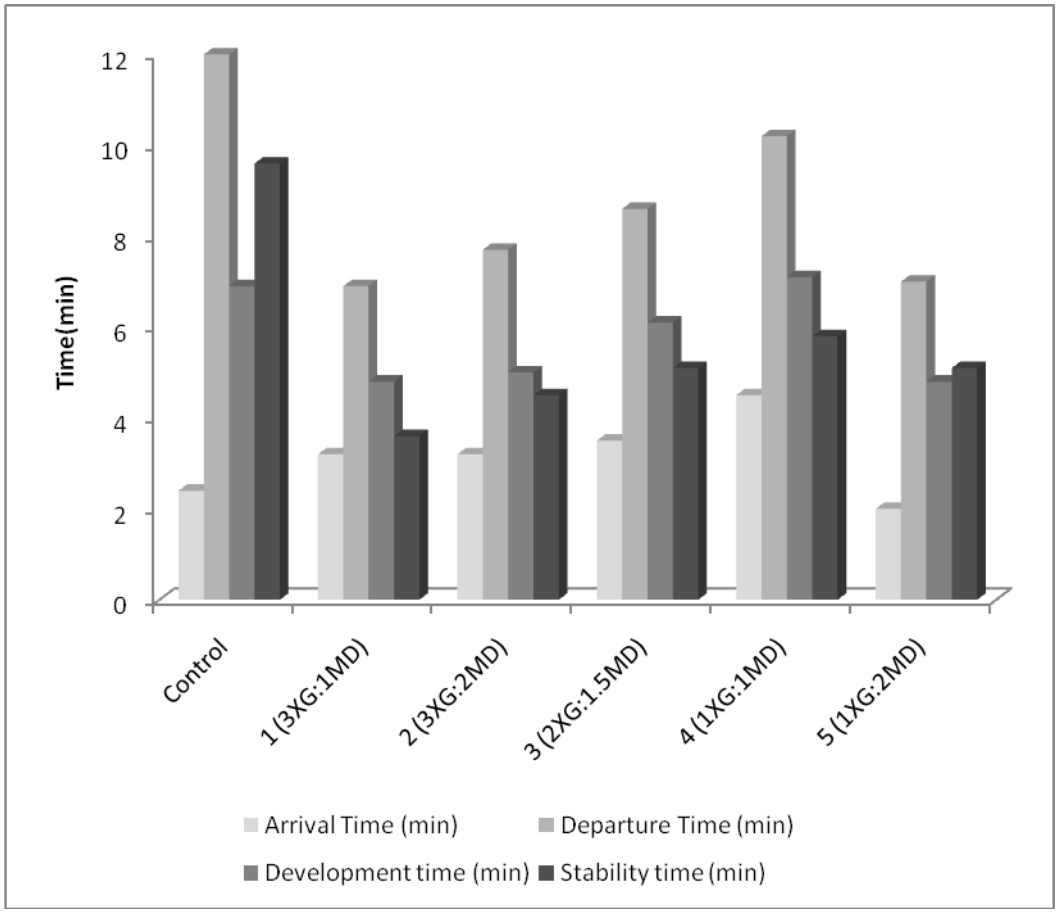


Figure 1. Effect of XG and MD on the rheological characteristics of wheat flour dough

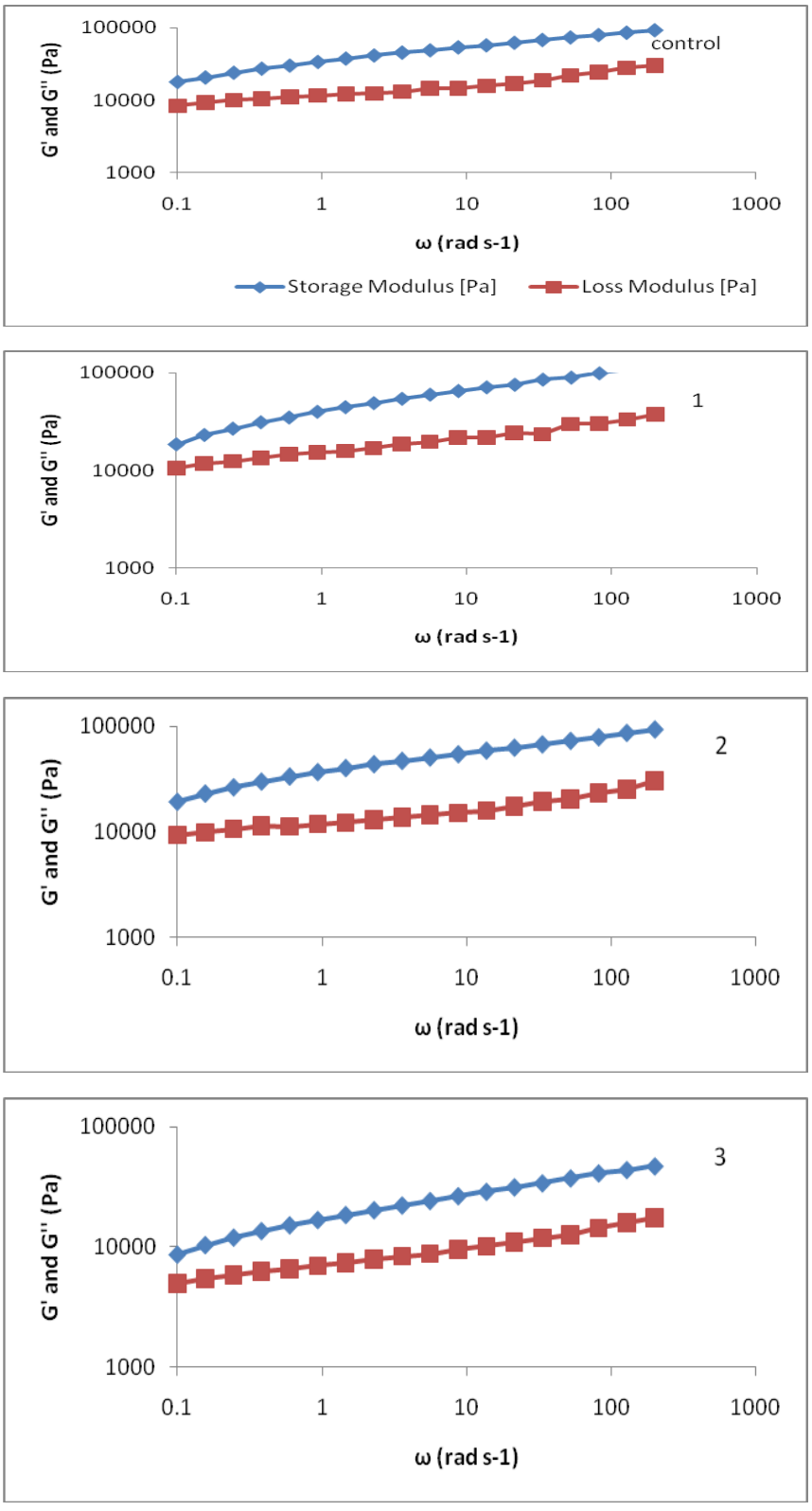
Both dynamic modulus G' and G'' showed almost linear and slightly parallel nature in the experimental frequency range. As per Winter's gel theory this linearity suggest that in the considered time scale the sample behaved like a critical gel. Flour-water dough is considered as gel-like network capable of flowing whose flow property follows power law as function of frequency. The power law constant were calculated using following equation:

$$G' = G'_0 \omega^n \quad (1)$$

where: G' represent the storage modulus and n is the power law exponent (dimensionless), ω is frequency and G'_0 is the intercept of the power law model for frequency sweeps. The constants were obtained from the linear regression analysis after a logarithmic transformation of the raw data. The value of n varied from 0.19 to 0.27 for G' and 0.14 to 0.20 for G''. The R^2 value for all the regression equation was more than 0.95. The slope of log G' vs log ω provides useful insight into the structure of biopolymer (Ross-Murphy, 1995). Material behaves like rubbery material when the magnitude of slope (n) of log G' vs log ω has value closer to 0 while liquid flow behaviour is exhibited when the slope approaches 2 (Upadhyay et al. 2012). Gabriele et al., 2001 suggested that when a 3D network is present the slope to be near zero can be expected. In the present work linear regression of log G' vs log ω data showed that the resulting values of n were low (<0.3) for all the sample, indicating the existence of a 3D network. The values of tanδ (damping factor), defined as the ratio of the loss (viscous) to storage (elastic) modulus (G''/G'), have been presented in Table 2. Material showing damping factor less than 1 (G' > G'') are considered to be dominantly gel orsolid-like else if damping factor is greater than 1 (G' < G'') then the material may be takento be more liquid like. Similar results have been reported by Georgopoulos et al. (2004), Tanner et al. (2008), Uthayakumaran et al. (2002), Phan-Thienet al. (2000), Berland and Launay (1995) and Upadhyay et al. (2012). The temperature sweep test results are shown in fig. 3. The temperature dependence of G' and G'' for temperature range of 30-90 ^{0}C showed that G' value was larger than G'' for all the samples. The value of G' and G'' increased sharply in the temperature range of 50-65 ^{0}C for all the samples. The difference in values of G' and G'' remained almost constant up to 50 ^{0}C for the control sample, sample 2, 3 and 5.As the temperature increased from 30 ^{0}C to 90 ^{0}C the damping factor or loss tangent (tanδ) decreased for all the samples. Damping factor is a measure of energy lost compared to energy stored in cyclic deformation (Steffe, 1996). The ingredient interaction such as gel formation makes the dough more elastic lowering the value of tanδ. (Tung and Paulson, 1995).The dynamic rheological parameters G', G'', tanδ and complex viscosity (η*) has been compared at constant frequency (0.934 rad s^{-1}).

Table 2. Effect of XG and MD addition on dynamic rheological property of dough at constant frequency 0.935 rad s^{-1}

Sample	G' (kPa)	G'' (kPa)	tanδ	η* (kPa.s)	n for G'	n for G''
Control	33.7	11.6	0.343	38.1	0.21	0.16
1 (3:1)	39.9	15.4	0.387	45.7	0.23	0.16
2 (3:2)	36.7	11.8	0.321	41.2	0.19	0.14
3 (2:1.5)	16.7	6.9	0.415	19.3	0.21	0.16
4 (1:1)	31.1	11.6	0.373	35.4	0.24	0.17
5 (1:2)	22.1	9.1	0.414	25.6	0.27	0.20


100000
10000
1000
G' and G'' (Pa)
0.1
1
10
100
1000
ω (rad s-1)
control
Storage Modulus [Pa]
Loss Modulus [Pa]
1
2
3

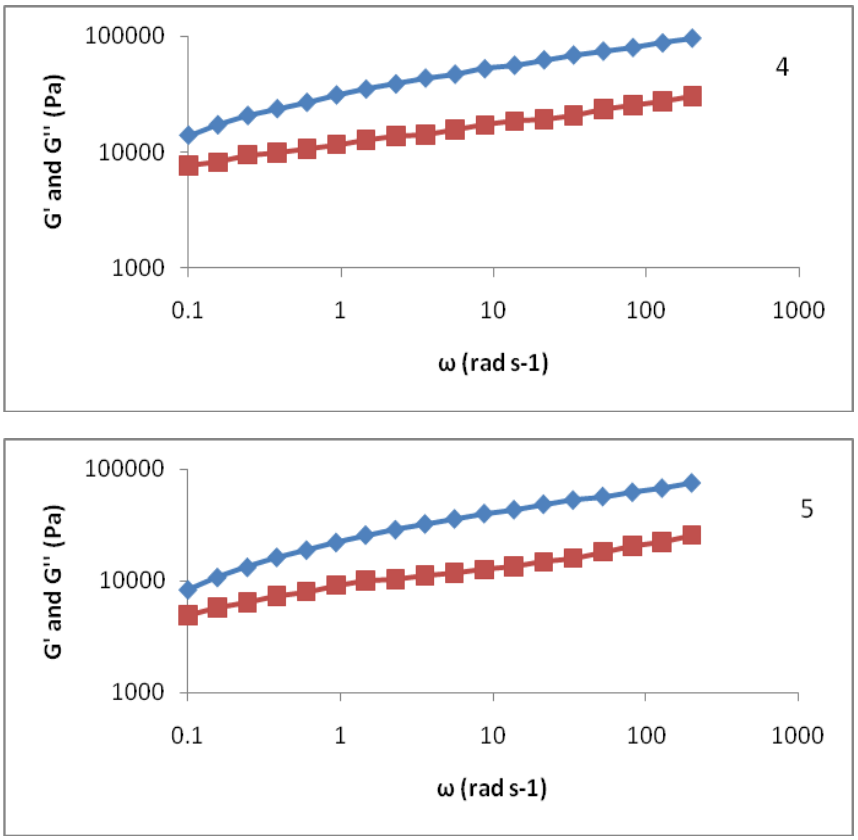


Figure. 2. Dynamic frequency sweep test of dough showing G' and G'' containing 0 (control), 3XG:1MD (1), 3XG:2MD (2), 2XG:1.5MD (3), 1XG:1MD (4) and 1XG:2MD (5) concentration ratio of XG and MD.

3.3. Effect of XG and MD on thermal property of wheat flour dough

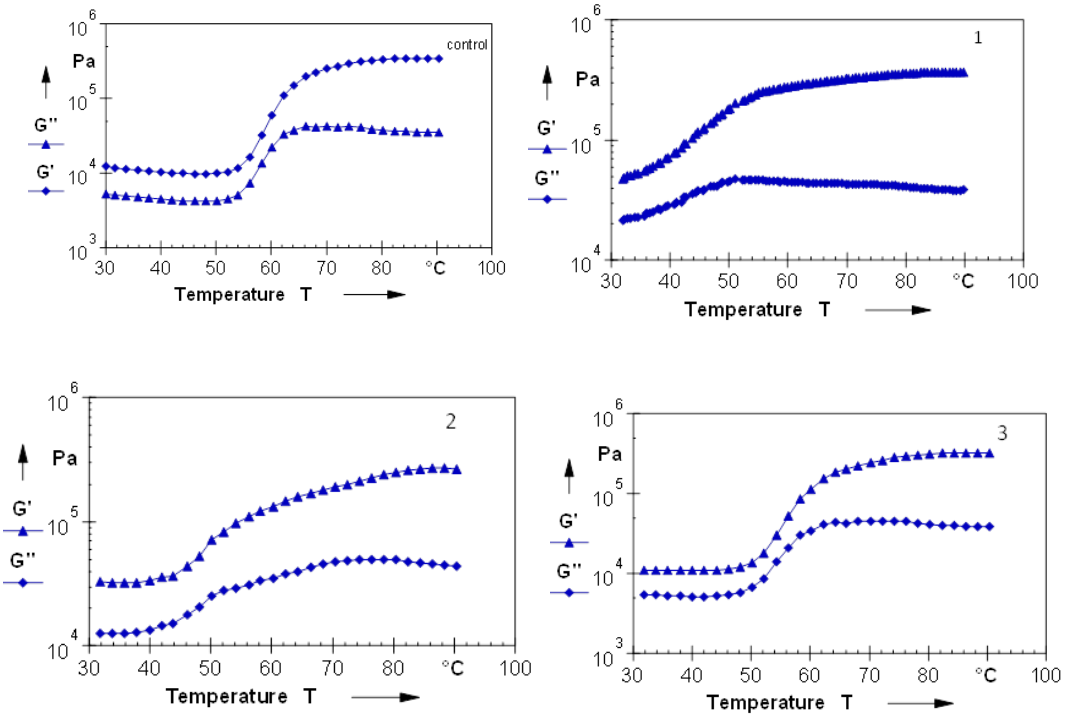
Two peaks were observed when the dough samples containing different concentrations of XG and MD were heated from 30 ^{0}C to 150 ^{0}C to imitate the conditions prevailing in oven. First peak was observed in the temperature range of 40-70 ^{0}C while second peak was observed in the temperature range of 113-123 ^{0}C for different samples. The first peak may be attributed to the gelatinization of starch while the second peak may be attributed to the dextrinization. Thermal parameters onset temperature (T_o), peak temperature (T_p) and endpoint temperature (T_c) as measured by DSC are shown in Table 3. Addition of XG and MD lowered the gelatinization parameters. Gelatinization is second order phase transition which involves loss in the crystalline structure of amylopectin, uptake of water molecules by starch granules, swelling, amylose leaching and loss of birefringence (Biliaderis, 1980). Amylopectin, the major component of starch forms crystalline structure of starch through hydrogen bonding. Diffusion of water molecules aided with heat leads to the breakdown in crystalline structure. The degree of breakdown in structure depends upon the distribution of water molecules among colloidal components in the system (Yuan et al.,1993). Interaction of hydrocolloid among themselves and with the starch effect the starch gelatinization by changing the chain mobility of the starch component. Hydrocolloid showing stronger interaction with starch than that present between water and starch increases the gelatinization parameters while those showing weaker interaction with starch decrease the parameters. Addition of XG and MD in different ratios delayed the starch

dextrinization. The onset temperature for dextrinization varied from 113.7-121.6 ^{0}C with the addition of XG and MD which was higher than the control sample onset temperature, 115 ^{0}C. Peak and end point dextrinization temperature was also delayed with the addition of XG and MD.

Table. 3. Thermal properties of wheat flour dough with the addition of XG and MD

Sample	Gelatinization				Dextrinization		
	T_0 (^{0}C)	T_P (^{0}C)	T_c (^{0}C)		T_0 (^{0}C)	T_P (^{0}C)	T_c (^{0}C)
Control	58.2±0.67^a	59.2±0.33=	59.8±0.65^a		115±1.28^a	116.9±1.23^a	119.2±1.06^a
1 (3XG:1MD)	46±0.47^b	54.8±0.76^b	63.5±0.71^b		113.7±1.09^b	116.1±1.49^a	116.3±1.41^b
2 (3XG:2MD)	47.1±0.61^b	57.7±0.98^c	68.3±0.34^c		114.6±1.12^b	117±1.35^a	121±1.26^c
3 (2XG:1.5MD)	44.7±0.49^b	47±0.49^d	56.6±0.88^d		118.7±1.20^c	119.2±1.28^b	119.7±1.29^a
4 (1XG:1MD)	42.3±0.87^c	48.3±0.37^d	54.4±0.50$_d$		120.6±0.79^d	120.9±1.87^b	121.2±1.03^c
5 (1XG:2MD)	40.1±0.36^d	43.1±0.77^e	46.2±0.18e		121.6±0.96^d	122.4±1.65^c	122.5±1.74^c

Values in bracket in sample column indicates the percentage of XG and MD added in the flour for dough preparation
Values are means ± SD of triplicate.Means with different subscript within the same column are significantly different ($p < 0.05$)



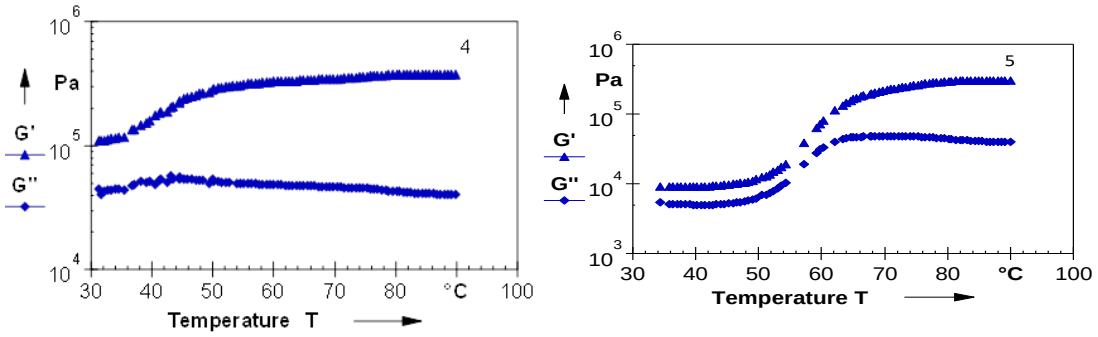


Figure 3. Dynamic temperature sweep test of dough showing G' and G'' containing 0 (control), 3XG:1MD (1), 3XG:2MD (2), 2XG:1.5MD (3), 1XG:1MD (4) and 1XG:2MD (5) concentration ratio of XG and MD.

4.Conclusions

Xyloglucan and maltodextrin had significant positive effect on the dough rheological properties. The XG/MD blend with the concentration of 1% w/v each exhibited maximum water absorption, arrival time, departure time, dough development time and stability. blend having 3% XG and 1% MD showed maximum elasticity and mixing tolerance index. Frequency sweep test showed that G' was larger than G'' for all the samples indicating a firm, elastic-like behaviour. Linear regression of log G' vs log ω data showed that the resulting values of n were very low signalling the presence of 3D network.

Gelatinization parameters were lowered while dextrinization parameters were delayed due to the presence of XG and MD. Knowledge of thermal and rheological parameters of wheat flour with the addition of XG and MD will help the food industry for the development of various bakery products.

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EFFECT OF TAMARINDUS COATING ON POST-HARVEST QUALITY OF APPLES AND PEARS STORED AT DIFFERENT CONDITIONS

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ABSTRACT
Edible coating on apples and pears was done using tamarind seed powder as a major source. Three storage conditions were designed for apples and pears i.e. ambient, dark and refrigerated. The storage period for apples lasted 28-34 days, whereas for pears it was 27-32 days. The weight loss percentage was increased for both the fruits in ambient and dark condition till fourth week, whereas in refrigerated condition it was found nearly stable. The sugar-acid ratio was decreasing week by week of all three conditions, but the Vitamin C content was increasing. The firmness of fruit was decreasing in every successive week as the force for penetration was decreasing, similarly TSS was also decreasing for both fruits in all three conditions. Dark condition stored apples were spoiled on 27 days. Similarly pears took 29 days to spoil completely. Controlled fruits found shelf life of 22-24 days, whereas coated fruit shelf life was extended to 27-29 days.

1. Introduction

In the world of globalization and increasing population it is becoming necessary to meet consumer demand in term of nutritional diet and food safety. Edible coatings (EC) are provided with a selective allowance to resist gas exchange, such as O_2, CO_2, and ethylene, which help in maintaining food product quality (Park, 1999). Such films or coatings give sterility to the surface and prevent other component losses. Any material which is used as a coating or wrapping material for fruits and vegetables to extend its shelf life and which can be safety consume is refereed as an edible coating (EC) (Raghav et al., 2016). It is stated that EC is the fine layer coating material which can be consumed and proves to be a good barrier against O_2, microbial contamination from foreign sources, moisture and solute movement for food (Lin and Zhao, 2007). With enhanced transportation and supply systems, storage needs, fruits and vegetables are no more consumed directly from the orchard or field like in the past. During the 15th century, an edible film called Yuba was prepared by using boiled soy milk's skin. Japan used it for food quality maintenance and improved physical appearance of fruits and vegetables (Biquet and Guilbert 1986). Today in recent time, many modern techniques, individually or in among combinations, such as CA storage, refrigeration and edible film (EF) coating are assured as one of the most economic ways for their quality and safety maintenance. Generally the thickness of EC is less than 0.3 mm. The EF and EC

development and application provided advancement and better development for the preservation of minimally processed products and fresh horticulture. EF and EC, like wax coating on various FV, are used from past many centuries to prevent moisture losses that creates a polished fruit surface making it more appealing. Wax coatings provides with better barrier against moisture as compared to other lipid based coating (LBC) and non-lipid coating (NLC) (Robertson, 2009). Oil, fat and wax based coatings are difficult to employ on the surface of fruits and vegetables due to its greasiness and thickness also it provides with rancid flavor. EF also helps in limiting the uptake of oil and fat during frying (Feeney et al., 1992; Polanski, 1993). Postharvest treatments using traditional synthetic waxes like thiabendazole (TBZ), imazalil (IMZ), sodium ortho-phenil phenate (SOPP) or other active ingredients that are used for many years and are still in current use of citrus packing houses for fresh fruit preservation, controlling postharvest decay, and extending shelf life of fruits (Palou, 2015). Repeated appliance of these treatments has raised serious issues for the citrus industry such as health and environmental issues associated with chemical residues or the procreation of pathogenic resistant strains (Palou,2015). Their symbiotic effect with many packaging materials and control, of environmental factors contribute to the physical-chemical, bioactive and other microbiological stability of products. Chitosan is derived from chitin, which is an animal based edible polymer. Chitin is mainly found in crustacean animal shells (Shahidi, 1999). Chitosan is the most common non-toxic and natural product after cellulose for formation of EC. Chitosan has a good characteristic feature without addition of any other type of additive and antioxidants like as it contains good O_2 barrier and CO_2 permeation and antimicrobial activity counter to microbes. Chitosan has excellent mechanical properties. Viscosity of chitosan is very high similar to the natural gums. Chitosan are composed of translucent and clear coatings that increase shelf life of fruits and vegetables. As maximum population from India belongs to vegetarian category and animal based products being a sensitive issue, it became a necessary part for food industry to introduce Indian market with plant based edible coating. EC provides with either translucent or opaque coatings, but consumers generally prefer invisible, clear coatings (Raghav et al., 2016). Coatings can also be obtained in either ways such as (I) by immersing the product into, or by spraying or brushing it with solution containing film ingredients, so as to deposit the film directly on food surface, (Gontard and Guilbert (1994) or (II) by creating stand-alone film from solution or through thermo-formation for subsequent covering of food surface. They just not only generate a modification in the gas atmosphere in function of their barrier properties, but also carry additives with definitive functions such as antioxidant, vitamins, antibacterial, antifungal, minerals, etc. (Olivas et al., 2007; Tapia et al., 2008). Tamarind being a multipurpose tree species has also found its way to the food industry. Tamarind not only acts as a nutritional source, but also act a cheap source of raw material in many forms (Mohite et al., 2016a). Tamarind decorticated seed kernels may contain up to 46 to 48% of a gel forming substance. Tamarind seeds are generally treated as waste and are discarded. Seed powder can easily be gelatinized with sugar concentrates even in milk or cold water (Kumar and Bhattacharya 2008). Tamarind gum or Tamarind Seed Polysaccharides (TSP) is a polysaccharide polymer (D-galactose, D-xylose and D-glucose) obtained from the endosperm of kernels of seeds (Mohite et

al., 2016b). Because of its ideal properties tamarinds can also be incorporated to form edible coatings. Edible coating from tamarind by-products will not only protect fruits from external hazards, but will also enhance it's the nutritional properties. Although tamarind has such an excellent properties but still not research literature is not available in edible coating fruits from it. Therefore the present investigation was designed to coat apples and pears using tamarind seed powder at different storage conditions.

2. Materials and methods

2.1. Materials and coat formulation

Apples and pears having uniform size, matured state and without defects were procured from the local market of NOIDA, India, and were stored at 4°C until processing. The fruits were phyto- sanitized by washing in chlorinated water (15ppm) thoroughly for 5 min and the fruits were allowed to dry naturally. Corn starch from the *Brown Corn Company* was procured from NOIDA, India. Chemicals such as L-ascorbic acid, HCl, meta-phosphoric acid, glycerol and acetic acid of LR grade, CDH brand were procured from R K chemicals Meerut, India. Tamarind seeds (*Variety: Mukteshwar)* were procured from SVPAU, Meerut it was used to prepare powder by removing seed coat from the seed and subsequently milling of seeds using hammer mill, and the powder was then sieved through BSS 100 mesh to get fine ground product for coating preparation.

The tamarind powder coating solution was prepared by dissolving 3 g of tamarind seed powder, 0.5 g of starch, 1.0 ml acetic acid in 100 ml distilled water with a continuous stirring using magnetic stirrer for 45 min at 55°C until the emulsion was homogenized. A 2 ml glycerol was added which play the role of plasticizer in it. After mixing, the solution for coating was filtered through a filter paper Whatman No.3 to remove any undissolved particles. Total six solutions were selected for the present study and each sample fruit was dipped for 1 min in the coating solution, 30 sec holding time was kept to remove excess coating materials on fruit surface and further they were stored at three storage conditions. These fruits were stored at different conditions (T1) tamarind solution coating on apple in ambient, (T2) tamarind solution coating on pear in ambient, (T3) tamarind solution coating on apple in refrigerated, (T4) tamarind coating on pear in refrigerated, (T5) tamarind coating of apple in the dark, (T6) tamarind coating of pear in the dark. Each sample solutions were prepared in five replications as the weekly study on conducted.

2.2. Physicochemical analysis of coated fruits

2.2.1 Weight loss percentage

Determination of weight loss percentage of coated apples and pears during the different storage condition was performed by placing the fruits on clean stainless trays at ambient, dark and refrigerated Conditions. On day 0, 7, 14 and 27 of storage, the weights of each fruit were recorded. The weight loss (WL, %) was calculated using the following equation:

$$WL(\%) = \frac{DW}{FDW}100 \qquad (1)$$

where: FDW is the weight of fruit at the first day, DW is the weight of fruit at the concrete storage day.

2.2.2 Determination of Vitamin C by titration method

In the absence of interfering substances that may reduce the dye or oxidize ascorbic acid during sample preparation, the capacity of a sample to reduce a standard dye solution, to determine dye factor by titration, is directly proportional to the ascorbic acid content in the sample.

$$\text{Dye factor (D.F)} = 0.5/\text{Titre} \quad (2)$$

10 ml Juice samples from fruits were taken and volume was made with 3% HPO_3 up to 100 ml and filtration was done. 10 ml filtrate was taken into a conical flask and titrate it with the standard dye solution to a pink colour end point. The dye is reduced by sulphur dioxide and thus interferes with the ascorbic acid determination, the following procedure was followed.0.1ml of HCl and 1 ml of 40% formaldehyde was added to 10 ml of filtrate, and allowed to stand for 10 minutes and then titrated(Hans, 1992).

2.2.3 Titratable acidity

The acid content is determined by titrating the sample with an alkali sodium hydroxide solution and phenolphthalein is added as an indicator of end point, then the titratable acidity of given sample is expressed as grams of tartaric acid per 100 ml (AOAC,1990).

2.2.4 Total Soluble Solids (TSS)

Sugar is the major soluble solid in fruit. Organic and amino acids, soluble pectin, etc. are the other soluble materials which are present. The soluble solids concentration (^{0}Brix) was determined by a small sample of fruit juice by using a hand held refractometer (Make Atago, India). This instrument measures the refractive index, which indicates how much a light beam is "bent" when it passes through the fruit juice. Clear juice from the fruits to be sampled was extracted. One drop of juice, which is obtained from fruit was placed on refractometer prism. A cover plate of refractometer was then lower and reading of sample was taken. Prisms of refractometer were rinsed between samples with distilled water and then dry with a soft tissue paper (Yaman and Bayoindirli, 2002).

2.2.5 Calculation of the Sugar acid ratio

The °Brix value of the fruit concerned must also be obtained before calculation of the sugar/acid ratio is possible. To calculate or determine the sugar/acid ratios of all produce are same, but some product contains different acids so the appropriate multiplication factor must be applied to each calculation. Some of the product may contain more than one type of acid; it is the primary acid that is tested. For the determination of sugar-acid ratio (SAR) we must divide the sugar concentration (°Brix) by the citric acid concentration. Acid factor was used as 0.0067 (malic acid).

$$\text{SAR} = \frac{Sugar\ concentration(brix)}{Acid\ concentration} \quad (3)$$

2.2.6 Penetration ratio

Firmness of edible coated fruits (three from every replication) was measured with the assistance of a 'Penetrometer' (Model FT-327, USA) utilizing 8 mm stainless steel test. Around 1 square centimeter of the skin in each natural product from the shoulder end of the two sides were expelled with the assistance of peeler and immovability of mash was recorded and communicated as far as weight (lb force).

2.3 Statistical analysis

Statistical analysis was performed by using Statistica software 6.0 to evaluate the effect of different storage level on coating on fruits.

3.Results and discussions

Edible coating of apples and pears using tamarind powder coating at ambient, dark and refrigerated condition was performed in the present study. The results for various parameters on coating materials used on fruits were analyses are given in below sub sections.

Table 1. ANOVA of variants used in edible coating of apples and pears

Weight loss%						
Source	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Intercept	106.16	103.05	114.64	101.49	113.50	100.7
Std Error Estimate	1.32	1.32	1.16	1.35	1.37	1.47
Std Error Predicted	5.87	5.87	1.14	8.31	4.32	14.35
R^2	0.56	0.55	0.67	0.51	0.54	0.35
F	0.94**	0.86**	1.66**	0.71**	0.89**	0.29**
P	0.43	0.45	0.67	0.48	0.54	0.64
Total Soluble Solids						
Intercept	123.50	126.50	98.06	127.00	118.50	113.36
Std Error Estimate	0.50	0.50	1.42	1.04	0.64	1.35
Std Error Predicted	3.54	4.25	14.86	10.69	3.17	5.80
R^2	0.95	0.94	0.85	0.77	0.91	0.51
F	18.01*	18.02*	14.86*	3.60*	10.00*	0.71**
P	0.05	0.05	0.055	0.77	0.04	0.48
Acidity %						
Intercept	84.23	77.20	57.21	114.08	65.23	91.65
Std Error Estimate	0.10	0.44	1.48	0.77	0.19	0.36
Std Error Predicted	1.17	6.54	97.30	87.78	3.78	2.82
R^2	0.99	0.95	0.34	0.87	0.99	0.97
F	430.2*	22.88*	0.22**	6.43**	134.43*	35.74*
P	0.002	0.04	0.65	0.12	0.007	0.001
Sugar acid ratio						
Intercept	117.82	120.08	100.7	118.08	122.43	113.29
Std Error Estimate	0.34	0.41	1.47	0.65	0.75	1.05
Std Error Predicted	1.50	2.25	14.35	3.07	5.46	3.08
R^2	0.98	0.96	0.35	0.91	0.88	6.74
F	38.93*	26.00*	0.29**	9.82**	6.98**	2.48**
P	0.02	0.03	0.64	0.08	0.12	0.25
Vitamin C						
Intercept	85.83	102.67	113.47	103.41	88.87	100.62
Std Error Estimate	0.51	0.52	1.40	1.09	0.21	0.37
Std Error Predicted	5.48	1.47	6.79	3.50	1.94	1.37
R^2	0.94	0.94	0.46	0.72	0.99	0.97
F	17.11*	16.18*	0.54**	2.15**	102.33*	33.59*
P	0.05	0.05	0.36	0.27	0.003	0.02
Penetration ratio						
Intercept	118.28	113.91	127.50	125.80	115.44	118.11
Std Error Estimate	0.18	0.43	0.33	0.25	0.30	0.25
Std Error Predicted	0.83	1.11	2.95	1.96	0.99	1.13
R^2	0.99	0.96	0.98	0.99	0.98	0.99
F	137.61	24.70*	41.41*	77.71*	50.15*	73.32*
P	0.007	0.03	0.02	0.01	0.01	0.01

** Significant at 5%, ** Non Significant*

3.1 Weight loss percentage

All of the tests were performed on week by week premise and the results were watched appropriately. Most extreme weight reduction percentage toward the end of the first week was seen if there should arise an occurrence of T_6 i.e., 3.04 though minimum was in T_4 i.e., 1.14. Toward the end of second week, T_5 with 3.54 percentage weight reductions was seen to have greatest loss percentage of different treatments incentive than the rest. T_1 has greatest weight reduction % i.e., 3.89 percentage toward the end of the fourth week ,though T_4 i.e., 1.34 has the slightest esteem (Figure 1).

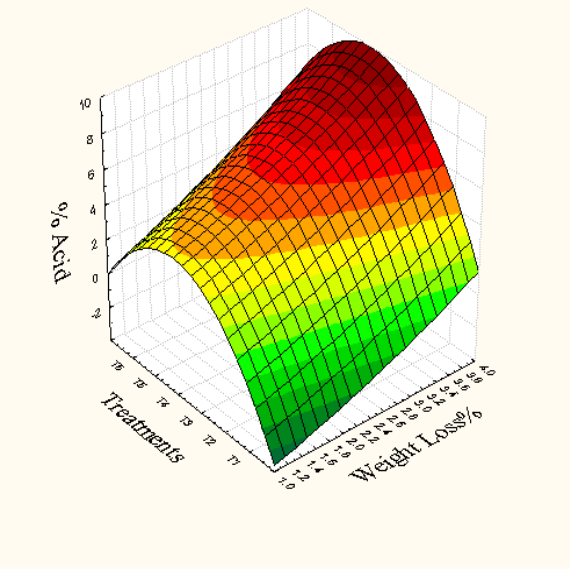

Figure 1. Percentage acid against weight

Whereas other differences that were observed were, T_1 showed a consistent decline in %weight loss up to the third week and an abrupt increase was noticed at the end of the fourth week. T_2, unlike T_1, showed a continuous inclination, till the end of the third week, whereas a sudden decrease was observed at the end of the fourth week (Assis and Pessoa, 2004). From the ANOVA table, it can be refined that weight loss percentage per week analysis was found non-significant ($P \leq 0.05$).

3.2 TSS (Brix°)

TSS in first seven day stretches of perception was most noteworthy in T_4 i.e., 14˚Brix while the minimum was in T_3 and T_5 i.e., 10˚Brix. There was a progressive abatement in TSS of the variations consequently greatest TSS toward the end of the second week was in T_2 and T_4 i.e. 12˚Brix and least was in T_6 i.e., 8˚Brix. TSS when seen in the third week, T_2 stayed same as in earlier week while a sudden increment in the TSS of T_6 was watched. Toward the end of fourth week T_2, T_3 and T_4 had a similar TSS i.e., 11˚ Brix. Despite the fact that T_2 has indicated diminish in TSS, T_3 demonstrated a decline in TSS while TSS of T_5 stayed unaltered. T_1 and T_2 were observed among their column and a decrease in TSS was observed from the end of the first week to the second week, whereas up to third week results remained unaltered and again a decline was noticed at the end of fourth week. T_1 and T_6 were seen to have least degree Brix among all variants, throughout the test. TSS found per week analysis was found significant ($P \leq 0.05$). Similar work was reported by Yaman and Bayoindirli (2002).

3.2 Acidity percentage

Acidity percentage in respect to malic acid was performed and the highest acidity percentage was found in T_5 i.e., 4.65 percentage and slightest were in 3.69 percentage toward the end of the first week. Acidity %was expanded a little and went between 3.97- 4.79 percentage. There was a slow increment in the acidity % of T_6 in the third week, i.e., 4.28 percentage as beforehand it was 3.97 percentage. Toward the end of the fourth week greatest acidic variation was T_5 and the minimum was T_3 i.e., 4.23 percentage. All the variants were observed to increase in acidity week after week, whereas only T_4 was noticed to have decreased in acidity at the end of the fourth

week. T_5 was seen to be more acidic among all variants throughout the testing period.

3.3 Sugar acid ratio

Sugar acid proportion toward the end of the first week was slight in T_5 i.e., 2.15 and most in T_2 i.e., 3.26 though there was further reduction saw toward the end of the second week in T_2 i.e., 2.85 and T_5 i.e., 1.88. There was very little distinct result seen in T_5 toward the end of third week while soluble solids for different treatments greatest sugar proportion in third week was in T_2 i.e., 2.78. Toward the end of fourth week most extreme sugar proportion was in T_3 i.e., 2.60 and the minimum was in T_5 i.e., 1.81. An observation of sugar-acid ratio, decrease in sugar value was observed week by week (Figure 2). Only T_3 was seen to have abrupt behavior. Initially there was an increase up to the second week, then a downturn at the end of the third week and lastly a stretch in value at the end of the fourth week. The statistical analysis refined that sugar-acid ratio per week analysis was found non-significant ($P \leq 0.05$).

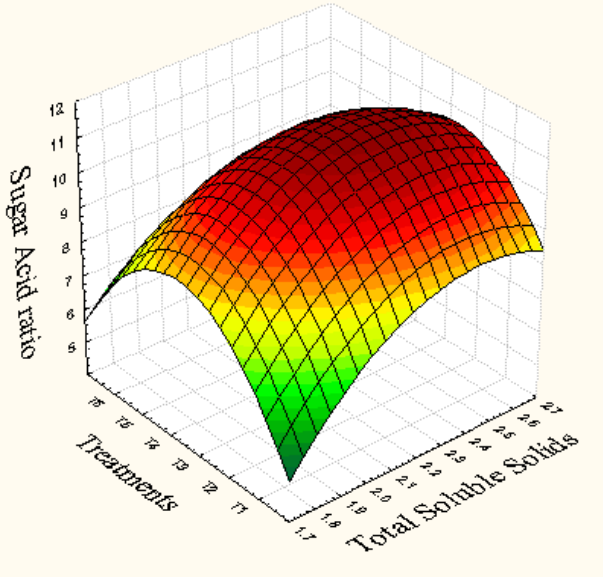

Figure 2. Sugar acid ratio against total

3.4 Vitamin C content

Vitamin C or ascorbic acid was resolved utilizing titration technique. The outcomes were ascertained as mg/100gm. Real wellspring of vitamin C was seen in variation T_1 i.e., 35.33mg/100gm and the slightest was T_2 i.e., 4.67mg/100gm. Toward the end of the second week T_4 demonstrated a lessening in ascorbic acid substance and has a minimal measure of ascorbic acid among all variations, i.e., 4.55mg/100gm while T_1 demonstrated a progressive increment in ascorbic acid substance, i.e. 38.35mg/100gm and turned out to be the healthiest source among all variations. After the end of third and fourth week an expansion in vitamin C content was seen in T_4 i.e., 6.57mg/100gm and 7.01mg/100gm individually. Values when observed at the end of consistent weeks showed an upturn except T_2, T_3 and T_4. At the end of the fourth week, vitamin C content in T_2 and T_3 displayed a slight decrease whereas T_5 exhibited the same at the end of first week itself (Figure 3). Vitamin C ANOVA analysis per week refined variants were mostly non-significant ($P \leq 0.05$).

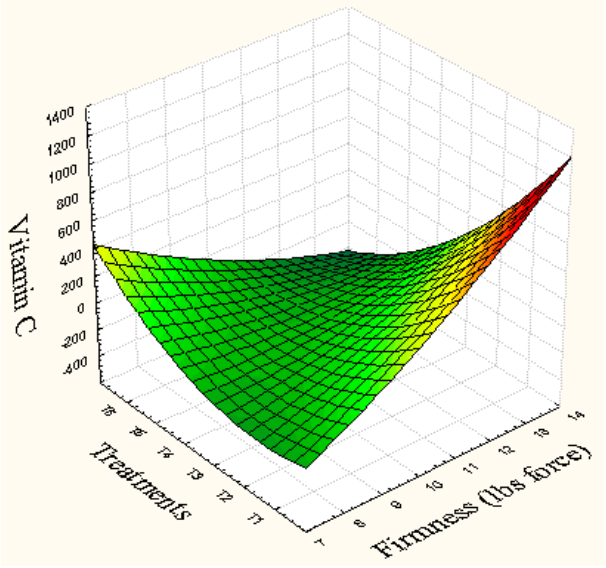

Figure 3. Vitamin C against Firmness for different treatments

3.5 Penetration ratio

Penetration proportion i.e., lb force was most extreme in T_4 i.e., 16.34 lb compel though slightest power was required in T_5 that has entranced proportion 11.72 lb drive. Penetration proportion was diminished toward the end of the second week in T_4 upto 1lb power. Least penetration toward the end of the third week was required in T_5 variation, i.e., 9.45 lb power and greatest was in T_4 i.e., 14.85 lb drive. Toward the finish of fourth week there was a steady reduction in the penetration proportion of T_5 i.e., 7.45 lb force (Khin *et al*, 2007). Penetration ratio per week analysis was found significant ($P \leq 0.05$) for all variants.

4. Conclusion

The present investigation of edible coating on apples and pears using tamarind byproducts was performed under ambient, dark and refrigerated condition. Coated fruits stored in dark condition were first spoiled then ambient and refrigerated condition. As week by week passed Vitamin C and titratable acidity increased in all three storage conditions. Fruit firmness and sugar acid ratios were decreasing in every successive week. There was a drop in TSS values for both fruits after the third week in all three conditions. The study stated that the shelf life of apples and pears can be successfully extended by 4 -5 days using tamarind seed powder edible coating.

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STUDY OF PHYSICOCHEMICAL PARAMETERS AND ANTIOXIDANT ACTIVITIES IN HONEY COLLECTED FROM DIFFERENT LOCATIONS OF ALGERIA

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ABSTRACT

In order to establish standards for the identification of the main honey in our country, we propose to determine its physicochemical characteristics, in addition to carrying out a thorough study of the antioxidant activity and mineral analysis of five types of honey from the north of Algeria. The characterization criteria used for the physicochemical parameters are: pH, acidity, electrical conductivity, ash as well as the moisture content. It is worthy of mentioning that the antioxidant activity of the different samples was evaluated by two methods: The Ferric Reducing Ability of Plasma (FRAP) and the trapping of the free radical DPPH. Next, determination of the mineral profile was carried out by atomic absorption spectrometry. The physicochemical results showed that there were differences from one sample of honey and another as they corresponded with the international standards. Positive correlations were found between total phenolics (R^2 = 0.937)/flavonoids (R^2 = 0.93) and the antioxidant power determined. The mineral analysis revealed the presence of K, Na, Fe, Cu, Co and Ni in the samples with mean values: 276.36, 86.196, 0.1461, 0.1279, 0.8174 and 0.0564 ppm respectively. The honey samples were not free of heavy metals, but their level was well below permitted levels. It is considered among the least polluted foods. The resultants showed that hives are usually located at a far distance from one or more of the possible sources of pollution.

1. Introduction

Honey is the sweet substance produced by Melissa bees from nectar and other sweet matter (honeydew) which these bees harvest and enrich from living plants and substances from their own bodies. Then, they transform the latter into their bodies once more, to be eventually ripened and stored in the shelves (Debbagh, 2000). Nutritionally, Honey is a first class food, high in energy value and with certain therapeutic properties. Honey contains at least 200 substances, mainly carbohydrates and water. It also contains minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals (Terrab et al., 2003). The composition of honey depends mainly on floral sources and some external factors such as environmental factors and treatment

methods (Terrab et al., 2003). Honey is a product that is not stable; it evolves continuously over time. Honey is used as a natural source of antioxidants that are effective in reducing the risk of disease (Meda, 2005). According to Bertoncelj et al. (2007), the compounds responsible for the antioxidant activity in honey are: flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase and carotenoids. Paramas et al. (2006) reported that honey phenolic compounds contribute significantly to its antioxidant power, as well as other, less important compounds. Antioxidants are endogenous or exogenous substances capable of neutralizing or reducing the damage caused by free radicals in the body. Several studies have also shown that the antioxidant activity of honey varies widely depending on the floral source (Dimitrova et al., 2007). For the needs of the colony, the bee harvest nectars, honeydews and pollens in the environment that are exposed to various bacteriological and chemical contaminants such as toxic elements. These elements can be found in the finished product that is consumed by humans (Fleche et al., 1997). On the other hand, elements such as cadmium, lead, and mercury are well known to be toxic to humans (Fredes and Montenegro, 2006). The sources of contamination of honey with heavy metals may be due to external environments such as plant emissions, non-ferrous metallurgy, leaded petrol, use of cadmium-containing pesticides, organic mercury, and pesticides-based Arsenic which are still in use in some countries (Pisani et al., 2007). Our study aims to characterize the physicochemical parameters, antioxidant activities and determination of trace elements and toxic elements of five varieties of honey from different Algerian regions (Batna, Medea, Ain Defla and Algiers).

2. Materials and methods

2.1. Honey samples

Five commercial honeys of different floral sources and geographical origins to the north of Algeria were purchased from local vendors: one sample from the area of ALGIERS, one sample from the area of BATNA, one sample from the area of AIN DEFLA, and two samples from the area of MEDEA. These honey samples were aseptically collected in sterile screwed cups and kept in a cool and dry place (at room temperature) before they were finally transported to the laboratory. The regions from which the samples of honey were collected are indicated in Table 1.

Table 1. Presentation of Studied Honey Samples

Sample N°	Date of Harvest	Harvesting Area	Presumed Floral Origin
S1	June 2015	Merouana (Batna)	Mountain Honey
S2	July 2015	Draa smar (Medea)	Wild carrots
S3	July 2015	Si mahdjoub (Medea)	Hawthorn
S4	August 2015	Rouina (Ain defla)	Polyfloral
S5	August 2015	Ain benian (Algiers)	Thyme

2.2. Physicochemical characteristics

The physicochemical characteristics are: moisture content, density, ash, total solids, electrical conductivity, free acidity, pH, lactone acidity, and total acidity. The measurements were performed according to The Association of Official Analytical Chemists, Inc (AOAC, 1990).

2.3. Estimation of total phenolic content

The total phenolic in the extracts of the different types of honey were assayed spectrophotometrically according to the method of Folin Ciocalteu (Singleton et al., 1999). The total phenolic content was expressed in mg of gallic acid equivalents (mg GAE/kg of honey).

2.4. Estimation of total flavonoids

The total flavonoid content is determined according to (Arvouet-Grand et al., 1994). This method is based on the formation of the flavonoid-alumium complex. Total flavonoid content was expressed as mg of rutin equivalents per 100 g of honey [mg RE/100 g].

2.5. Total protein content

The determination of the total protein in the extracts of the different types of honey was carried out spectrophotometrically according to Lowry et al. (1951); it's based on the formation complex present in Folin-Ciocalteau reagent. Bovine serum albumin (BSA) (0–50 mg/mL) was used as a standard for preparing the calibration curve.

2.6. Analysis of antioxidant activities

2.6.1. DPPH radical scavenging activity

Indeed, DPPH is characterized by its ability to produce stable free radicals. This stability is due to the delocalisation of the free electrons within the molecule. The presence of these DPPH radicals gives rise to a dark violet coloration of the solution according to the method Velazquez et al. (2003); with some modifications. Honey samples were dissolved in ethanol at a concentration of 100 mg/mL, and 0.75 mL of each sample was mixed with 1.5 mL of DPPH in methanol (0.02 mg/mL), with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and the absorbance then measured at 517 nm. Ascorbic acid (0 – 100 mg/mL) was used as positive controls. The radical scavenging activity was calculated following the Equation:

% Inhibition = [(blank absorbance - sample absorbance)/blank absorbance] * 100

The mean of three IC_{50} (concentration causing 50% inhibition) values of each honey sample was determined graphically.

2.6.2. Reducing power

The reducing power of an extract is associated with its antioxidant power. This technique is developed to measure the capacity of the extracts tested to reduce ferric iron (Fe^{3+}) present in the $K_3Fe(CN)_6$ complex of ferrous iron (Fe^{2+}). An aliquot (0.5 mL) of each honey extract (10 %) was mixed with 0.5 mL of 200 mmol/L sodium phosphate buffers (pH 6.6) and 0.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 0.5 mL of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 3000 rpm for 8 min. The upper layer (0.5 mL) was mixed with 0.5 ml of deionized water and 0.1 mL of 0.1 % of ferric chloride and the absorbance was measured spectrophotometrically at 700 nm: higher absorbance indicates higher reducing power (Oyaizu, 1986). Acid ascorbic was used as a standard.

2.7. Minerals and heavy metals

The mineral elements studied were assayed in the calcination of honey product residue at 500 ° C for 5 hours for ash, with a test portion of 10 g for the five honey samples. Then the calcined ash of honey was dissolved in 100 ml of 2N hydrochloric acid. The determination of the Minerals and heavy metals was carried out by two methods: The analysis of the micronutrients studied (Fe, Cu, Co, and Ni) was measured by atomic absorption spectrometer with the

appropriate lamp for each ion measured. Approximately a 2300°C was used for temperature. Standard stock solutions were also prepared. The K and Na contents were determined using a flame photometer. All the samples were analyzed in triplicates (AOAC, 1990; Ioannidou et al., 2005).

2.8. Statistical analysis

All measurements were carried out in triplicate, and presented as mean ± SD. Correlation and linear regression analyses were performed using Microsoft Office Excel 2010. Statistical analyses were performed by one-way ANOVA, followed by Tukey multiple comparisons test. A difference was considered to be statistically significant when $p < 0.05$.

3. Results and discussions

3.1. Physicochemical characteristics

The data obtained are presented in Table 2 and they show the good quality of the honey samples studied.

Table 2. Physicochemical characteristic for different types of honey samples

Physicochemical characteristics	Sample N°				
	S1	S2	S3	S4	S5
Moisture content (%)	19.63±0.16^{c}	21.85± 0.08	18.58±0.06^{d}	16.44±0.09^{e}	20.81±0.06^{b}
Total solids (%)	80.36±0.16^{c}	78.1 ±0.08	81.42±0.06^{b}	83.55 ±0.09^{a}	79.18±0.06^{d}
Density	1.46±0.01	1.44 ± 0.7	1.48 ± 0.08	1.49 ± 0.01	1.45±0.7
pH	3.64±0.04^{d}	3.73 ± 0.02^{c}	3.32 ± 0.01^{e}	3.84 ± 0.02^{b}	3.93±0.02^{a}
Electrical conductivity (mS/cm^{1})	0.70±0.03^{b}	0.65±0.01^{b,}	0.80 ± 0.04^{a}	0.61±0.03^{c}	0.24±0.02^{d}
Ash content (%)	0.58±0.02^{b}	0.54±0.01^{b,c}	0.66 ± 0.03^{a}	0.50 ± 0.02^{c}	0.19±0.02^{d}
Free Acidity (meq.kg^{-1})	18.83±0.76^{b}	18.5 ± 1^{b}	11.5 ± 1^{c}	21.5 ± 1^{a}	23.66±0.57^{a}
Lactonic acidity (meq/kg)	6.33±1.04^{c}	14.33±1.25^{b}	3.166±0.29^{d}	19.16±1.04^{a}	15.16±0.29^{b}
Total acidity (meq/kg)	25.16±1.5^{c}	32.83±2^{c}	14.66 ±1.25^{d}	40.66±1.89^{a}	38.83±0.3^{b}

Values represent means ± SD (standard deviations) for triplicate experiments. Values in the same row followed by a different letter are significantly different ($p < 0.05$).

3.1.1. Moisture content

Moisture content is a parameter related to the degree of maturity. It is responsible for the stability of the honey during storage. The moisture content (%) in the investigated samples ranged from 16.44 ± 0.09 to 21.85 ± 0.08. These values are less than or equal to 21%, the maximum recommended by European standards (Official Journal of the European Communities). There were significant differences, using the Tukey test ($P < 0.05$), between humidity values obtained for the five honey samples. The lowest moisture content was 13.4% in the sample S4, this honey is polyfloral and comes from the AIN DEFLA region which is characterized by a warm and dry climate. This confirms that the risk of fermentation is very low in this sample. It is concluded that our samples can be stored without risk of alteration of their physicochemical properties. The moisture content is a highly important factor because it allows the estimation of the degree of maturity of

honey and can provide information on stability against fermentation and crystallization during storage; therefore it conditions the conservation of the product (De Rodriguez et al., 2004; kuçuk et al., 2007). These results are indicative of good storage of the honey studied. According to Khalil et al. (2012), moisture content is very important for the shelf life of honey and may lead to undesirable fermentation. The variation in moisture content is due to different environmental conditions such as climate, floral origin of honey samples, water content of nectars (Nandaa et al., 2003; Bogdanov et al., 2004) and processing techniques and storage (Ozcan and Arslam, 2006).

3.1.2. Total solid

Total solid is a measure of dissolved solids in honey like fructose, glucose, and sucrose as well as organic compounds such as acids and minerals also. The results of the total solids are presented in Table 2. The total solids of the honey ranged from (83.55 ± 0.09%) to (78.14 ± 0.08%) which were significantly different ($p < 0.05$). The sample S4 has the highest soluble solid, unlike sample S2. The difference of soluble solid might be due to the difference in chemical composition of honey. We observed that there was an inverse relationship between total solids and moisture content of honey in all samples.

3.1.3. pH

The pH will influence the stability of honey and its storage conditions. It also important for knowing the type of honey (Amri et al., 2007). The pH values of five honey samples were measured and the obtained results confirmed that, all tested samples were acidic (pH 3.32 ± 0.01 to 3.93 ± 0.02) (Table 2). Among all honey types, S3 was the most acidic (pH 3.32 ± 0.01) followed by S1 (pH 3.64 ± 0.04), S2 (pH 3.73 ± 0.02) and (pH 3.84 ± 0.02) for S4 honey. Lowest acidity was detected in S5 (pH 3.93 ± 0.02). Our results are consistent with those reported by Bogdanov et al. (1999) who reported that honey derived from nectar has a pH between 3.5 and 4.5. According to Doukani et al. (2014) all Algerian honeys were acidic in nature with a pH of between 3.70 and 4.05. These values are similar to those reported for other honey samples from India, Brazil, Spain and Turkey with a pH between 3.49 and 4.70 (Azeredo et al., 2003; Ouchemoukh et al., 2007; Saxena et al., 2010[(a)]). None of our studied samples exceeded the allowable limit, which can be considered as a sign of freshness. Khalil et al. (2012) indicate that honey is naturally acidic regardless of its geographic origin, which may be due to the presence of organic acids that contribute to its flavor and stability against microbial deterioration. The variation in pH is due to the buttered flora, the salivary secretion of the bee and the enzymatic and fermentative processes during the processing of the raw material (Doukani et al., 2014).

3.1.4. Electrical conductivity

The Conductivity is a good criterion for the determination of the botanical origin of honey and is now designated as routine checks of honey and replaces the ash content. The specific conductivity of honey is correlated with the levels of mineral salts, organic acids and protein substances (Terrab et al., 2004). The honeys studied have electrical conductivities varying between (0.80 ± 0.04ms/cm and 0.24 ± 0.02ms/cm) which were significantly different ($p < 0.05$). According to studies by (Achour and Khali, 2014; Doukani et al., 2014), the electrical conductivity of the Algerian honey also meets the standards (0,240 to 560 mS/cm) for the first and (0,267 and 0,729

mS/cm) for the second. The electrical conductivity expresses the ability of the aqueous solution to conduct an electric current. It is positively correlated with the content of soluble salts. The content of the latter in diluted solutions is proportional to the conductivity (Amellal, 2008). According to Belhaj et al. (2015), the electrical conductivity is influenced by the pH of the solution, the valence of the ions and the degree of ionization. It is a good criterion related to the botanical origin of honey, and very often used in honey control routines instead of ash (Terrab et al., 2003). The Conductivity measurement gives valuable information on the botanical origin and makes it possible, in particular, to differentiate between flower honey and honeydew honey (Bogdanov, 2003).

3.1.5. Ash content

The ash content is a criterion of quality which depends on the botanical origin of the honey. Mineral content is a criterion used in international standards. The percentage of ash content in the honey samples analyzed varies from 0.19 ± 0.02 to 0.66 ± 0.03 % ($p < 0.05$) (Table 2). The results obtained were close to the Algerian honey results, which were in the range of 0.06 to 0.54% (Ouchemoukh et al., 2007). Nandaa et al. (2003), report that the permissible limit of ash content of nectar honey is 0.6%. The ash values found were below or equal to 0.6% and these results are therefore in line with the limit allowed by (Codex Alimentarius, 2001) for nectar honeys. The variation in ash content can be explained by harvesting techniques, beekeeping techniques and bees collecting techniques Finola et al. (2007) and mainly determined by soil and characteristic climate (Acqarone et al., 2007).

3.1.6. Free, lactonic and total acidity

Acidity is an important criterion of quality; it gives very important indications of the state of the honey (Doukani et al., 2014). Free acidity is an important criterion during extraction and storage because of its influence on the texture and stability of honey. This acidity comes from organic acids some of which are free and others combined in the form of lactones. Free acidity ranged was from 11.5 ± 1 to 23.66 ± 0.57 meq/kg (below 50 meq/kg). Lactonic acidity ranged was from 3.16 ± 0.25 to 19.16 ± 0.41 meq/kg. The values of the total acidity of the honey analyzed vary from 14.66 ± 1 to 40.66 ± 1 meq/kg. Total acidity values were found to be within the normal range set by (Codex Alimentarius, 2001) of 50 meq/kg. This indicates the absence of undesirable fermentations. These values are close to those found by Doukani et al. (2014): 19.56 to 38.91 meq/kg and (Achour and Khali, 2014):10 to 40 meq/kg. The acidity of honey is mainly due to gluconic acid. This acid is present in all honey. It is an enzyme of the bee, the glucose oxidase that is at the origin of it (Karabagias et al., 2014). According to (Schweitzer, 2004), the natural acidity of honey increases when the honey ages, when it is extracted from the rays with propolis and especially when it is altered by fermentation. The Variation in acidity in different honey can be attributed to floral origin or variations due to the harvest season (Doukani et al., 2014).

3.2. Total phenolic contents

The phenolic compounds or polyphenols are one of the most important classes of compounds found in honey (Khalil et al., 2012). The total polyphenol dosage gives us an overall estimate of the content of different classes of phenolic compounds contained in the samples analyzed (Pawlowska et al., 2006). The determination

of the total phenolic content is also considered as a promising method of studying the floral origins of honey. It is collected from the botanical and geographical origin which affects the concentration of phenolic compounds, the distribution of pollen and the antioxidant activity of honey (Alvarez-Suarez et al., 2009). The results obtained showed that the concentration of polyphenols recorded in the honey varied considerably ($p < 0.05$) from (71.60 ± 1.4 to 134.97 ± 0.80 mg GAE/100 of honey). These values vary according to the type of honey. The lowest value was recorded in honey S1 (71.60 ± 1.4 mg GAE/100 of honey) and the highest concentration of polyphenols was established at (134.97 ± 0.80 mg GAE/100 of honey) for the sample S3. The high antioxidant potential of sample S3 may be due to the phenolic content of Hawthorn. For Algerian and Slovenian honey, the content of phenolic compounds varied from (64 to 1304) and from (448 to 2414 mg GAE / 100 g), respectively (Bertoncelj et al., 2007 and Ouchemoukh et al., 2007). The phenolic content of the five samples of honey we analyzed is similar to the mean values found for some Algerian honey. Recent studies have shown that the concentration and type of phenolic substances depend on the floral origin of the honey; they are the main factors responsible for the biological activities of honey (Al-Mamary et al., 2002 and Wei and Zhirong, 2003).

3.3. Total flavonoids content

In honey, most phenolic compounds are in the form of flavonoids: the most common are: Narginia, Pinobanksin, Pinobankcine-3-acetate, Pinobankcine-3-butirate, Pinobankcine -3-hexanoate, 7-dihydroxu-5-methoxyflavanone, 2,5-dihydroxy-7-methoxyflavanon, whose concentration depends on various factors, including plant species used by bees, plant health, season and environmental factors (Küçük et al., 2007). The amount and type of flavonoids found in honey vary depending on the source. Generally, the darkest honey, like those derived from sunflower and buckwheat; contain quantities of flavonoids greater than the paler honey, as well as greater antioxidant capacity (Medic Sanic et al., 2004). The total flavonoid content of honey samples (mg of QE/100 g) varied from (15.96 ± 1.25 to 72.53 ± 0.69) is shown in Table 3 with the highest and the lowest levels observed in S3 and S1 respectively. The flavonoid content of the five honey samples that we analyzed are close to the average values found as it the results obtained for honey from the northeast of Brazil, which ranged from 2.5 to 83.8 mg of quercetin/kg of honey (Liberato et al., 2011).

3.4. The protein content

As regards the protein content in honey varieties, it is recorded that the protein content (mg/g of honey) is between 3.75 ± 2.25 and 10.81 ± 0.50 ($p < 0.05$) (Table 3), which was determined using the bovine serum albumin (BSA) as standard (R^2 = 0.9934). The protein content in Algerian honey was higher than to that found in Brazilian honey where it varies from 0.199 to 2.236 mg/g (Azeredo et al., 2003). The results of this study are close to that found by (Wei and Zhirong, 2003) where it varies from 3.7 to 9.4 mg/g. The protein content in honey can be attributed to the presence of enzymes, some of which are introduced by bees themselves, and others are thought to be derived from the nectar. The richness of honey in proteins mainly peptones, albumins, Globulins and nucleoproteins are derived from the plant and / or the bee and which differs depending on the botanical

origin of the honey (Amri et al., 2007). The level of protein is dependent on the type of flower. Proline is the predominant amino acid found in honey (Saxena et al., 2010 [(b)]).

Table 3. Total phenolic, flavonoids and protein contents for different types of honey samples

Sample N°	Total phenolic content mg of GAE/(100 g)	Total flavonoids content mg of RE/(100 g)	Total protein content mg/g
S1	71.60 ± 1.41^{e}	15.96 ± 1.25^{e}	3.75 ± 2.26^{d}
S2	113.90 ± 2.02^{b}	48.5 ± 1^{b}	$6.94 \pm 0.21^{c,d}$
S3	134.97 ± 0.80^{a}	72.53 ± 0.69^{a}	10.81 ± 0.50^{a}
S4	97.72 ± 3.18^{c}	39.14 ± 0.69^{c}	$7.97 \pm 1.03^{a,c}$
S5	78.35 ± 2.06^{d}	19.06 ± 0.73^{d}	$9.88 \pm 1.09^{a,c}$

Values represent means ± SD (standard deviations) for triplicate experiments. Values in the same column followed by a different letter are significantly different ($p < 0.05$).

3.5. Analysis of antioxidant activities

3.5.1. DPPH radical scavenging activity

The DPPH radical is one of the substrates most commonly used for the rapid and direct evaluation of antioxidant activity because of its stability in radical form and the simplicity of the analysis (Bozin et al., 2008). The antioxidant activity is determined by the reduction in the absorbance of an alcoholic solution of DPPH at 515 nm, which is due to its reduction to a non-radical form DPPH-H by the hydrogen-forming antioxidants present in the Sample (Maisuthisakul et al., 2007 and Da Silva et al., 2008). The results of DPPH radical scavenging activity (RSA) of different honey samples are summarized in Table 4. The IC50 values ranged from 0.50 ± 0.47 to 12.66 ± 0.52 mg/ml where there were significant differences ($p < 0.05$) between these values. The IC50 value was 0.017 ± 0.00087 mg/ml, for ascorbic acid. A lower IC50 value in honey indicates a high free radical scavenging capacity (Habati et al. 2017). The results of DPPH RSA analyses demonstrated that the most active radical scavengers were found in S3, followed respectively by S2, S4, and S5. In a study conducted by Ferreira et al. (2009), the antioxidant values ranged from 106.67 to 168.94 mg/mL, and according to data from Beretta et al. (2005), they ranged from 1.63 to 47.62 mg/mL. These results showed that the honey samples collected in the present study have greater antioxidant potential compared to the results reported in the literature. Many authors have demonstrated that honey is a source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, immune system and inflammatory processes (Gheldof et al., 2002). In honey, the components responsible for the antioxidant effect are flavonoids and phenolic acids. The quantity of these components varies widely depending on the floral and geographical origin of the honey. In addition, the processing, handling and storage of honey can influence its composition (Gheldof et al., 2002).

3.5.2. Reducing power

The FRAP test directly assesses the presence of antioxidants in various samples including honey (Khalil et al., 2012). The reducing power is measuring the conversion of a Fe^{3+}/ ferricyanide complex to the ferrous form. This method is a simple, rapid and reproducible test (Bougandoura and Bendimerad, 2012). It is universal can be applied to both plants and plasmas and in

organic and aqueous extracts (Li H-B et al., 2008). Absorbance values of honey samples ranged from 0.44 ± 0.03 (AEAC= 0.10 ± 0.0003mg/g) to 1.009 ± 0.03 (AEAC= 0.26 ± 0.01 mg/g) (Table 4). There were differences between the antioxidant activity values of different types of honey, suggesting that they have different antioxidant potentials. Our values are close to those found by Doukani et al. (2014) on Algerian honeys (AEAC = 0.083 mg / g at AEAC = 2.4 mg / g). By comparing our results with the work of Sagdic et al. (2013) carried out on certain types of honey from Turkey we found antioxidant activity values between (AEAC = 70.09 mg/g and AEAC = 86.19 mg/g) for Astragalus honey and meillat honey, we found that these values were higher compared to our results. This can be explained by the botanical, geographical origin and nature of the phenolic compounds of honey. According to Doukani et al. (2014), the antioxidant activity of the different samples of honey analyzed depends mainly on the floral source of honey. However, they suggested that the botanical species is the main source of honey, but is not the only factor that contributes to its antioxidant propertie.

Table 4. Reducing power and IC_{50}value for different types of honey samples

Sample N°	Reducing power		DPPH IC_{50} (mg/ml)
	Absorbance [700 nm]	AEAC	
S1	0.44 ± 0.02^{d}	0.10 ± 0.0003^{c}	NA
S2	0.89 ± 0.01^{b}	0.23 ± 0.015^{b}	1.31 ± 0.07^{c}
S3	1.03 ± 0.03^{a}	0.26 ± 0.01^{a}	0.50 ± 0.34^{d}
S4	0.86 ± 0.01^{b}	0.21 ± 0.013^{b}	4.31 ± 0.19^{b}
S5	0.61 ± 0.006^{c}	0.13 ± 0.006^{c}	12.66 ± 0.37^{a}

AEAC: Ascorbic acid equivalent antioxidant capacity. IC50: concentration causing 50% inhibition. NA: not active. Values in the same column followed by a different letter are significantly different ($p<0.05$).

3.7. Correlations

Correlation between antioxidant activity and total phenolics had a correlation coefficient of R^2 = 0.937 (Figure1), while correlation between antioxidant activity and flavonoids had a correlation coefficient of R^2 = 0.93 (Figure 2); both indicating that a greater percentage of the antioxidant activity of the studied honey from the North Region of Algeria may be attributed to the presence of phenolic compounds.

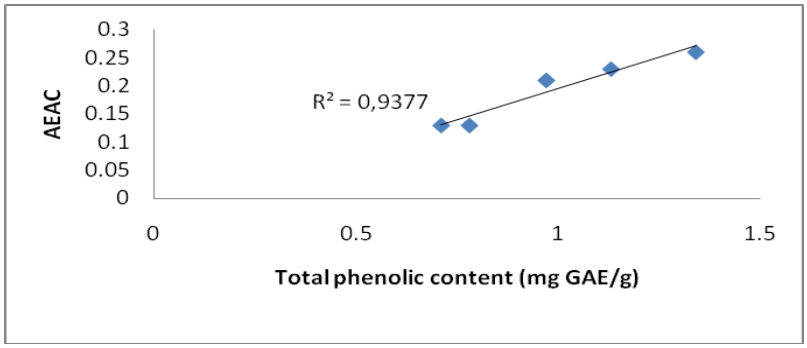

Figure 1. Correlation between antioxidant activity and total phenolic content in honey samples

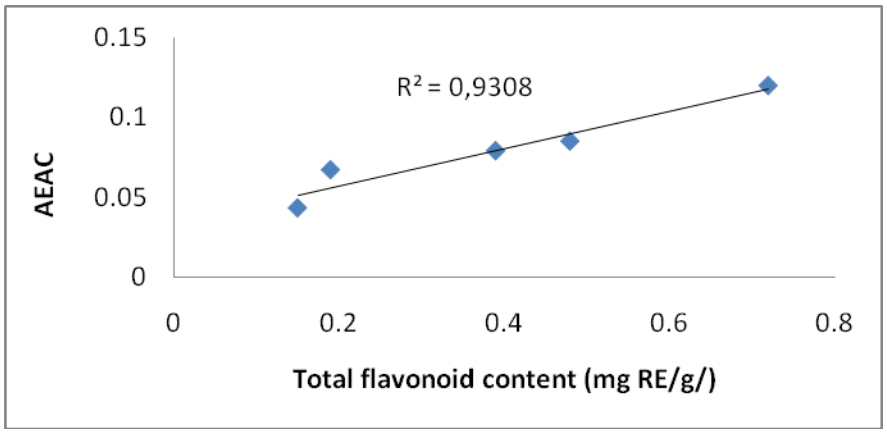

Figure 2. Correlation between antioxidant activity and flavonoid content in honey samples

Several studies have shown that antioxidant activity is strongly correlated with the content of total phenolic compounds. Beretta et al. (2005) Found that dark colored honeys have a high content of total phenolic compounds and hence a high antioxidant capacity. In honey, the components responsible for the antioxidant effect are flavonoids and phenolic compounds (Gheldof et al., 2002). Alongside this, a strong correlation was found between the antioxidant activity and the flavonoids content in the honey (Al-Mamary et al., 2002). According to Doukani et al. (2014), the antioxidant activity of the different honey samples analyzed depends mainly on the floral source of honey. However, they suggested that the botanical species is the main source of honey, but is not the only factor that contributes to its antioxidant properties. In addition, the processing, handling and storage of honey can influence its composition (Gheldof et al., 2002).

3.6. Minerals and heavy metals

All honey analyzed contains minerals and heavy metals elements at different concentrations (Table 5).

Table 5. Minerals and heavy metals concentrations in analyzed honey samples

Minerals and heavy metals concentrations (ppm)	Sample N°				
	S1	S2	S3	S4	S5
K	97.94 ± 3.51	252.52 ± 2.19	353.15 ± 3.28	374.65 ± 6.31	279.86 ± 3.94
Na	18.81 ± 3.51	129.03 ± 2.18	126.34 ± 3.28	82.43 ± 6.31	74.37 ± 3.94
Fe	0.09075 ±0.84	0.2051 ± 1.35	0.1807 ± 0.17	0.09075 ± 0.84	0.1633 ± 0.68
Cu	0.1113 ± 4.9	0.1633 ± 3.1	0.1225 ± 2.32	0.1203 ± 2.01	0.1225 ± 2.32
Co	ND	0.721 ± 3.01	1.441 ± 1.23	0.1029 ± 5.9	1.005 ± 2.08
Ni	0.05135 ± 4.8	ND	ND	0.06155 ± 1.2	ND

Values represent means ± SD (standard deviations) for triplicate experiments. ND: not detected.

Concerning minerals, potassium appeared in the greatest content with an average (271.62 ± 1.52 ppm) followed by sodium (86.20 ± 1.52 ppm). Noticeable contents of tested heavy

metals (as environmental pollutants) were detected:

Nickel is present in small quantities for two varieties of honeys S1 and S4 with an average of (0.0564 ± 3 ppm). In general, nickel concentrations in honey are between 0.3 and 1.3 ppm. These concentrations may be accidental or mostly natural (Bogdanov, 2004). The mean value observed for copper is (0.1279 ± 2.93 ppm). These values were close to those that have been reported between 0.05 and 1.8 ppm ranged from various types of Moroccan honey (Chakir et al., 2011). Copper can contaminate the environment through the use of pesticides against pests that damage crops (Provenzano et al., 2010). The mean value observed for iron and cobalt is (Fe: 0.1461 ± 0.77 ppm, Co: 0.8174 ± 3.05 ppm). The variability of mineral content can be attributed to environmental, botanical, or geographic factors (Bogdanov, 2006). The results indicate that samples of honey taken from five different regions of Algeria were, for the most part, of good chemical quality, meeting the required standards. The trace elements that are present in our moderate honey samples present no health hazards as long as they are in low doses. On the other hand, they contribute to the good functioning of the organization. The decomposed toxic elements in the honey studied present no risk because they are below the maximum residual limit. Therefore, these results indicate that the production areas of this honey are unpolluted by the toxic elements.

4. Conclusions

This is the first study to investigate the Natural substances occupy an increasingly prominent place in therapy. Indeed, the honey and his composition vary from hive to hive, from district to district and from season to season. This work is a contribution to the study of the physicochemical properties, quantification of the phenolic content and antioxidant activity evaluation of five samples of honey from different regions of the Algerian north ; it has allowed us to understand that the field products of the hive is still a valid field of scientific research. The present study concludes that, the quality, physicochemical properties and antioxidants activities of honey were varied based on the geographical and botanical origins, handling, and transportation and storage conditions.

The resultants showed that samples of honey from different locations in Algeria were not free of heavy metals, but were well below permitted levels. Honey from Algeria is considered among the least polluted foods. It shows that apiaries are located at a distance that is far from one or more of the possible sources of pollution. The results of this first regional survey show the good quality of the honey analyzed in relation to the parameters studied.

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COMPARISON OF PROXIMATE COMPOSITION, AMINO ACID, VITAMIN, AND MINERAL CONTENTS OF WHOLE FISH POWDER AND FISH PROTEIN CONCENTRATE FROM LOCAL INDONESIAN SNAKEHEAD FISH (*Channa striatus)*

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ABSTRACT

The gabus *(Channa striatus)* is an Indonesian indigenous predatory freshwater fish that traditionally known to have medicinal benefits in wound healing and energy boosting for sick people. The aim of this study was to investigate the nutritional content of two processed products made from the gabus fish: (1) whole fish powder (WFP) and (2) fish protein concentrate (FPC). Proximate analysis revealed that protein content was higher in FPC (85.85%) than in WFP (67.93%). Lipid, crude ash, and water contents of WFP were 5.74%, 10.76%, and 3.60% respectively, while those of FPC were 4.48%, 4.74%, and 4.61% respectively. Both products contained all essential amino acids ranging from 8.14 g (histidine) to 2.19 g (methionine) per 100 g of WFP protein and from 7.08 g (leucine) to 2.81 g (methionine) per 100 g of FPC protein. Non-essential amino acids contents was from 7.20 g (arginine) to 0.23 g (aspartic acid) in WFP protein and from 10.45 g (glutamic acid) to 2.7 g (serine) in FPC protein. Both products contained vitamin A, B2, E, D3, and B12. Calcium and phosphor were higher in WFP (2401 and 18.51 mg per 100 g respectively) than in FPC (178 mg and 7.1 g per 100 g respectively). FPC and WFP of *Channa striatus* have relatively high protein contents and complete amino acids composition and they can be considered as food supplement, especially as protein and amino acids sources.

1. Introduction

Gabus (*Channa striatus)* is a snakehead fish found in abundance in Asian countries. It is an indigenous predatory fresh water fish that can be found in all bodies of water, from small ditches, rice fields, to large rivers in Asian tropical countries such as Indonesia, Malaysia, India, and Pakistan (Mohsin and Ambak, 1983). In these countries, this particular fish has long been regarded as valuable food for its medicinal benefits. People suffering from severe diseases have been traditionally suggested to include this fish in their diet to boost their immunological states. Post-natal women and post-surgery patients are often recommended to consume this particular fish in order to promote faster wound healing (Shafri and Manan, 2012). Several researchers have reported that gabus fish (*Channa striatus)* is rich in nutrients such as amino and fatty acids that are essential for wound healing and promotion of immune system in the body

(Zuraini et al., 2006; Dahlan and Daud, 2010; Schlenker and Long, 2007). However, due to its physical characteristics, fresh snakehead fish impractical and inconvenient to be used directly as food supplement for sick people. In addition, the protein content of fresh fish (around 20%) is much lower than that of fish protein concentrate (ranges from 60-90%) (Zuraini et al., 2006; Windsor, 2007; Murueta et al., 2007). Therefore, product innovation which can increase the usability of snakehead fish as a raw material for production of supplemental foods that are rich in protein and essential amino acids is timely important. This study was conducted to determine proximate composition, amino acids, vitamin, and mineral contents of two products from snakehead fish; namely, Whole Fish Powder (WFP) and Fish Protein Concentrate (FPC).

2. Materials and methods

2.1. Material

Samples of *Channa striatus* (1.0-2.0 kg/fish) were obtained from Bili-bili watershed in South Sulawesi. Analytical grade chemicals and solvents used in this study were procured from Merck and Sigma Aldrich, US.

2.2. Sample preparations

2.2.1. Whole fish powder (WFP)

Ten kg of fresh *Channa striatus* samples were gutted, weeded, and washed thoroughly with running tap water. The cleaned fish were then cooked in a pressure cooker with 8 L of clean water (all pieces were covered with water), at low heat. The cooking was continued for approximately 30 minutes after the initial boiling occurred. After that, the head and bones were removed from the flesh then the flesh was homogenized using a high speed mixer (Philips, Netherland) and dried at 60^0C for 6 hours using an air dryer. The dried sample was ground using an electric grinder and sieved using a 100 mesh sieve. The sample was stored in an air-tight container at refrigerated temperature until used for analysis.

2.2.2. Fish protein concentrate (FPC)

Samples of snakehead fish were gutted, weeded, and washed thoroughly, and then deboned and cut into small pieces. One hundred grams of fish meat sample was mixed with 100 ml HCl (ratio 1:1 w/v) and homogenized using a high speed mixer (Philips, Netherland) at 50-60 ^{0}C. The mixture was then filtered. The liquid obtained (filtrate) was mixed with 200 ml of hexane solvent and centrifuged for 30 minutes to separate the fat from the filtrate. The oil and the hexane solvent were separated using a separation funnel. The liquid extract of protein concentrate obtained from the fat separation process was then dried at 60-70^{o}C. The fish protein powder obtained from the drying process was stored in an air-tight container at refrigerated temperature until used for analysis.

2.3. Proximate analysis

Proximate analysis was performed in duplicate for samples of whole fish powder and fish protein concentrate. Moisture, ash, and fat contents were analyzed using the Association of the Official Analytical Chemists (AOAC, 1984) methods 14004 (1984), 14009 (1984) and 14006 (1984), respectively. Nitrogen was determined using the Kjeldahl method. The quantity of protein contained in each sample was calculated as 6.25 x N (Method 7015, AOAC, 1984).

2.4. Amino acids analysis

Amino acids content of whole fish powder and fish protein concentrate were analyzed using Waters Acquity UPLC H-Class and H-Class Bio amino acid analysis method (Waters Company, USA, 2012) and amino acid analysis (Nollet, 2004). Briefly, 0.1 g of powdered sample was transferred into a tube containing 5 ml of 6 N HCl and then vortexed. The mixture was then hydrolyzed for 22 hours at 110^0C. After the hydrolization process, the solution was cooled to room temperature and transferred into a 50 ml flask and added with aquabidest until the total volume reached 50 ml. The

diluted solution was filtered through a 0.45 µm filter. The filtrate was pipette 500 µl and mixed with 40 µl of AABA and 460 µl of aquabidest. Ten µl of the solution was mixed with 70 µl of AccQ-fluor borate and vortexed. After that, 20 µl of fluor A reagent was added and kept for 1 minute and then incubated for 10 minutes at 55^0C. The solution was then injected into the UPLC system. The net height of every peak produced by the chart recorder of the analyzer (each representing an amino acid) was measured and calculated.

2.5. Vitamin & Mineral

2.5.1. Fat Soluble Vitamins

Fat soluble vitamin contents of snakehead whole fish powder and fish protein concentrate were estimated by using the Association of the Official Analytical Chemists method 2002.05 (AOAC, 2007). By this method, 2 grams of sample was placed into a 50 ml tube. Meanwhile, 50 mg each of vitamins A, D3, and E were placed into a 50 ml centrifuge tube. Both tubes were added with 5 ml of ethanol-ascorbic acid 0.1% and 4 ml of 50% KOH solution. The tubes were heated at 70^0C for 30 minutes and then vortexed for 10 minutes. After the heating, the tubes were cooled to room temperature and 5 ml of hexane was added into each tube and shaken for 5 minutes. The tubes were then put aside until separation occurred. The solution of h-hexane was separated and transferred into a flask and added with 1 ml of methanol-ascorbic acid 0.1% and also add with 2 x 10 ml of n-hexane (in a centrifuge tube). After that, the solution was evaporated in a dark room until dry and then added with HPLC grade methanol. The diluted solution was then put into a 50 ml flask, homogenized, and filtered with 0.45 µm filter. The filtrate was pipetted into autosampler vial and injected about 20 µl into the chromatography system. The Chromatography used was equipped with octadecyl silane (RP-18) column with a flow rate of 0.7 ml/ minute. λ for vitamin A, D, and E were 325, 264, and 292 nm, respectively. The vitamin standards were prepared in mobile phase. The concentrations of the vitamin in the WFP and FPC samples were calculated in relation to the peak of the standard vitamins.

2.5.2. Water Soluble Vitamins

Vitamin contents (water soluble vitamins) of WFP and FPC of snakehead fish were estimated using ultra performance liquid chromatograpy (UPLC) system (Waters Company, USA 2012). Two grams of sample was diluted with a solution of acetonitrile: formic acid 2% in methanol (75:25) in a 25 mL flask and homogenized. The diluted solution was then filtrated through a 0.2 µm polytetrafluoroethylene (PTFE) membrane. About 5 µl of the filtrate was injected into the UPLC system and measurement was conducted using the following chromatography conditions: detector (photodiode array (PDA) 265 nm, λ range 3D 190-400 nm; 2D 265 nm, resolution 1.2 nm), column (Amide 1.7 µm, 2.1 x 100 mm), temperature 30^0C, and flow rate (0.3 mL/min). Vitamin standards were prepared in mobile phase. Vitamin concentrations was calculated in relation to the peak of standard vitamins.

2.5.3. Minerals

Mineral contents were analyzed using the Association of the Official Analytical Chemists (AOAC, 2013) official methods 2011.14. Samples (0.5 g) were mixed with 5 mL nitric acid and heated in a closed-vessel microwave digestion system (MDS) from ambient to a temperature of 150^0C in 10 minutes and hold at that temperature for 10 minutes. After that, the ash was cooled to room temperature and added with H2O to a total volume of 25 mL and homogenized. The mineral contents were measured using an inductively coupled plasma-optical emission spectrometry (ICP-OES) instrument.

2.6. Statistical analysis

Experiments were carried out in duplicate with mean values and standard deviation (SD) were calculated.

3.Results and discussions

3.1. Proximates

Proximate analysis results presented in Table 1 show that both of the products produced from snakehead fish contained a high amount of protein. The protein content was found to be higher in the FPC (85.85%) compared to that in the WFP (67.93%). The protein contents observed in this study were in the range of an ideal fish protein concentrate (Windsor, 2001). Moreover, the protein content of 85.85% for the FPC used in this study was higher than the average of 57-79% protein contents of several FPC produced from bycatch fish species reported previously (Murueta, 2007). In addition, the protein contents of the WFP and FPC used in this study were higher than the protein content of fresh snakehead fish (±20%) (Zuraini, 2006).

The crude ash component was much higher in whole fish powder (10.76%) than that in fish protein concentrate (4.74%). This may indicate that some of fine bones were present in the whole fish powder and contributed to the higher crude ash component found from proximate analysis. Total fat content was relatively low in both products, even though it was moderately higher in the whole fish powder (5.74%) than in the fish protein concentrate (4.48%). The lower fat content of the fish protein concentrate may significantly increase the stability of the product against lipid oxidation which may also enhance product quality (Windsor, 2001).

The moisture contents of WFP and FPC were 3.6% and 4.61% respectively. The two parameters (moisture and fat) are important for the storage quality of the products. High moisture content can increase the possibility of fat hydrolysis and the growth of microorganisms which will result in the reduction of quality and safety of the product. The lower the amount of fat and moisture in the WFP and FPC, the better the quality and the longer the shelf life of the products.

Table 1. Proximate composition of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus)**

	Whole fish powder	Fish protein concentrate
Protein (%)	67.93±0.44	85.85 ±0.22
Fat (%)	5.74±0.03	4.48±0.02
Moisture (%)	3.6±0.01	4.61±0.01
Crude ash (%)	10.76±0.01	4.74±0.01

*Value are means of duplicate samples

3.2. Amino acids

The amino acids composition of WFP and FPC of *Channa striatus* are shown in Table 2. It can be seen from the table that the two products contain 17 of the 22 amino acids found in nature. All essential amino acids were present in both WFP and FPC. The highest concentrations of essential amino acids per 100 g product were histidine (8.14 g) for WFP and leucine (7.08 g) for FPC and the lowest were Methionine (2.19 g and 2.81 g for WFP and FPC respectively). For non-essential amino acids, the highest concentration found was arginine (7.20 g/100 g) for WFP and glutamic acid (10.45 g/100 g) for FPC. It can also be seen from the values in the table that for every 100 grams of fish protein concentrate from snakehead fish, the amount of histidine, isoleucine, leucine, lysine, methionine, valine, and threonine contained were higher than the daily recommended intake (DRI) for children (WHO/FAO/UNU, 2007). Only phenylalanine and tryptophan was found to be lower than the recommended daily intake. The amount of histidine, isoleucine, lysine, and valine contained in 100 grams of whole fish powder were higher than the DRI for children while leucine, methionine, phenylalanine, and threonine contents were lower than the DRI for

children. However, the concentrations of all essential amino acids per 100 grams of WFP and FPC were higher than the WHO/FAO/UNU recommendation for amino acids intake in adult. Although several of the amino acids concentrations found in the WFP and FPC of snakehead fish were slightly lower than those found in protein hydrolisates from Herring fish *(Clupeaharengus)* (Liceaga-Gesualdo & Li-Chan, 1999), the overall figures were generally similar. Since the amino acids found in the WFP and FPC were present in just the right balance for human nutrition, both products might be potentially used as functional foods for protein supplementation for human in need.

Table 2. Amino acids composition (g/100 g protein) of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus)**

Amino acid (g/100 g protein)	Whole fish powder	Fish protein concentrate	Herring *(Clupeaharengus)* protein hydrolysates***	Daily recommendation WHO/FAO/UNU***	
				Child (g)	Adult (g)
Essential amino acids					
Histidine	8.14±0.011	3.70±0.010	1.22	1.90	1.60
Isoleucine	3.33±0.013	3.94±0.003	3.15	2.80	1.30
Leucine	5.70±0.019	7.08±0.002	8.42	6.60	1.90
Lysin	6.56±0.040	6.63±0.004	8.46	5.80	1.60
Methionine	2.19±0.018	2.81±0.019	4.94	2.70	1.70
Phenylalanine	3.25±0.016	4.84±0.008	3.39	6.30	1.90
Valine	3.74±0.004	4.39±0.006	4.72	3.50	1.30
Threonin	3.39±0.004	4.56±0.006	4.74	3.40	0.90
Tryptophan	0.55±0.004	0.64±0.005	-		
Non-essential amino acids					
Tyrosine	2.21±0.012	3.27±0.005	2.64		
Aspartic acid	0.23±0.002	7.66±0.008	10.72		
Glutamic acid	4.37±0.005	10.45±0.014	15.87		
Serine	4.19±0.005	2.7±0.002	4.87		
Glycine	5.04±0.007	5.94±0.061	7.59		
Arginine	7.20±0.009	9.35±0.011	7.06		
Alanine	3.80±0.004	3.94±0.004	7.54		
Proline	3.29 ± 0.003	3.90±0.006	4.54		

* Values are means of duplicate samples

** Liceaga-Gesualdo & Li-Chan, 1999

***WHO/FAO/UNU, 2007

3.3. Vitamins and Mineral

The compositions of vitamins and minerals contained in WFP and FPC are presented in Table 3 and Table 4. From Table 3, vitamin A, E, B2, and B12 were present in relatively small amounts in both WFP and FPC compared to the DRI. On the other hand, vitamin D

concentrations were relatively high, which were 10.78 µg and 14.92 µg in WFP and FPC respectively. The adequate intake of vitamin D is 5 µg/day (Schlenker & Long, 2007).

Table 3. Vitamins content of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus)**

Vitamin per 100g	Whole fish powder	Fish protein concentrate
Vitamin A (µg)	7.81±0.65	30.1±18.3
Vitamin D (µg)	10.78±1.82	14.92±4.03
Vitamin E (mg)	1.78±0.25	2.52±1.44
Vitamin B1	ND	ND
Vitamin B2 (mg)	0.68±0.01	0.69±0.00
Vitamin B6	ND	ND
Vitamin B12 (µg)	0.98±0.01	1.08±0.01

* Values are means of duplicate samples
ND: Not detected

Mineral contents of both WFP and FPC are shown in Table 4. The highest mineral content was Calcium in Whole fish powder (2401 mg/100 g), whereas in FPC calcium concentration was only 179 mg/100 g. The high concentration of calcium in WFP was the result of the inclusion of fine and small fish bones in the product, which on the other hand was absent in FPC. Other minerals such as Phosphor, Magnesium, Zinc, and Iron were also present but in relatively low concentrations.

Table 4. Mineral contents of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus)**

Mineral (mg/100g)	Whole fish powder	Fish protein concentrate
Calcium	2401.20±10.52	178.91±9.33
Phosphor	18.51 ± 0.15	7.58±0.85
Magnesium	108.87±0.00	137.35±5.21
Zinc	0.032±0.000	0.026±0.00
Iron	0.086±0.001	0.154±0.003

*Values are means of duplicate samples

4. Conclusions

Whole fish powder (WFP) and fish protein concentrate (FPC) from snakehead fish (*Channa striatus)* are rich sources of protein and amino acids. Both products contain all essential amino acids and most of non essential amino acids. Furthermore, the amounts of essential amino acids present in both products are relatively high and meet or exceed the recommended daily intake per 100 grams of the products. In comparison to fresh snakehead fish, both WFP and FPC are more practical and convenient to use. In addition, the amounts that need to be consumed to achieve the daily requirements for protein and amino acids are relatively small. Therefore both products might be used for protein supplementation for sick people and others in need, particularly in tropical developing countries where *Channa striatus* are abundant.

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NUTRITIONAL, FUNCTIONAL, SENSORY AND MICROBIAL QUALITIES OF WHEAT-TOMATO SEED FLOUR BREAD

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ABSTRACT
Wheat-tomato seed flour bread in the ratios (100:0, 95:5, 90:10, 85:15, and 80:20) were produced using straight dough method, packaged, stored on shelf for 9 days at ambient (27±2 ºC, 70±3 % RH) and assessed for sensory, nutritional and microbial qualities. The microbiological assessment was based on Nigeria Industrial Standards of Standard Organisation of Nigeria/Food Drug and Administration standards. Loaf weight increased significantly with tomato seed flour incorporation while loaf volume and specific volume decreased significantly with increased levels of tomato seed flours inclusion. High protein in tomato seed reflected positively in the composite proportions. The sensory results revealed that crumb colour, texture and taste decreased significantly ($p < 0.05$) with increased tomato flour at 1 day. There were no significant differences in acceptability between 100% wheat flour bread (100:0) and 5 % (95:5) tomato flour inclusion at the third (3) day of production. Increase in the levels of tomato seed flour reduced bacterial and yeast/mould counts. There was mould growth on control in day 5, while the composite breads were microbiologically safe at when compared with standards. *Staphylococcus aureus* and *Bacillus cereus* were isolated but within safety limit. It was revealed that tomato seeds have antimicrobial properties and can also be used as composite flour in bread.

1. Introduction

Bread may be described as a fermented confectionary produced mainly from wheat flour (Bhise and Kaur, 2014). Traditional read production involves a mixture of wheat flour, yeast (*Saccharomyces cerevisiae*), salt, and water followed by a series of processes such as kneading, proofing, shaping and baking. The consumption of bread in Nigeria is on a steady increase because it is a convenient and ready-to-eat (RTE) food normally consumed as breakfast, lunch, and sometimes dinner.

There is a growing interest in using composite flour for bread making owing to some economic, social, and health reasons. Composite flours are mixture of flours from tubers rich in starch (e.g. cassava, yam, sweet potato) and /or protein rich flours (e.g. soybean, peanut) and/or cereals (e.g. rice, maize, millet), with or without wheat flour. It brings about better overall use of domestic agricultural produce. The shelf life of bread is between 2 – 3 days without any preservative if the qualities required are still to be maintained. Shelf life of bakery

product is mostly characterised by onset of staling and ropiness formed due to microbial invasion. Other factors affecting shelf life of bread are rancidity, crystallisation, grittiness, development of off flavours, structural weakness, fade colour and moisture migration (Bhise and Kaur, 2014). Bakers make use of divers of hemical preservatives to extend the shelf life of bread for weeks and months. Some of these chemical preservatives are carcinogenic and increase body residual toxicity (Saeed et al., 2013). However, some of these preservatives limit of addition are abused and such is dangerous to human health. The current trend adopts the use of natural preservatives; a promising alternative to chemical methods in extending the shelf life of products (Adegoke et al., 2014). The potential sources of these natural preservatives are spices, herbs, fruits, seeds, leaves, roots, and bulbs. It has been currently discovered that tomato (*Solanum lycopersycum L*) contains alkaloids compound such as tomatine which shows antimicrobial activity towards various microbial pathogen (Kuzukue et al., 2004). Thus, it was deduced that tomato has the potential to provide antimicrobial and antifungal compounds. Nigeria is the second largest producer of tomato in Africa and 16th in the World with current production of 1.8 million tonnes of fresh tomato per year, but 50 % of these are lost due to poor storage system and lack of processing enterprises (Ugonnu et al., 2015). Therefore, harnessing the potential of tomato as preservative and composite flour would reduce loss and health problem due to consumption of overdose chemical preservatives in foods and also improve the value chain of tomato. The study investigated quality of wheat-tomato seed composite bread and effectiveness of tomato seed as natural preservative in bread.

2. MATERIALS AND METHODS

2.1. Raw materials

The tomato fruit was 'elite' variety procured from a local market in Gashua, Yobe State. Other ingredients such as wheat flour ('Dangote^{TM}), yeast ("Royal^{TM}"), salt, margarine, and sugar were purchased from supermarket in Abeokuta, Ogun State.

2.2.1. Preparation of tomato seed flour

The tomato fruits were processed to seed as described by Persia *et al*, 2003. Tomato seeds were removed from tomato fruits. The seeds were washed, dried in oven at 60 °C to constant weight, milled, and sieved using mesh 60 μm to obtain tomato powder.

2.2.2.Treatment

Bread was prepared with composite flour of wheat and tomato seed blends. The tomato seed flour was incorporated into wheat flour in the ratios of (wheat: tomato seed) 100:0, 95:5, 90:10, 85:15 and 80:20.

2.2.3. Bread preparation

The bread was produced by straight dough method as described by Bhise and Kaur (2014), without addition of preservative with little moderation. The proportions of the ingredients were not changed except proportion of composite flour, yeast (1 g) and sugar (3.0 g). The amount of the yeast Royal^{TM} was based on manufacturer's direction and experimental trial at optimum level for bread production. The ingredients were mixed using a spiral mixer with water and continued until soft dough was formed. The dough was kneaded manually by hand for 20 min and the whole mass was divided into small sizes, moulded into desired shape. The dough was set in baking pan, and proofed for 55 min at 31°C, RH 75 % in electric proofing machine. The loaves were baked in an oven at 200 °C for 25 min. The baked bread was allowed to

cool, packaged in Low Density Polyethylene bags and stored on shelf for 9 days for microbial analysis while the chemical and baking qualities were carried out on day 1 (one).

2.3.1. Chemical properties

Chemical characteristics of wheat flour, tomato seed flour and composite bread namely protein, fat, ash, crude fibre, and moisture were analysed by standard procedures (Bhise and Kaur, 2014).

2.3.2. Baking quality

The functional baking quality of bread; loaf weight, loaf volume, and specific volume were determined using the method of Bhise and Kaur (2014).

2.3.3. Microbial analysis

Bacterial enumeration was done using pour plate technique as described by Akhigbemidu et al., (2015). 25 g of each sample of bread was aseptically transferred into 225 ml of sterile distilled water, mixed for 5 min. I ml of the sample was pipetted into another sterile diluents containing 9 ml to obtain 10^{-2} with serial dilution continued till 10^{-5}. 0.1 ml of the diluent was inoculated into sterile petri dishes and molten nutrient agar was added. The samples were incubated at 37 ºC for 24 h. Yeast and moulds were enumerated using the spread plate method as described by Tournas et al. (2001). 0.1mlof serial diluents prepared were aseptically pipetted on pre-poured, solidified Dichloran rose bengal chloramphenicol (DRBC) agar plates and inoculums were spread with sterile bent glass rod. The bacteria and mould/yeast were analysed periodically at 1, 3, 5, 7, and 9 days; Staphylococcus *aureus, Bacillus cereus, Coliform, Esterenchia coli,* and *Salmonella* presence were investigated and compared with standard Organisation of Nigeria and Food and Drug Administration standard minimum limit for microbial quality of ready-to-eat and bakery products. This was only done at day 5 since fungi growth have been found on control. Direct plate count with Baird-Parker medium was used for enumeration of *Staphylocuccus aureus* followed by coagulase test; Mannitol-egg yolk-polymixin for Bacillus and tyrosine decomposition test for isolation; Xylose lysine deoxycholate agar for Salmonella followed by urease and methyl test for isolation; Violet red bile agar for Coliform. The analysis on isolation and identification were only done at day 5 when cream/green colour growth was observed on control sample.

2.4. Sensory analysis

Sensory attributes: colour, taste, texture, and overall acceptability based on a nine point hedonic scale, where 1 is dislike extremely and 9 is like extremely was assessed. The sensory analysis was limited to 1 and 3 days because of appearance of green and cream colour which is believed to be an indication of microbial invasion on control sample of bread at 5 day. The result was subjected to statistical analysis.

2.5. Statistical analysis

Data obtained were analysed statistically using techniques of analysis of variance (ANOVA) and least significant difference (95% significance).

3. Results and discussions

3.1. Proximate quality of tomato seed and bread

The proximate composition of tomato seed and bread are presented (Tables 1 and 2) respectively. Protein content of the tomato seed flour indicated (27.11 %) is related to the report of Parsia et al., (2003). High protein content in tomato seed flour may contribute to nutrient and baking

quality of bread when compare with other low protein composite flours. The increased percentage of fat in tomato seed flour may have positive influence on bread texture. The protein and fat contents of bread with tomato seed flour substitutions were higher than the control sample (100 % wheat bread). This may be due to high percentages of protein and fat in tomato seed. However, aside from moisture other proximate contents were also related to the result recorded by Ijah et al., (2014) in their study on nutritional quality of bread produced from wheat and potato flour blends and also similar to study of Monji et al., (2011), on cocoyam-wheat composite breads. The nutritional quality of the bread is adequate and comparable to other breads.

3.2. Functional properties of bread

Loaf weight increased significantly with increase in tomato seed flour blends, but loaf volume and specific volume decreased significantly ($p<0.05$) with increased levels of tomato seed flours inclusion (Table 3). There was no significant difference ($p > 0.05$) between 5 % tomato seed flour substitution and the control (100 % wheat flour bread). There exist no correlation between specific volume of bread and the protein content. The decrease in loaf volume was found precedent to increase in tomato seed flour substitution. This may be due to lack of gluten in tomato seed; reduced flour strength and a lower ability of the dough network to enclose carbon dioxide produced during fermentation, and such partial substitution of wheat flour may impair the quality of the bread. A similar finding on loaf volume of composite flours was reported (Dhingra and Jood, 2004). It has been observed that consumers prefer average weight bread in Nigeria. Weightless or less weight bread are usually been considered as shoddy quality bread with poor patronage.

3.3. Microbial assessment of bread

The potency of tomato seed flour in reduction of microbial growth (aerobic and yeast/mould count) increased with proportion of the treatments (Figure 1 and 2). The microbial growth of both aerobic bacteria and yeast/mould were lower in tomato seed flour bread compare to the control. The microbial load increased with storage duration (days) for both the aerobic and mould counts. Yeast/mould were not detected on fresh bread at day 1, this might be as a result of baking temperature (200 ºC) which might have eliminated all the yeast in the dough (Adeboye et al., 2015). The 5 % and 10 % tomato seed flour potency against bacteria growth was found related and comparable to 15 % and 20 % tomato seed flour substitutions at 5 day, however, the latter showed better potency at day 7 and 9. Silva-Beltrán et al., (2015).reported that phenols contained in alkaloid compounds in tomato fruits are related to germ and microbial-resistance in tomato leaves. It was also deduced that tomato extract have the potential to provide new antimicrobial and antifungal compounds. The amounts and types of antifungal compounds in tomato extracts vary among different tomato cultivars (Silva-Beltrán et al., 2015). There was an appearance of cream and green colour moulds on control (100% wheat bread) on day 5 and cream yellow colouration on 5 % Tomato seed flour substitution on day 7 while other levels of substitutions (10, 15 and 20 % Tomato seed flour) did not show any colour throughout the period of the experiment. This is an indication of effectiveness of tomato seed in reducing mould growth in bread. The enumeration and isolation of microbiological quality of the bread samples was compared to Standard Organisation of Nigeria revealed safety of the bread at day 5 for all the samples (Table 4). However, the scanty growth on control was observed on 4

day, this might prevent it consumption. This confirmed 3 days for shelf-life of bread without any preservative. The thermal treatment during processing decreases microbiological load to acceptable level which also might have aided low growth on bread and less contamination (Martinez-Gomez, 2017). *Staphylococcus aureus* as isolated from the samples except on 20 % Tomato seed bread. However, the colony count population did not exceed minimum standard limit. The presence of *Staphylococcus aureus* is an indication of poor sanitation and it is highly vulnerable to destruction by heat treatment. However, processed foods may contain relatively small numbers of debilitated viable cells. *Bacillus cereus* as isolated in 20 % Tomato seed bread only.

Table 1: Proximate composition of tomato seed and wheat flour

Parameters	Tomato seed	Wheat flour
Moisture,%	8.91±0.06	9.96±0.11
Protein, %	27.11±0.12	13.04±0.08
Ether extract, %	20.82±0.18	1.21±0.51
Ash, %	7.69±0.014	1.62±0.12
Crude fibre, %	19.63±0.03	2.14±0.02

Dry basis mean value ± standard error of three replicates

Table 2: Proximate composition of bread

Samples	Protein,%	Ether extract,%	Ash, %	Crude fibre,%	Moisture,%	Carbohydrate,%
100 % Wheat	11.15±0.04	1.90±0.13	1.27±0.05	0.99±0.14	33.82±0.41	84.69±0.01
95%W: 5%T	11.65±0.02	2.04±0.05	1.76±0.02	1.26±0.58	30.06±0.09	83.29±0.06
90%W:10%T	12.42±0.01	3.13±0.01	1.81±0.02	1.31±0.13	29.51±0.13	81.33±0.01
85%W:15%T	13.89±0.03	3.84±0.09	2.14±0.01	1.43±0.05	28.22±0.04	79.70±0.02
80%W:20%T	15.07±0.04	4.01±0.11	2.39±0.03	1.84±0.21	26.17±0.57	77.69±0.01

Values are means ± standard error of three replicates

Table 3: Baking quality of bread incorporated with tomato seed

Samples	Loaf volume, cm^3	Loaf weight, g	Specific volume cm^3/g
100 % W	458.91±0.02	239.22±0.22	2.27±0.47
95%W:5%T	451.42±0.07	248.41±0.65	2.09±0.19
90%W:10%T	424.76±0.15	253.79±0.09	1.96±0.06
85%:15%T	381.83±0.91	261.68±0.03	1.41±0.01
80%W:20%T	359.07±08	279.04±0.11	1.28±0.31

Values are means ± standard error of three replicates

Table 4: Isolation and enumeration of microbes

Samples	Organism	Count, cfu/g	Standard (NIS/FDA)	m	M
			n		
100 % W	*Bacillus cereus*	0	0	10	
	Coliform	0	2	10	10^2
	Staphylococcus aureus	$3x10^1$	1	10	10^2
	E. coli	0	0	0	
	Salmonella	0	0	0	
	Mould/Yeast	4.3×10^3	2	10^4	10^5
	Aerobic count	5×10^3	2	10^4	10^5
95%W:5%T	*Bacillus cereus*	0	0	10	
	Coliform	0	2	10	10^2
	Staphylococcus aureus	$5x10^1$	1	10	10^2
	E. coli	0	0	0	
	Salmonella	0	0	0	
	Mould/Yeast	6.1 x 102	2	10^5	10^6
	Aerobic count	1.3×10^3	2	10^4	10^5
90%W:10% T	*Bacillus cereus*	0	0	10	
	Coliform	0	2	10	10^2
	Staphylococcus aureus	1	1	10	10^2
	E. coli	0	0	0	
	Salmonella	0	0	0	
	Mould/Yeast	1.6×10^2	2	10^5	10^6
	Aerobic count	$1.7x10^3$	2	10^4	10^5
85%:15%T	*Bacillus cereus*	0	0	10	
	Coliform	0	2	10	10^2
	Staphylococcus areus	2	1	10	10^2
	E. coli	0	0	0	
	Salmonella	0	0	0	
	Mould/Yeast	1.1×10^2	2	10^5	10^6
	Aerobic count	1.6×10^3	2	10^4	10^5
80%W:20% T	*Bacillus cereus*	1	0	10	
	Coliform	0	2	10	10^2
	Staphylococcus areus	0	1	10	10^2
	E. coli	0	0	0	
	Salmonella	0	0	0	
	Mould/Yeast	0.4×10^2	2	10^5	10^6
	Aerobic count	$7.1 x10^2$	2	10^4	10^5

Legend: n- normal amount, m-minimum amount, M-maximum amount

3.4. Sensory results of the bread

The sensory evaluation was limited to day 1 and 3 due to appearance of green colour on control sample (100 % Wheat) on day 5. The sensory results (Table 5) revealed that crumb colour, texture and taste decreased significantly ($p < 0.05$) with increased tomato flour inclusion at 1 day. The insignificance in colour of samples on day 3 (Table 6) may be due to formation of complex between native flour lipids and proteins from tomato seed and wheat which might have been responsible for stability of colour in tomato seed flour-wheat incorporated bread (Chung et al., 1981). There were no significant difference (p0.05) in acceptability between 100% wheat flour bread (100:0) and 5% (95:5) tomato flour inclusion at the first (1) and third (3) day.

Table 5:Sensory evaluation of bread at 1 day

Samples	Colour	Taste	Texture	Acceptability
100 % W	8.33b	8.00d	8.17d	8.00b
95%W:5%T	7.83ab	7.90cd	7.78cd	7.83b
90%W:10%T	7.50ab	6.85bc	7.00ab	6.50a
85%:15%T	7.17ab	6.03ab	6.84ab	6.00a
80%W:20%T	6.83a	5.36a	6.26a	5.83a

Values are means ± standard error of three replicates
Different superscripts in the same column indicates significant differences at $P < 0.05$

Table 6: Sensory evaluation of bread at 3 day

Samples	Colour	Taste	Texture	Acceptability
100 % W	7.17a	6.33a	6.34ab	6.83b
95%W:5%T	6.67a	5.67a	7.00b	7.17b
90%W:10%T	6.33a	6.00a	6.17ab	5.67ab
85%:15%T	7.00a	5.37a	6.33ab	5.67ab
80%W:20%T	7.33a	5.83a	5.67a	4.82a

Values are means ± standard error of three replicates
Different superscripts in the same column indicates significant differences at $P < 0.05$

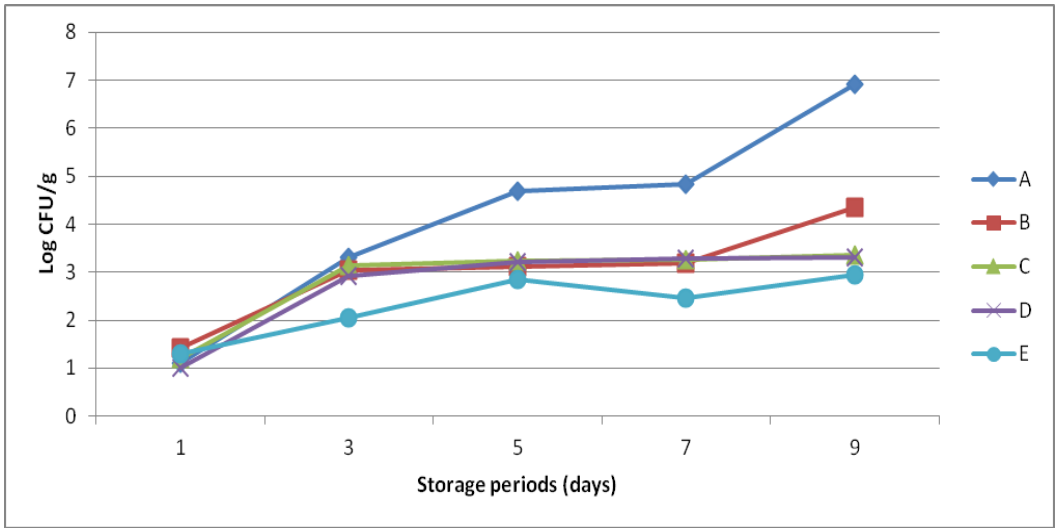

Figure 1. Total aerobic count on bread, cfu/g; A-100% W, B- 95%W: 5% T, C- 90%W:10% T, D- 85%: 15%T, E- 80%W:20%T

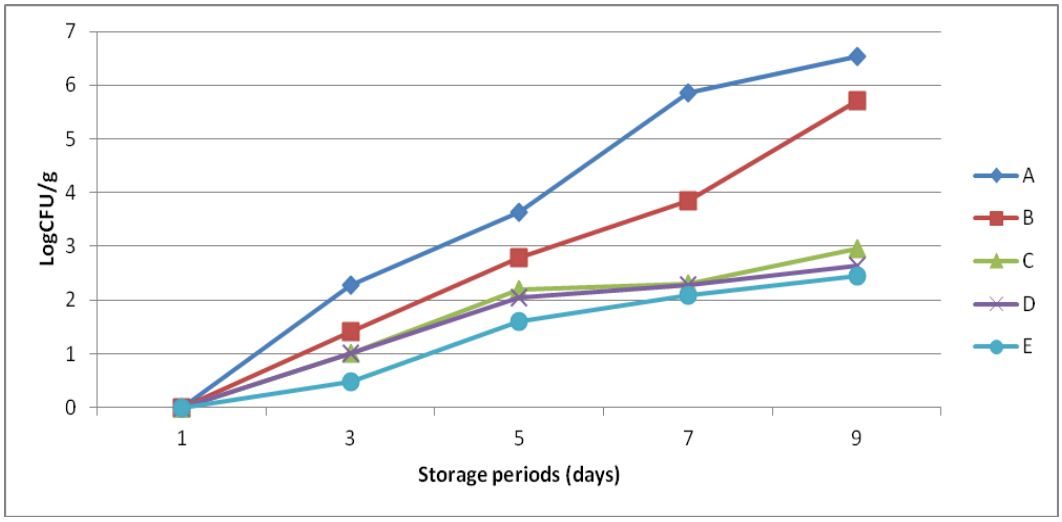

Figure 2. Total yeast/mould count on bread; A-100% W, B- 95%W: 5% T, C- 90%W:10% T, D- 85%: 15%T, E- 80%W:20%T

4. Conclusions

The tomato seed flour has shown to be potent antimicrobial agent against bacteria and mould in bread. Low percentage substitution of tomato seed in wheat flour could be recommended to serve dual purpose as preservative and composite flour in production of bread with acceptable nutritional, functional and sensory qualities. There is a need to examine the potency of other varieties of tomato seeds against microbial growth in foods.

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PROCESS OPTIMIZATION FOR THE DEVELOPMENT OF LOW FAT FRIED INDIAN TRADITIONAL SNACK USING RESPONSE SURFACE APPROACH

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ABSTRACT

The present study was conducted to optimize the process for the development of low fat fried Indian traditional snack. The effect of hydrocolloids (carboxymethyl cellulose, methyl cellulose, guar gum and gum tragacanth) on quality attributes of snack were also investigated in this study. Frying temperature (150-170 ^{0}C) and frying time (1.5-3 min.) were taken as independent variables. The optimized process parameters were found to be 150 ^{0}C of frying temperature and 1.5 minute of frying time. The maximum oil uptake reduction against control snack was observed 64.22 %, 58.59 %, 51.26 % and 49.57 % in 1% methyl cellulose, 1 % guar gum, 1 % gum tragacanth and 2% carboxymethyl cellulose added snack, respectively. Texture analysis showed that 1.5 % carboxymethyl cellulose added snack was found best crispy among all samples based on fracturability. Guar gum and methyl cellulose added snack had higher L values than control snack while reverse trend were observed in all concentrations of carboxymethyl cellulose and gum tragacanth added snack except 2 % gum tragacanth.

1. Introduction

Indian fried snacks are being popular world over. Indian snack market is highest in demand and growing very fast. It is one of the largest snack market in the Asia pacific region (MOFPI, 2011). The global market for Indian snack is huge and enormous growth is estimated up to 2020 due to increasing urbanization and gain in per capita income. The reason of popularity may be attributed to the unique taste, texture and colour of the food items. Deep-fat frying is one of the oldest and popular methods of food preparation (Varela, 1998 and Gertz, 2000). It is being used over the last 3000 years (Stier, 2004). Deep fat frying gives desired texture (crispy) and flavour to the final product, along with a reasonable shelf life enhancement due to removal of water.

Water removal during deep-fat frying was studied by Farkas, 1994. He investigated the four phases of water removal during deep fat frying viz. initial heating, surface boiling, falling rate, and bubble end point. Although deep fat frying looks simple process but it involves very complex phenomenon. Food is fully immersed in hot cooking oil (above 100°C) for a span of time. In deep fat frying, heat and mass transfer operations involve during cooking that alters the food surface and forms the crust (Ngadi and Xue, 2009). Heat transfer occurs in two different modes: conduction and convection. Convection heat transfer occurs between the oil and the food

surface whereas conduction heat transfer occurs within the food (Totosaus and Perez-chabela, 2004). Mass transfer occurs during deep-fat frying as moisture evaporation takes place in product and pores are formed (Irudayaraj, 2001). These pores are filled by oils and the resulting products absorb large amount of oil. The mass transfer phenomenon is a function of frying time and temperature (Ngadi et al., 2008). Consumption of high fat food products are said to cause obesity and more importantly many heart diseases linked with increased cholesterol level (Albert and Mittal, 2002 and Shih et al., 2005). People prefer low fat fried foods due to health issues. During deep fat frying, the oil uptake of food reaches up to 45% (Saguy and Dana, 2003). Hence, there is a broader scope of research for reduction of oil uptake in deep fat fried foods and snacks. Fat uptake is reduced in deep fried foods either by removal of surface oil before the cooling (Ouchon and Pyle, 2004) or by addition of hydrocolloids or pretreatment with coating of edible films (Albert and Mittal, 2002 and Rimac-Brncic et al., 2004). Nowadays hydrocolloids are being widely used in many food products to improve quality attributes and shelf-life (Saha and Bhattacharya, 2010). Hydrocolloids have wide range of functional properties in foods and have been used as multifunctional additives in food processing (Rimac-Brncic et al., 2004). They are used as an emulsifier, stabilizer and gelling agent (Albert and Mittal, 2002; Puvanenthiran et al., 2003; Rimac-Brncic et al., 2004 and Philips and Williams, 2009). They enhance oxidative stability (Pranoto et al., 2009), improves viscosity, water binding capacity and emulsion stability (Abd Karim et al., 1999; Aguilera and Gloria-Herna´ndez, 2000 and Bravin et al., 2006). Hydrocolloids play an important role in reducing fat uptake during the frying process. (Annapure et al., 1999; Albert and Mittal, 2002 and Singthong and Thongkaew, 2009). Texture is an important parameter for the fried food and may be affected by addition of hydrocolloids along with other variables like frying time, oil temperature, moisture content and nature of starch (Kilincceker and Hepsag, 2012). Texture plays an important role for acceptance or rejection of snacks.

The aim of this study was to optimize the process parameters for the development of low fat fried Indian traditional snack (Mathri) and investigate the effect of different hydrocolloids such as carboxymethyl cellulose (CMC), methyl cellulose (MC), guar gum (GG) and gum tragacanth (GT) on quality attributes of deep fried Indian traditional snack (Mathri).

2. Materials and methods

2.1. Raw Materials

Refined wheat flour and soybean oil (commercial brand, "Fortune") used in the experiment was procured from local market of Greater Noida, U.P., India. Carboxy methyl cellulose, methyl cellulose, guar gum and gum tragacanth were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India.

2.2. Preparation of hydrocolloids solutions

Hydrocolloids namely carboxymethyl cellulose (CMC), methylcellulose (MC) guar gum (GG) and gum tragacanth (GT) were dissolved in hot (55 to 60 ˚C) distilled water at three different concentrations of 1%, 1.5%, and 2% (Albert and Mittal, 2002).

2.3. Preparation of snack sample (Mathri)

Refined wheat flour samples were weighed and mixed with already prepared hydrocolloids solutions (carboxymethyl cellulose, guar gum, methyl cellulose and gum tragacanth) of different concentrations

(1.0%, 1.5% and 2.0%) and dough samples were made. Appropriate amount of salt and spices were added in dough samples and mixed properly. A uniform sheet of different dough samples were prepared. Sheet thickness of 2.5 mm was maintained and cut into many rectangular pieces (3x2 cm). Thus, a total of 12 samples were prepared. The rectangular pieces were fried in soybean oil at optimized conditions (by RSM) in a thermostatically temperature controlled fryer. The oil was changed after frying of each batch. Excess oil from all fried samples were drained off and allowed to cool at room temperature for further analysis.

2.4 Optimization of process parameters

A central composite rotatable design (CCRD) with two factors (frying temperature and frying time as independent variables) was selected that produced thirteen combinations of experiments. The results of these combinations of experiments were used to optimize the process parameters for the development of snack. The outline of experimental design along with corresponding results is shown in Table 1. The second order polynomial equation was used to examine the statistical significance of the model as given in equation 1. The responses (moisture, oil uptake, colour L value, colour a value, colour b value and overall acceptability) for different experimental conditions were performed and fitted in following equation as given below:

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

Where: Y is response variable; β_0 is a constant; x_i are independent variables; $\beta_{i,}$ is linear effects of regression coefficient; β_{ii} is quadratic effects of regression coefficient; β_{ij} is interaction effects of regression coefficient.

Design expert software was used for statistical analysis of experimental data.

2.5 Determination of oil uptake reduction in snack

Oil content of control and hydrocolloid added samples were determined by the soxhlet extraction unit. Reduction of oil uptake was calculated by following formula.

$$\text{Oil reduction (\%)} = \frac{A - B}{A} \times 100 \quad (2)$$

Where: A is the oil in control sample; B is the oil in hydrocolloid added sample.

2.6. Texture analysis

Penetration test of snack samples was conducted using texture analyzer (TA-Hdi), Stable Micro Systems (U.K.). A 0.25 inch diameter spherical probe was moved down onto a snack sample, which was centrally located over a circular support, up to the point of fracture. Snack samples were tested in three batches and the average of the 3 maximum peak force and distance at break/fracture values were calculated to give a measure of fracturability. The peak force from the resulting curve was considered as breaking hardness of the snack. TA settings were kept as pre-test speed 1 mm/s, test speed 0.5 mm/s, post test speed 10 mm/s, distance 7 mm, trigger force 5 g, data acquisition rate 500 pps and 50 kg load cell.

2.7 Colour analysis

Hunter lab colorimeter (Hunter lab, Reston, USA) was used for the colour determination of snack samples, which gave L, a and b values. Where; standard values referred to the $BaCl_2$ plate (L= 96.9, a=0, and b=7.2) used for calibrating the colorimeter.

3. Results and Discussions

3.1 Effect of independent variables on moisture content

Moisture is an important factor for deep fried snacks. Moisture of deep fried snack varied from 1.32 to 6.52 (Table 1). Statistical attributes are shown in Table 2. Model F-value (137.37) was found significant in the Regression model. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. The Lack of Fit F-value (2.46) implies the Lack of Fit is not significant relative to the pure error ($P > 0.05$). Non-significant lack of fit is good. There is a 20.22% chance that a "Lack of Fit F-value" this large could occur due to noise. The model (Eq. 3) as shown below was obtained for representing the variation of moisture.

$$\text{Moisture} = 2.87 - 1.28\,A - 1.34\,B + 0.26\,A^2 + 0.29\,B^2 + 0.42\,AB \quad (3)$$

Where: A is frying temperature (^{0}C); B is frying time (min.)

It was observed from the equation (3) that interaction term of frying temperature and frying time (AB) had significant positive effect on moisture and indicates concave shape variation with the change in value of variables. In this study, frying temperature and frying time both have significant negative impact on moisture content. As the frying temperature increased, there was a gradual decrease in moisture content because water reaches to its boiling point and vaporizes rapidly with increased temperature and it is transferred through the surface of the product due to pressure and concentration gradients (Achir et al., 2008). There was decrease in moisture content with increase in frying time (Figure 1a). The reason of decrease in moisture content of fried snack with increase in frying time is due to the replacement of moisture by frying oil (Prakash et al., 2015). Similar results have been reported by Rahman and Uddin (2008) in papads.

3.2 Effect of independent variables on oil uptake

Oil uptake is a crucial factor for deep fried snacks. The oil uptake of deep fried snack was found in the range of 35.32 to 45.05 % (Table 1). Significant Model F-value (93.20) of Regression model shows the fitness of model for oil uptake. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. The "Lack of Fit F-value" of 3.67 implies the Lack of Fit is not significant. There is a 12.09 % chance that a "Lack of Fit F-value" this large could occur due to noise. The model (Eq. 4) as shown below was obtained for representing the variation of oil uptake.

$$\text{Oil uptake} = 41.87 + 2.94\,A + 2.09\,B - 0.69\,A^2 - 0.22\,B^2 - 0.89\,AB \quad (4)$$

Where: A is frying temperature (^{0}C); B is frying time (min.)

It was observed from the equation (4) that interaction term of frying temperature and frying time (AB) had significant negative effect ($P < 0.05$) on oil uptake, indicates convex shape variation with the change in value of variables. Oil uptake is an important parameter for the development of low fat snack and both process variable (frying temperature and frying time) play an important role. Figure 1b depicts the significant effect on the oil uptake of fried snack. Oil uptake increased significantly ($P < 0.05$) with increasing frying temperature. This observation is consistent with previous studies (Krokida et al., 2000 and Rahman and Uddin, 2008). With increasing frying time, oil uptake increased significantly ($P < 0.05$). This is in agreement with previous studies (Pinthus et al., 1995 and Krokida et al., 2000).

Table 1. Experimental design and corresponding results using response surface methodology (RSM)

Run	Independent Variables		Responses					
	Frying temp. (^{0}C)	Frying time (Min.)	Moisture (% wb)	Oil uptake (Kg oil/kg dry solids)	Colour L value	Colour a value	Colour b value	Overall acceptability
1	150	1.5	6.52	35.32	64.26	8.08	38.63	6
2	170	1.5	3.31	42.28	53.03	12.41	36.16	8.5
3	150	3	2.88	41.58	60.72	7.8	37.7	5.8
4	170	3	1.36	44.98	50.21	14.17	31.18	7
5	145.86	2.25	5.24	35.76	62.04	8.85	37.63	6
6	174.14	2.25	1.32	45.05	45.28	15.98	32.87	8
7	160	1.19	5.16	38.6	59.05	8.75	37.28	7.5
8	160	3.31	1.52	44.06	52.9	11.33	33.6	6
9	160	2.25	2.84	41.86	58.97	9.99	36.39	6.5
10	160	2.25	3.1	41.36	60.92	10.28	37.45	6.7
11	160	2.25	2.76	41.78	59.52	9.3	36.8	6.3
12	160	2.25	2.95	42.16	60.77	10.71	36.85	6.2
13	160	2.25	2.69	42.17	59.8	10.1	36.29	6.4

Table 2. Anova for different responses (quadratic model) of snack

Source	Moisture		Oil uptake		Colour L value		Colour a value		Colour b value		Overall acceptability	
	F-value	Prob >F	F-value	Prob > F	F-value	Prob > F	F-value	Prob > F	F-value	Prob > F	F-value	Prob > F
Model	137.3655	< 0.0001	93.19867	< 0.0001	34.39549	< 0.0001	37.96947	< 0.0001	34.61579	< 0.0001	42.53102	< 0.0001
A	309.9088	< 0.0001	291.3585	< 0.0001	129.5475	< 0.0001	154.9336	< 0.0001	98.36021	< 0.0001	138.048	< 0.0001
B	338.5355	< 0.0001	146.8385	< 0.0001	14.22364	0.0070	9.434621	0.0180	49.15724	0.0002	47.2977	0.0002
A^2	10.86624	0.0132	13.87503	0.0074	23.7583	0.0018	20.62077	0.0027	8.237333	0.0240	13.88101	0.0074
B^2	13.54156	0.0079	1.485008	0.2625	7.351935	0.0301	0.584553	0.4695	5.870953	0.0459	4.192132	0.0798
AB	16.77189	0.0046	13.37502	0.0081	0.065044	0.8060	2.985418	0.1277	13.05458	0.0086	10.94787	0.0130

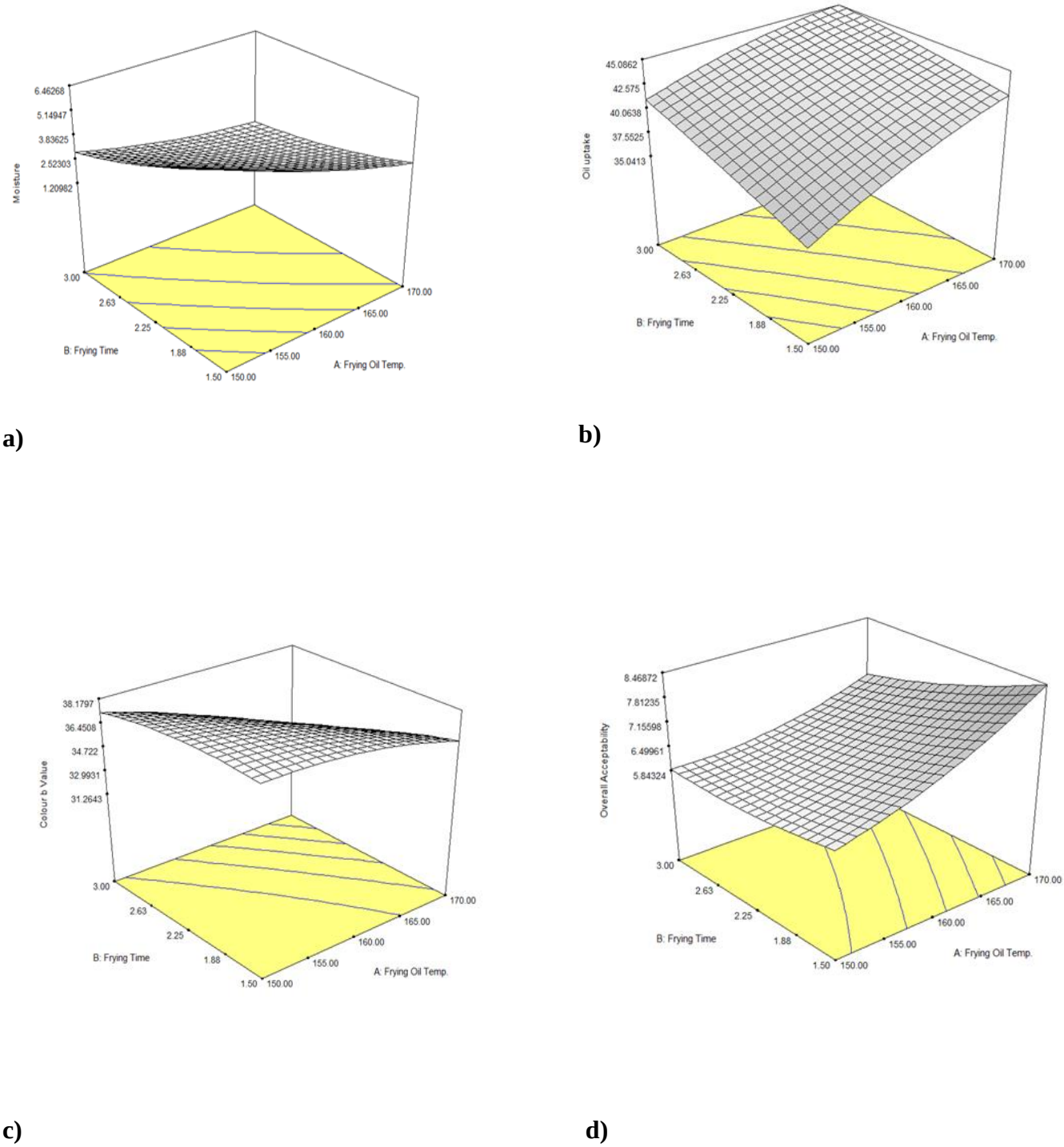

Figure 1. Response surface plot showing the effect of frying time & frying temperature on **a)** Moisture; **b)** Oil uptake; **c)** Colour b value; **d)** Overall acceptability

The increase in oil content of snack with an increase in frying temperature and time is due to the mass transfer phenomenon as water evaporates rapidly with increasing

temperature and time and frying oil replaces the moisture.

3.3 Effect of independent variables on colour L value

Colour is an important factor for deep fried snacks. The evaluated colour L value of the deep fried snack varied from 45.28 to 64.26 (Table 1). Statistical attributes of colour L value are shown in Table 2. Regression model fitted to experimental results for colour L value (Table 2) shows that Model F-value of 34.40 is significant. The "Lack of Fit F-value" of 5.38 implies the Lack of Fit is not significant. The model (Eq. 5) as given below was obtained and representing the variation of colour L value.

$$\text{Colour L value} = 60.00 - 5.68\,A - 1.88\,B - 2.61\,A^2 - 1.45\,B^2 + 0.18\,AB \quad (5)$$

Where: A is frying temperature (^{0}C); B is frying time (min.)

It was observed from the equation (5) that interaction term of frying temperature and frying time (AB) was not found significant ($P > 0.05$) on colour L value.

3.4 Effect of independent variables on colour a value

The colour a value of the deep fried snack was found in the range of 7.8 to 15.98 (Table 1). Regression model fitted to experimental results for colour a value (Table 2) shows that Model F-value of 37.97 is significant ($P < 0.001$). There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. The "Lack of Fit F-value" of 1.75 implies the Lack of Fit is not significant relative to the pure error ($P > 0.05$). There is 29.45% chance that a "Lack of Fit F-value" this large could occur due to noise. The model (Eq. 6) was selected for representing the variation of colour a value. The quadratic model obtained from regression analysis for colour a value analysis in terms of coded levels of the variables was as follows:

$$\text{Colour a value} = 10.08 + 2.60\,A + 0.64\,B + 1.02\,A^2 - 0.17\,B^2 + 0.51\,AB \quad (6)$$

Where: A is frying temperature (^{0}C); B is frying time (min.)

It was observed from the equation (6) that interaction term of frying temperature and frying time (AB) was not found significant ($P > 0.05$) on colour a value.

3.5 Effect of independent variables on colour b-value

The assessed colour b value of the deep fried snack varied from 31.18 to 38.63 (Table 1). Regression model fitted to experimental results for colour b-value (Table 2) shows that Model F-value of 34.62 is significant ($P < 0.001$). The "Lack of Fit F-value" of 2.14 implies the Lack of Fit is not significant relative to the pure error ($P > 0.05$). There is a 23.76% chance that a "Lack of Fit F-value" this large could occur due to noise. The quadratic model (Eq. 7) obtained from regression analysis for representing the variation in colour b value was as follows:

$$\text{Colour b value} = 36.76 - 1.97\,A - 1.39\,B - 0.61\,A^2 - 0.51\,B^2 - 1.01\,AB \quad (7)$$

Where: A is frying temperature (^{0}C); B is frying time (min.)

It was observed from the equation (7) that interaction term of frying temperature and frying time (AB) had significant negative effect ($P < 0.05$) on colour b value which indicate convex shape variation with the change in value of variables. Colour is an important physical characteristic of the fried snack and directly related to the acceptability of food products. Figure 1c shows the effect of frying time and frying

temperature on colour b-value. The results suggested that there was decrease in colour b-value as frying temperature and frying time increased. Similar results were also described by Sunisa et al. (2011) in fried chicken and Krokida et al. (2001b) in deep fat frying of potato strips.

3.6 Effect of independent variables on product sensory characteristics

The overall acceptability of the deep fried snack measured in the range of 5.8 to 8.5 (Table 1). Regression model fitted to experimental results for overall acceptability (Table 2) shows that Model F-value of 42.53 is significant ($P < 0.001$). There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Lack of Fit is not significant. There is a 44.61% chance that a "Lack of Fit F-value" this large could occur due to noise. The model (Eq. 8) was selected for representing the variation of oil uptake. The quadratic model obtained from regression analysis for overall acceptability in terms of coded levels of the variables was as follows:

$$\text{Overall acceptability} = 6.42 + 0.82\,A - 0.48\,B + 0.28\,A^2 + 0.15\,B^2 - 0.32\,AB \quad (8)$$

Where: A is frying temperature (^{0}C); B is frying time (min.)

It was observed from the equation (8) that interaction effect of frying temperature and frying time (AB) had significant negative effect ($P < 0.05$) on overall acceptability which indicate concave shape variation with the change in value of variables. Sensory evaluation indicates the overall acceptability of the product. Frying temperature and frying time both have significant impact on overall acceptability of the product. The figure 1d shows that there was decrease in overall acceptability as frying time increased. Absorption of excess fat, bitter taste and dark colour formation might be the reason of unacceptability. Overall acceptability slighly increased with incresed frying temperature. This might be due to development of crispness as temperature increases.

3.7 Optimization of process parameters and verification of results

A numerical multi-response optimization technique (Kashudhan et al., 2017) was applied to determine the optimized conditions of frying temperature and frying time for the development of Indian fried snack (Mathri). The optimized parameters were 150 ^{0}C of frying temperature and 1.5 minute of frying time. The responses predicted by the design expert software for these optimum process conditions resulted 6.46 % of moisture, 34.45 % of oil uptake, 63.68 of colour L value, 8.19 of colour a value, 37.97 of colour b value and 8.47 of overall acceptability with desirability 0.997. The experimental sample under the optimum process conditions resulted moisture: 3.22, oil uptake: 35.50, colour L value: 59.39, colour a value: 15.02, colour b value: 32.23 and overall acceptability: 8.0.

3.8 Effect of hydrocolloids on oil uptake reduction of snack during deep frying

Table 3 and 4 show data on moisture in fried product, absolute oil uptake during frying and relative oil uptake reduction in hydrocolloid containing fried snack as compared to control. Moisture content, absolute oil uptake and relative oil uptake reduction of hydrocolloids at different concentrations were found varying in the range of 1.52 ± 0.52 to 6.55 ± 0.12 (%), 0.127 ± 0.006 to 0.291 ± 0.02 (kg oil/kg dry solids) and 18.02 ± 0.64 to 64.22 ± 0.27 (%), respectively.

Table 3. Effect of carboxy methyl cellulose, methyl cellulose, guar gum and gum tragacanth on product moisture and oil uptake of snack

	Product moisture* (%, wb)				Oil uptake* (kg oil/kg dry solids)			
Control Sample	3.22				0.355			
Experimental Samples	CMC	GG	GT	MC	CMC	GG	GT	MC
1% HC	3.27 ± 0.05	6.55 ± 0.12	4.32 ± 0.07	4.84 ± 0.07	0.236 ± 0.005	0.147± 0.006	0.173± 0.006	0.127± 0.006
1.5% HC	3.61 ± 0.07	4.71 ± 0.08	3.21± 0.03	1.97 ± 0.32	0.223 ± 0.006	0.163± 0.006	0.183± 0.006	0.266± 0.04
2% HC	4 ± 0.02	4 ± 0.02	2.83± 0.15	1.52 ± 0.52	0.179 ± 0.009	0.217± 0.005	0.234± 0.008	0.291± 0.02

*Mean ± SD

Table 4. Oil uptake reduction due to carboxy methyl cellulose, methyl cellulose, guar gum and gum tragacanth

	Reduction in oil uptake (%)			
Experimental Samples	CMC	GG	GT	MC
1% HC	33.52 ± 1.44	58.59 ± 2.05	51.26 ± 1.60	64.22 ± 0.27
1.5% HC	37.18 ± 1.12	54.08 ± 0.95	48.45 ± 0.71	25.07 ± 0.41
2% HC	49.57 ± 0.81	38.87 ± 1.10	34.08 ± 0.85	18.02 ± 0.64

*Mean ± SD

Table 5. Effect of hydrocolloids on textural value of snack

	Textural Parameters							
	Fracturability* (g.sec)				Hardness* (g)			
Control Sample	5029.76				4734.84			
Experimental Samples	CMC	GG	GT	MC	CMC	GG	GT	MC
Level of HC 1%	4673.16	4901.01	5664.88	5263.46	4527.61	4112.89	3425.96	2919.43
Level of HC1.5%	7379.97	3494.11	6374.36	2896.30	2677.96	2420.53	4363.57	2528.26
Level of HC 2%	5910.90	3850.75	7124.74	4071.63	4164.54	3229.96	5126.24	1424.58
ANOVA (Row)	Significant F(Calculated) = 3.49 < F (Critical) = 3.31, p > 0.05				Significant F (Calculated) = 4.20 > F (Critical) = 3.40, p < 0.05			
ANOVA (Column)	Significant F (Calculated) = 17.69 > F (Critical) = 2.68, p < 0.05				Significant F (Calculated) = 19.45 > F (Critical) = 3.0, p < 0.05			
ANOVA (Interaction)	Significant F (Calculated) = 6.45 > F (Critical) = 2.26, p < 0.05				Significant F (Calculated) = 6.51 > F(Critical) = 2.50, p< 0.05			

*Mean of three replications

Table 6. Effect of hydrocolloids on colour value of snack

	Colour Parameters											
	L value*				a value*				b value*			
Control Sample	59.3933				15.0266				32.23			
Experimental Samples	CMC	GG	GT	MC	CMC	GG	GT	MC	CMC	GG	GT	MC
Level of HC 1%	57.38	62.38	54.36	61.20	15.32	13.55	14.32	13.74	32.42	34.15	23.25	32.40
Level of HC1.5%	45.80	62.26	53.17	67.46	12.36	12.43	16.53	9.9	16.62	26.09	31.61	30.10
Level of HC 2%	54.88	65.23	66.38	60.79	15.74	12.78	13.50	13.35	31.95	35.35	31.77	29.40
ANOVA (Row)	Significant F(Calculated) = 64.52> F (Critical) = 3.40, p< 0.05				Significant F (Calculated) = 35.44 > F (Critical) = 3.40, p< 0.05				Significant F(Calculated) = 55.89 > F (Critical) = 3.4, p<0.05			
ANOVA (Column)	Significant F (Calculated) =221.50> F (Critical) = 3.0, p< 0.05				Significant F (Calculated) = 68.91> F (Critical) = 3.0, p< 0.05				Significant F (Calculated) = 19.40> F (Critical) = 3.0, p< 0.05			
ANOVA (Interaction)	Significant F (Calculated) = 82.57 > F (Critical) = 2.50, p< 0.05				Significant F (Calculated) = 45.89 > F (Critical) = 2.50, p< 0.05				Significant F (Calculated) = 44.74 > F (Critical) = 2.5, p< 0.05			

*Mean of three replications

All the carboxymethyl cellulose added samples were found able to retain higher moisture than control sample. A significant reduction in fat absorption was observed with increased concentration of carboxymethyl cellulose. The fat uptake reduction was found maximum 49.57±0.81 % in 2% CMC containing snack and best moisture retention (4±0.02 %) was also found in same sample. Garmakhany et al. (2011) obtained a maximum fat reduction of 65.1±0.05 % with 1 % CMC coated potato french fries which could be due to different product. Guar gum added snack samples were found to be effective in moisture retention than control sample. As the concentration of guar gum increased, the reduction of fat uptake was found decreased. This might be due to film formation of hydrocolloids on the product which helps to retain the natural moisture and prevents the absorption of oil in deep fried samples (Annapure et al., 1999 and Sakhale et al., 2011). Best moisture retention was found in 1 % guar gum (6.55±0.12 %) containing snack and maximum oil uptake reduction (58.59±2.05 %) was also found in 1 % guar gum containing snack sample as expected. Sakhale et al. (2011) also reported 43.72% reduction in oil uptake in casing of samosa with 1.5 % guar gum, which could be caused by different frying temperature and time. Gum tragacanth was also found an effective fat replacer. It was observed that with increase in level of hydrocolloid, there was significant decrease in moisture content as shown in Table 3. That is why oil uptake reduction was also found decrease with increase of hydrocolloids. The maximum moisture retention (4.84±0.07 %) and fat uptake reduction (51.26±1.60 %) was found in 1% gum tragacanth containing snack sample. This could be due to lower moisture loss of hydrocolloid containing samples during frying hence, lower oil uptake. Zolfaghari et al. (2013) obtained 33% oil uptake reduction with (1% w/w) gum tragacanth coating in donuts. All the methylcellulose added snack samples were found to be effective in moisture retention except 1.5%. Maximum fat uptake reduction was found in 1% methylcellulose added snack (64.22±0.27 %) because of having maximum moisture content (4.84±0.07 %). Albert and mittal (2002) reported similar result with 58.2 ± 6.3% reduction in fat uptake in methyl cellulose coated deep-fried cereal products. This is in agreement with Williams and Mittal (1999) who also reported the effect of methyl cellulose on oil uptake reduction of fried cereal products, fried potato balls and deep-fat fried poultry product respectively.

3.9 Effect of hydrocolloids on textural attributes of snack

Effect of different concentrations of hydrocolloids on hardness and fracturability of developed snacks are given in Table 5. Hardness and fracturability were analyzed for the carboxymethyl cellulose, methylcellulose, guar gum and gum tragacanth added snack samples at different concentrations. Hardness of carboxymethyl cellulose, methylcellulose, guar gum and gum tragacanth added snack samples varied between 2677.96 to 4527.61, 1424.58 to 2919.43, 2420.53 to 4112.89 and 3425.96 to 5126.24, respectively as against 4734.84 for control snack samples. Fracturability of carboxymethyl cellulose, methylcellulose, guar gum and gum tragacanth added snack samples varied between 4673.163 to 7379.973, 2896.303 to 5263.46, 3494.11 to 4901.013 and 5664.887 to 7124.747, respectively as against 5029.76 for control snack samples. Hardness value of all carboxymethyl added samples were

observed less than control samples. 1.5% carboxymethyl added sample had the lowest hardness value among all carboxymethyl added samples. Methyl cellulose added snack samples showed the less hardness value than control sample. Results indicate the decreased hardness with increased hydrocolloid concentrations. 2% methyl cellulose added snack sample had the lowest value among all methyl cellulose added snack samples. The effect of addition of guar gum on textural attributes of snack samples was also observed. The hardness of guar gum added samples were also found less than control sample. Gum tragacanth added snack samples showed the lower hardness value than control sample except 2% gum tragacanth added sample.

Thus, 2% methylcellulose was found the most fracturable and crispy among all hydrocolloids containing snack samples. Final product texture and quality could be affected by interactions between proteins and starch fractions (amylose and amylopectin) found in food composition of product (Rovedo et al., 1999).

3.10 Effect of hydrocolloids on colour attributes of snack

The effect of different hydrocolloids on colour quality of snack samples are given in Table 6. Lightness (L), redness (a) and yellowness (b) were observed to determine colour changes during frying. The results showed that redness among all carboxymethyl cellulose added samples were not significantly different ($p \leq 0.05$). Likewise, lightness and yellowness of all carboxymethyl cellulose added samples were not different significantly ($p \leq 0.05$), except for 1.5%. Yellowness of all methylcellulose added samples were not found significantly different with control. The values of Lightness and redness were also not significantly different with control except for 1.5% methylcellulose. Lightness and redness values of guar gum added samples were not found significantly different with control sample. Redness value of gum tragacanth added samples were also not significantly different, while lightness value for 2% gum tragacanth and yellowness value for 1% gum tragacanth sample was found significantly different with control sample. The highest L value was observed with 1.5% methylcellulose, 2% gum tragacanth and 2% guar gum while lowest L value was observed with 1.5% carboxymethyl cellulose added samples. Highest and lowest a value was found with 1.5 % gum tragacanth and 1.5% methylcellulose, respectively. Highest and lowest b value was observed with 2% gum tragacanth and 1.5% carboxymethyl cellulose, respectively.

4. Conclusion

In the first part of this study, process parameters (frying temperature and frying time) were optimized to develop low fat fried Indian snack using response surface methodology. The optimized conditions were found to be 150 ^{0}C of frying temperature and 1.5 min. of frying time. In the second part of this study, snacks were developed using above mentioned optimized conditions and the effect of different hydrocolloids (carboxymethyl cellulose, methylcellulose, guar gum and gum tragacanth) were studied in terms of oil uptake reduction, textural attributes and colour attributes of the snack. Maximum oil uptake reduction was observed in 1 % methyl cellulose (64.22 %) followed by 1 % guar gum (58.59 %), 1 % gum tragacanth (51.26 %) and 2 % carboxymethyl cellulose (49.57 %) added snack. Hydrocolloids added snack were studied for texture analysis in terms of fracturability and hardness. 1.5 % carboxy methyl cellulose added snack was concluded best crispy among all samples based on fracturability.

1.5% GT added snack showed highest redness among all hydrocolloid added samples. All concentrations of gum tragacanth added snack samples were found to be best in sensory test. This might be due to high crispness and low hardness. It can be concluded that 2 % gum tragacanth added snack was best in textural properties, colour and sensory attributes because gum tragacanth provided highest crispness, good colour value and excellent overall acceptability but in terms of oil uptake reduction 1% methyl cellulose and 1% guar gum produced best result.

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THE PRODUCTION OF PROBIOTIC SCALLION YOGURT: VIABILITY OF *LACTOBACILLUS ACIDOPLILUS* FREELY AND MICROENCAPSULATED IN THE PRODUCT

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ABSTRACT

Two forms of probiotic Scallion yogurt as a new dairy probiotic product, with free and microencapsulated bacteria, were produced in the same circumstances. Extrusion method was used for microencapsulation. Pour plate method was used to culture bacteria in MRS-S-agar medium in two replications. The pH value, acidity, fat percentage, protein percentage and dry matter of products were measured according to Iran national standards. Sensory properties were measured by using 32-member taste panel. The results showed that the viability of the free and microencapsulated bacteria reached a 4.61 log cfu g-1 from 8.48 log cfu g-1 and 6.11 log cfu g-1 from 7.82 log cfu g-1 respectably during 42 days. The reduction of bacterial count in the Scallion yogurt containing free cells was significantly ($p<0.05$) higher than the one containing microencapsulated cells. The acidity and pH measurements showed that the most acidity and the lowest pH value (108.1; 3.88) belonged to the free bacteria sample, and microencapsulated sample had the lowest acidity and the highest pH (99.48; 4.21). Sensory examinations showed no significant differences between two types of Scallion yogurt ($p>0.05$). Microncapsulation of *L. acidophilus* with calcium alginate and resistant starch is able to effectively maintain the count of this probiotic high enough for the therapeutic minimum in the scallion yogurt (10^7 cfu g-1).

1. Introduction

Consumption of functional foods, type of foods which possess medicinal and nutritional value and improve intestinal flora and lead to their increased health effects on the body, is favored greatly nowadays. To attain this goal, it is necessary for a Probiotic-like product to maintain the probiotic which is present in it at the defined level and possess proper textural properties. Today, acceptability and consumption of probiotic products are extremely widespread in the world, especially in Europe, America and Japan (Larisch et al., 1994; Mohan et al., 2016; Tomás et al., 2015; Li et al., 2016). Thus, dairy products such as yogurt and other dairy probiotic products have

been increasingly studied and produced (Krasaekoopt et al., 2003). Foodstuffs containing probiotics fall inside the ‘‘functional foods’’ collection and these foodstuffs must contain as a minimum 107 cfu g-1 probiotic microorganism and consumed at levels higher than 100 g/day to have supportive outcomes on healthiness (Mirzaei et al., 2012; Sultana et al., 2000). Probiotic yogurt is known as a good functional food for all age groups, and adding some beneficiary edible vegetables like Scallion in it can improve its nutritional value and acceptability. Scallion (Allium fistulosum) is a species of perennial onion originated in Southeast Asia, which was spread to Europe and North America (Janick, 2001). This root plant used both as a food and medicinal applications. Scallion is rich in thiosulfinates, volatile sulfur compounds and phenolic and steroidal origin compounds which are used in the treatment and prevention of several kinds of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (Lanzotti, 2006). Nowadays Scallion yogurt is produced industrially as a popular and favorable kind of yogurt in Iran. Homemade scallion yogurt is also consumed in Europe and North America.

Low viability of probiotics in different conditions of food products and acidic-biliary conditions of the digestive system has encouraged researchers to find ways to improve these indicators. From the perspective of microbiology, microencapsulation is a process in which tiny alive cells are surrounded by a coating of hydrocolloid in microscopic scale and enclosed in order to be separated from the environment and finally these cells have a targeted release in the appropriate time and place (Mirzaei et al., 2012; Tamnak et al., 2016; Krasaekoopt et al., 2003). This release can happen by changing factors such as pH, mechanical stress, thermal, enzymatic activity, time, osmotic pressure, slow release of moisture inside the capsule and the presence of chemical components. Therefore, microcapsules act as the factors to separate cells from environmental and immunize them from inappropriate conditions such as high activity, bile salts, severe freezing or freeze-drying, molecular oxygen, bacteriophages, and chemical antibacterial components and consequently prevent damage and cell loss, also other objectives can be mentioned such as stabilization or improvement in the sensory properties of products and making the cells immovable (Kailasapathy, 2002). It should be noted that Lactobacillus has the most survivability in the fermented dairy products (Vinderola and Reinheimer, 2000). Different types of yogurt can be used as carriers of probiotics because they are widely used and popular products are used by all segments of society. So far, some studies have been conducted in connection with the design and production of different types of probiotic yogurt (Sultana et al., 2000; Krasaekoopt and Watcharapoka, 2014; Bosnea et al., 2017b). Generally, the main goal of microencapsulation of probiotics in yogurt is to enhance survivability of probiotics in the products and to provide the possibility of transferring and safely releasing them in the appropriate place in the gastrointestinal that it has been studied about different types of probiotic bacteria such as *Lactobacillus casei* (Ohashi et al., 2004; Krasaekoopt and Watcharapoka, 2014; Li et al., 2016). Recent developments about microencapsulation of probiotics are in the direction of both industrial consumption and mentioned goals and solve many problems about viability of probiotic organisms in the products and also in the digestive system and increase the safety of these products. Low survivability of probiotic bacteria in yogurt is attributed to low pH in yogurt and post-acidification during the storage period. It seems that microencapsulation is the most promising technology to protect bacteria cells from environmental effects (Kailasapathy, 2002;

Marshall and Tamime, 1997; Alver et al., 2017; Pitigraisorn et al., 2017; Krasaekoopt and Watcharapoka, 2014; Ghorbani-Choboghlo et al., 2015). The aim of this research is to study the viability of probiotic *Lactobacillus acidophilus* and its effect on acidification after production, textural stability and overall acceptability of scallion yogurt for the first time. Probiotic Scallion yogurt can be introduced as a beneficial dairy product for all age groups.

2. Materials and methods

2.1. Essential materials

MRS agar and MRS-broth (scharlau) culture medium, Salicin (merk, Germany), Tween 80 (merk, hohenbrunn, Germany), and other required materials for chemical tests were purchased from Merk Company. Commercial bacterial culture used for YC-X11 yogurt including combined culture (CHR-HANSEN) containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and one strain culture of probiotic bacteria *L. acidophilu*s were procured from Iran Scientific and Industrial Organization (PTCC 4356: 1643).

2.2. Microencapsulation of probiotic bacterium

Extrusion method was used for microencapsulation. Starter culture is usually offered to market as a freeze-dried culture. In order to activate it, 1 g starter uniformly mixed in 100 ml MRS-agar and at 37 °C was incubated for 24 hours, then by absorbing water, it exits from incubation and enters exponential growth phase. Then it was used to purify the desired bacteria. For this purpose, the culture medium was thoroughly stirred to be homogeneous state. Then, under the hood and sterile conditions, it was transferred by 1ml pipette into each of 20 microtubules and then was centrifuged at 5000 rpm for 10 min. After centrifuging, surface liquid was removed. Sediment of bacteria was centrifuged twice by saline to be rinsed thoroughly (Pourjafar et al., 2016; Kailasapathy, 2002).

From these 20 microtubes containing emulsion, 10 microtubes were used for direct inoculation to scallion yogurt and 10 microtubes for use in microencapsulation. 8 g alginate sodium was added to distilled water, after 24 hours 8 g resistant starch was added to it. Then 10 microtubes which had 0.5 McFarland *L. acidophilus* bacteria were used for encapsulation. About 0.5 ml tween 80 was added and then the mixture slowly was added into 0.1 mol L-1 calcium chloride solution. Alginate with exposing to calcium ions caused to shape capsule walls and droplets are deposited in the form of beads in calcium chloride solution (Mirzaei et al., 2012; Pasukamonset et al., 2016).

2.3. Preparation probiotic scallion yogurt containing free and microencapsulated *L. acidophilus* bacteria

Cow milk was prepared from Pars Moghan dairy farming and homogenized in two phases with 1.5 percent cream with 75% fat, 2% skimmed milk free of antibiotics (prepared from Tehran Pegah factory) preheated in the tank at 60 °C and a pressure of 150 bars. Then it is heated at 90 °C for 30 min and after cooling to 45 °C mixed with starter culture and incubated to reach a desired acidity to 80 degrees Dornic and pH 4.5 (usually 3-4 h). Free and encapsulated bacteria was separately inoculated into three pounds cast at 42 °C and stirred, after 15-20 min incubation, salt and granular shallots added and then at 32 °C packaged and transferred to cold storage (Standard, 2008; Krasaekoopt et al., 2003; Ansari et al., 2017).

In order to examine viability of *L. acidophilus,* three types of scallion yogurt were produced in the same conditions that the first sample contains free *L. acidophilus* bacteria, the second sample contains microencapsulated *L. acidophilus* bacteria, and the third sample consider as control sample and without

bacteria. The produced yogurt sample was kept at 4 °C for 6 weeks and its pH, acidity, score of *L. acidophilus* was tested within seven days, but its dry weight, fat percentage, protein and sensory properties were measured every two weeks for 6 weeks about each three yogurts.

2.4. Evaluation of viability of *L. acidophilus*

In this research, the count of *L. acidophilus* in two groups of Scallion yogurt containing free and microencapsulated bacteria was evaluated within 42 days of storage every 7 days by pouring plate method in MRS-S-agar culture medium (37 °C for 72 h). Evaluation of viability of *L. acidophilus* in free form 1 gr of yogurt containing free *L. acidophilus* was diluted in 9 ml sterile peptone water (0.1%) to reach a concentration of 10-3 to 10-12. After dilution, pour plate method was used to culture in MRS-S-agar medium in two replications (0.01 g salicin for per 100 ml medium) (Mirzaei et al., 2012; Shah, 2000).

For counting live microencapsulated bacteria in yogurt, at first bacteria was released from bead and then cultured. For this purpose, 10 g yogurt sample was stirred in 100 ml PBS (Phosphate Buffered Saline) 0.1 mol L-1, with pH 7 for 3 min until the beads were solved and bacteria were released and 1 ml of them were incubated to reach desired dilution and cultured in MRS-S-agar medium (at 37 °C for the period of 72 h) (Pourjafar et al., 2012; Mirzaei et al., 2011).

2.5.Evaluation of physical-chemical properties

The pH value of samples was determined using a pH meter and acidity was measured by Dornic degree using 0.1 normal sodas and phenolphthalein. Fat percentage measured by using Gerber method. Measuring was according to Iran National Standard to the number of 366.384 (Standard, 2008). In determination of dry weight, measuring was according to Iran National Standard to the number of 637 (Standard, 2008).

Protein percentage measured by using Kjeldahl method. First, foodstuff was boiled in sulfuric acid in the presence of two catalysts. The heat accelerated digestion. Then the present nitrogen in the solution (produced in the first stage) was released as ammonia gas which is in the form of steam. After passing through the refrigerant as it became liquid and entered to the flask containing buric acid, and ammonium borate was formed. Finally, the ammonium borate formed in the previous stage was titrated with hydrochloric acid (0.1 normal) (Standard, 2008). Amount of protein percentage was calculated by following formulas.

(14×100×volume of consumer acid×acid normality) / (sample weight (g)×100) (1)

(0.14×6.38 (V1-V2)) / P= the percentage of total nitrogen (2)

2.6. Evaluation of sensory properties

Sensory properties were measured by using panel taste of 32 people under the terms of the same place, light and containers and in similar days with bacterial counting. In this method, a questionnaire with three aspects for scoring on color and appearance (1-5 scores), texture (1-5 scores), smell and taste (1-10 scores), and total score (1-20) was applied (Hekmat and Reid, 2006; Kailasapathy, 2006).

2.7. Statistical analysis

This experiment was conducted by using CRD (Completely Randomized Design) with three treatments and seven replications. Statistical model used in the test is as follows:

$$y_{ij} = \mu + CA_i + R_j + \varepsilon_{il} \quad (3)$$

All of the collected data related to the test were transferred to Excel software and classified and then analyzed by SPSS 19 software. The results of test analyzed statistically using GLM (General Linear Model). T test and F test were used for comparison groups. Also, in section

3.3., data obtained by using One-way ANOVA were tested.

3.Results and discussions

3.1 The results of comparing viability between free and microencapsulated bacteria

Viability of *L. acidophilus* was evaluated in two groups of scallion yogurt containing free and microencapsulated forms of this bacterium. The statistical difference between the two groups of data was evaluated using t-test. There is a significant difference between the two groups with 95% confidence. Thus, the null hypothesis is rejected. The results showed that the algorithm for the viability of bacteria was decreased in both groups of yogurt containing free probiotic and encapsulated bacteria during 6 weeks (Fig. 1). Free bacteria were decreased 4 logs compared to the first day while encapsulated bacteria were decreased just 1 log. Comparing these two groups showed that encapsulated bacteria has significantly higher viability than free bacteria ($p<0.05$).

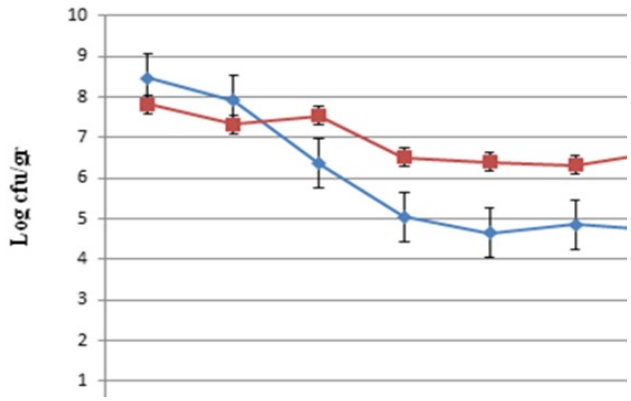

Figure 1. Comparing survivability of free and microencapsulated *Lactobacillus acidophilus* (A= Free Bacteria, AC= Microencapsulated bacteria).

Bosnea et al. (2017), Shori (2017), Kailasapathy (2006), Krasaekoopt et al. (2003) reported that the viability of encapsulated bacteria in yogurt is more than free ones. On the other words, microencapsulation improves viability of probiotic bacteria in the acidic conditions of yogurt. Therefore, it is the best way to transfer bacteria in probiotic food. At present, the evidences show that microencapsulation have an important role to protect bacteria in acidic food and conditions like yogurt (Shori, 2017; Krasaekoopt et al., 2003; Kailasapathy, 2006; Bosnea et al., 2017a).

3.2 Physical and chemical properties of scallion yogurt

In this section data of the three groups was compared in order to determine that physical and chemical characteristics of the three groups had a significant difference during 6 weeks of scallion yogurt storage.

The results of comparing pH between three different groups

There is a significant difference between free bacteria sample with encapsulated and control samples ($p<0.05$) but there is not a significant difference between encapsulated and control samples ($p>0.05$). On the other hand, during 42 days of storage of scallion yogurt, the acidity and pH measurements showed that the most acidity and the lowest pH value (3.88-108.1), respectively belonged to the free bacteria sample and the lowest acidity and the highest pH (4.21-99.48), respectively, belonged to the microencapsulated sample. (Fig. 2 and 3). In the free sample, *L. acidophilus* cooperated with the starter of yogurt and caused to enhance pH compared to other samples. In the microencapsulated sample, bacteria trapped inside the capsule had no effect on pH of yogurt. The control sample had no probiotic bacteria, consequently had no bacteria which produced *L. acidophilus* and had lower pH than free sample.

The results of comparing the fat percentage, protein percentage and dry weight between three groups of scallion yogurt

The results showed that there is no significant difference between two groups with 95%

confidence. Thus, the null hypothesis is confirmed.

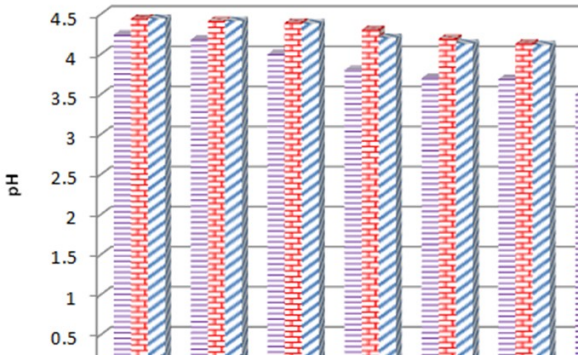

Figure 2. Evaluating the pH for free, microencapsulated and control samples during 6 weeks (A=Samples with free bacteria, AC= Samples with microencapsulated bacteria, O= Control samples).

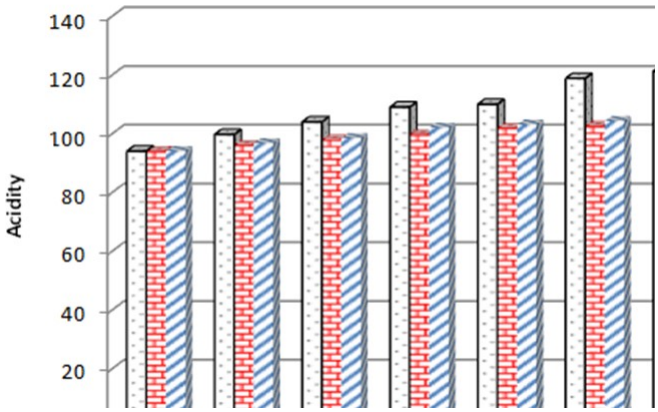

Figure 3. Evaluating acidity for free, microencapsulated and control samples during 6 weeks (A=Samples with free bacteria, AC= Samples with microencapsulated bacteria, O= Control samples).

3.3 Sensory properties of scallion yogurt

During 42 days of yogurt storage, there was a significant difference in total sensory properties in three groups. But after 5 weeks,

the sample scallion yogurt containing microencapsulated bacteria evaluated more desirable (Fig. 4 and 5). Scallion yogurt containing microencapsulated *L. acidophilus* has the most desirable texture and the control sample without *L. acidophilus* has the most desirable flavor and color during 42 days.

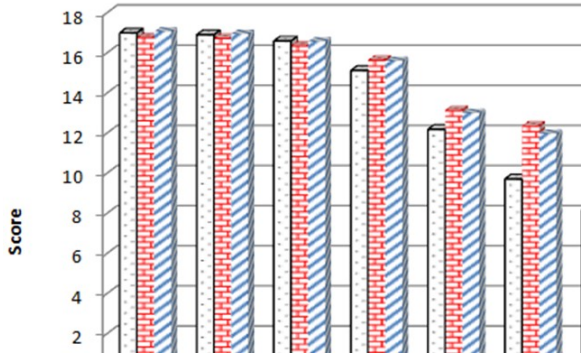

Figure 4. Evaluating the effect of *Lactobacillus acidophilus* on sensory properties of yogurt during6 weeks of storage (A=Samples with free bacteria, AC= Samples with microencapsulated bacteria, O= Control samples).

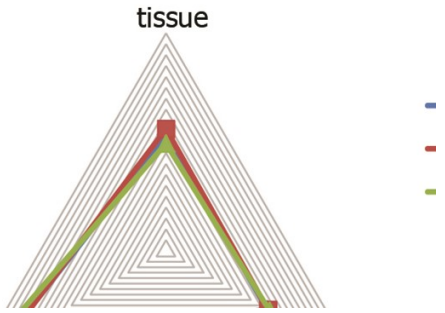

Figure 5. Sensory properties of yogurt during6 weeks of storage (A=Samples with free bacteria, AC= Samples with microencapsulated bacteria, O= Control samples).

Kailasapathy (2006) in a study about yogurt reported that viability of microencapsulated *L. acidophilus* was increased 2 logarithmic cycles and viability of microencapsulated *Bifidobacterium lactic* was increased 1 logarithmic cycle. These bacteria were staying away from acidic conditions of yogurt due to protect bacteria in capsules. The encapsulated bacteria were decreased only 2 logarithmic

cycles. Generally, it could be said that microencapsulation plays an important role in the survivability of probiotic bacteria while it has not much impact on sensory properties ($p<0.05$). There was not a significant difference between free and microencapsulated samples while the smoothness of free sample evaluated more desirable (Kailasapathy, 2006).

4. Conclusions

In this study, probiotic *L. acidophilus* bacterium was used to produce probiotic scallion yogurt. According to the consequences of this study, encapsulation of *L. acidophilus* with calcium alginate and resistant starch is able to effectively maintain the count of this probiotic microorganism high enough for the therapeutic minimum in the scallion yogurt and can serve as an excellent carrier for delivering the probiotic bacterial cells into the human being gut. Also, the results of this study could be used by the dairy product factories for optimizing the quality of their products.

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EFFECTS OF POLYETHYLENE AND BIODEGRADABLE STARCH-BASED MULCHING FILMS ON EGGPLANT PRODUCTION IN A MEDITERRANEAN AREA

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ABSTRACT

Traditional plastic films are used for eggplant cultivation in open field in Mediterranean environments. However, disposal and/or recycle of mulches contaminated with soil and/or vegetation represent an environmental concern. We compared two traditional plastic mulches (transparent and black polyethylene films) with five black biodegradable mulching films (Mater-Bi T12A, Mater-Bi T12B, Mater-Bi T15A, Mater-Bi T15B and Mater-Bi T15C) having different thickness and synthetic composition. 'Birgah' eggplant F_1 hybrid plug-plants were transplanted on 2nd May, 2016 in the north-western coast of Sicily. Air temperature in the soil-mulch film gap in the plots with transparent PE was higher than in the plots with the black PE film, biodegradable mulches and control. However, air temperature in the plots with Mater-Bi T12A and Mater-Bi T15B were 0.5-9.7 °C higher than those of the bare soil between 11.00 and 18.00 HR. Percent soil exposure (PSE) for transparent and black PE films were 6.7 and 7.7%, respectively by the end of the cultivation period. While, PSE for Mater-Bi T12A and Mater-Bi T15B was lower than other biodegradable mulching treatments and achieved 63.3 and 63.3%, respectively. Transparent PE film significantly increased plant height at 50 DAT by 14.9%, marketable yield per plant by 6.1%, average fruit weight by 11.1% compared with black PE film, Mater-Bi T12A and Mater-Bi T15B. No significant differences were found among transparent PE, black PE, Mater-Bi T12A and Mater-Bi T15B treatments for TSS and CA content. Mater-Bi T12A and Mater-Bi T15B mulches should be taken in consideration for eggplant production in Mediterranean environments.

1. Introduction

Eggplant (*Solanum melongena* L.) ranks among the world top ten most important vegetable crops with a global production of about 50 million tons per year, and a net value of more than US$10 billion a year, which makes it the fifth most profitable Solanaceous crop after potato, tomato, pepper, and tobacco (FAO, 2016). Sicily is one of the main eggplant production area within the Mediterranean Basin that is considered a secondary center of diversification (D'Anna and Sabatino, 2013). In the Mediterranean region eggplant is mostly cultivated in open field during spring-summer or under unheated greenhouses for early production. Since, improving quantity

and quality fruit yield is one of the main objective for vegetable growers, different agricultural practices have been proposed for improving eggplant production such as grafting (Rouphael et al., 2010; Sabatino et al., 2013; Sabatino et al., 2016; Sabatino et al., 2018; Rouphael et al., 2018), soilless cultivation (Kittas et al., 2006) and soil mulches (Adamczewska-Sowińska et al., 2016). Polyethylene mulching has been used in agriculture for more than 60 years to improve plant performance thanks to its benefits in reducing weed pressure and herbicide use, moderating soil temperature, and conserving soil moisture (Emmert, 1957; Ibarra-Jimenez et al., 2006; Kasirajan and Ngouajio, 2012; Lamont, 2005). Recently, Adamczewska-Sowińska et al. (2016), in a five years experiment, demonstrated that mulching the soil with polyethylene black or transparent films, increased eggplant fruit cropping compared to mulching the soil with black polypropylene textile or control (bare soil). However, despite its benefits, used polyethylene mulch is difficult to dispose and/or recycle at the end of cultivation as it is contaminated with soil and/or vegetation (up to 50% by weight) (Kasirajan and Ngouajio, 2012). A solution to this problem has been the introduction in agriculture of films produced with biodegradable raw materials such as starch (Bastioli, 1998; Lörcks, 1998), that is biodegraded in the soil due to the action of micro-organisms such as bacteria, fungi and algae. On the other hand such materials must be functional during the period of crop cultivation (Briassoulis, 2004; Tocchetto et al., 2002). Thus, the objective of the current work was to compare black and transparent polyethylene films (traditional plastic mulches) with five biodegradable mulching films in an eggplant cultivation conducted in open field during spring-summer season in the western coast of Sicily.

2. Materials and methods

2.1. Plant material and growing conditions

The experiment was conducted at the experimental farm of the Department of Agricultural, Alimentary and Forest Sciences of Palermo (SAAF) (longitude 13°19′E, latitude 38°09′N) in the northern coast of Sicily (Italy). The trail was carried out in a sandy clay loam soil (46.5% sand, 22.3% silt, 31.2 clay) at pH 7.2.

Table 1. Mulch treatment tested in 'Birgah' eggplant grown at the experimental farm of the Department of Agricultural, Alimentary and Forest Sciences of Palermo (SAAF) in the northern coast of Sicily (Italy).

Mulch treatment	Manufacturer	Color	Thickness (mm) (*)	Key product ingredient(s)(*)
Transparent PE	Agripolyane, Saint-Chamond, France	Transparent	0.015	Polyethilene
Black PE	Agripolyane, Saint-Chamond, France	Black	0.015	Polyethilene
Mater-Bi T12A	Novamont, Novara, Italy	Black	0.012	Mater-Bi (starch-copolyester blend)
Mater-Bi T12B	Novamont, Novara, Italy	Black	0.012	Mater-Bi (starch-copolyester blend)
Mater-Bi T15A	Novamont, Novara, Italy	Black	0.015	Mater-Bi (starch-copolyester blend)
Mater-Bi T15B	Novamont, Novara, Italy	Black	0.015	Mater-Bi (starch-copolyester blend)
Mater-Bi T15C	Novamont, Novara, Italy	Black	0.015	Mater-Bi (starch-copolyester blend)

(*)Information was obtained from each mulch manufacturer.

The soil was prepared by making a medium-deep plowing (35 cm) and a reduction of the earth aggregates achieved by mechanical rotating means. On 2nd May, 2016, five biodegradable mulching films and two traditional plastic mulches (black and transparent polyethylene films) (Table 1) were installed and eggplant F_1 hybrid (violet globose shape) plug plants were transplanted in single row 1.0 m apart.

In row spacing was 0.50 cm (2 plants m^{-2}) and drip irrigated. Plots were eight rows wide and 10 m long. During the cultivation period the crop received, by drip irrigation system 250 kg nitrogen ha^{-1}, 150 kg phosphorous pentoxide ha^{-1} and 250 kg potassium oxide ha^{-1}. The fertilization was calculated on the basis of theoretical uptake, expected yields and mineral elements in soil. All cultural practices recommended for eggplant cultivation in Mediterranean environment were adopted uniformly according to crop needs (Baixauli, 2001). In order to evaluate the mulching effects on growth, yield and fruit quality of 'Birgah' eggplant, microchip thermometer (Hanna Instruments, R.I., USA) with probe were used to collect data of the air temperature in the soil-mulch film gap (Figure 1).

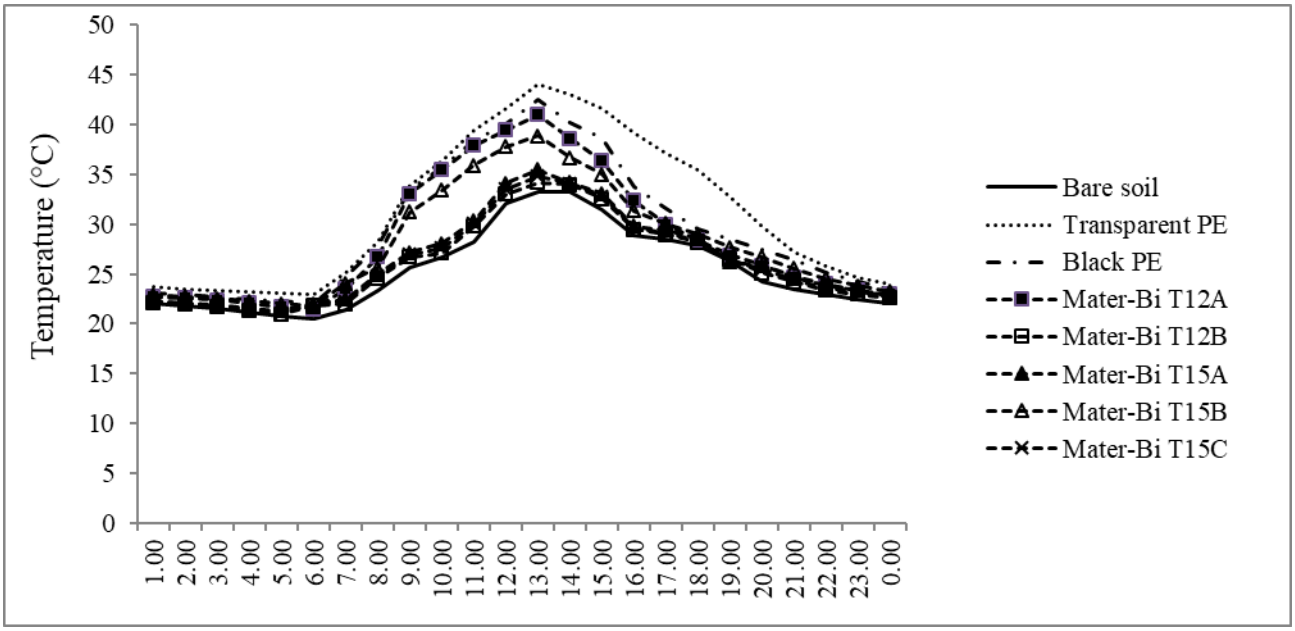

Figure 1. Average hourly air temperature in the soil-mulch film gap.

2.2 Plant vigor, yield and fruit quality

Plant vigor was assessed by plant height at 50 day after transplanting (DAT), number of leaves at 50 DAT and stem diameter at 50 DAT. Immediately after harvesting, total yield per plant, marketable yield per plant, number of marketable fruit per plant and the data of first harvest as DAT were recorded. Average fruit weight was also calculated. Sample of the fruit pulp were squeezed by hand with a garlics queezer. The juice was filtered and soluble solids content (SSC) was measured using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd. Taiwan). Fruit firmness was determined by measuring its resistance to the plunger of a digital penetrometer (Trsnc, Italy). Each fruit was subjected to a compression in two opposite point in the equatorial part using a 6 mm diameter stainless steel cylinder probe. The mean peak force was calculated in Newton (N). Fruit dry matter percentage was determined by drying fruits in a thermo-ventilated oven at 105 °C for 72 hours (until

constant weight) as 100%×(dry weight/fresh weight). Polyphenols were extracted and analyzed according to Stommel and Whitaker (2003) with minor modifications. Quantification of chlorogenic acid (CA), carried out after a RP-HPLC separation, was based on absorbance at 325 nm relative to the sesamol internal standard and an external standard of authentic CA (Sigma-Aldrich, St.Louis, MO). The results were expressed as mg100 g^{-1} of dw.

2.3. Mulching film biodegradation rates

In order to evaluate mulch biodegradation during the crop season, the percent soil exposure (PSE) of each mulch treatment was recorded in the center of the row of each replication two times per month.PSE was determined such that 0% represented soil that was completely covered and 100% represented fully exposed soil. Ratings were in 1% increments up to 20% PSE and in 5% increments thereafter (Ghimire et al., 2018).

2.4. Experimental design and statistical analysis

Mulching treatments were defined by a completely randomized design with four replications per treatment, each consisting of ten plants. Statistical analyses were performed using a one-way ANOVA and mean separation was conducted by Tukey HSD test. Percentages were subjected to angular transformation prior to perform statistical analysis ($\Phi = \arcsin(p/100)^{1/2}$).

3. Results and discussion

Transparent PE foil showed the highest air temperature in the soil-mulch film gap over a 24 h period (Figure 1),whereas, lower temperature were recorded in the soil-mulch film gap of the black PE film.Air temperaturein the plots with Mater-Bi T12A and Mater-Bi T15B biodegradable mulching films were 0.5-9.7 °C higher than those of the bare soil between 11.00 and 18.00 HR (Figure 1). During the experiment period (from 2nd May, 2016 to 30th August, 2016), no rainfall occurred. The mean plant height at 50 DAT among different treatments varied between 37.8 and 47.8 cm for the Mater-Bi T15C and Transparent PE, respectively (Table 2). Plants cultivated on the transparent PE film were significantly taller than those cultivated on the black PE film, Mater-Bi T12A and Mater-Bi T15B biodegradable films, which in turn were significantly taller than those growing on bare soil, Mater-Bi T12B, Mater-Bi T15A and Mater-Bi T15Cbiodegradable foils (Table 2).

Table 2. Effects of mulching treatments on plant vigour traits of 'Birgah' eggplant.

	Plant height at 50 DAT (cm)		No. leaves at 50 DAT (No.)		Stem diameter at 50 DAT (mm)	
Bare soil	38.2	c	13.2	d	10.2	c
Transparent PE	47.8	a	18.9	a	12.9	a
Black PE	41.6	b	15.6	b	10.9	b
Mater-Bi T12A	42.0	b	15.2	b	11.2	b
Mater-Bi T12B	38.6	c	14.3	c	9.2	d
Mater-Bi T15A	39.0	c	14.7	bc	8.9	d
Mater-Bi T15B	41.0	b	15.6	b	11.0	b
Mater-Bi T15C	37.8	c	14.0	c	10.1	c

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test.

Table 3. Effects of mulching treatments on yield parameters of 'Birgah' eggplant.

	Total yield plant^{-1} (kg)		Marketable yield plant^{-1} (kg)		No. marketable fruits plant^{-1} (No.)		Average fruit weight (g)		First harvest (DAT)	
Bare soil	2.7	d	2.4	d	5.7	b	420.8	d	75.6	a
Transparent PE	3.9	a	3.5	a	6.3	ab	558.6	a	59.1	d
Black PE	3.5	b	3.3	b	6.6	a	502.6	b	65.2	c
Mater-Bi T12A	3.4	b	3.2	b	6.4	a	498.6	b	66.8	c
Mater-Bi T12B	3.1	c	2.8	c	6.6	a	426.9	cd	69.8	bc
Mater-Bi T15A	3.2	c	2.8	c	6.5	a	430.9	cd	72.9	b
Mater-Bi T15B	3.5	b	3.2	b	6.3	ab	510.3	b	65.7	c
Mater-Bi T15C	3.0	c	2.9	c	6.2	ab	466.5	c	72.7	b

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test.

The assessments on number of leaves at 50 DAT and stem diameter at 50 DATsupported the trend established for plant height (Table 2). The positive effects of mulching on growth and plant vigour have been studied in different crops (Brown et al., 1992; Moncada et al., 2008; Kosterna, 2014; Iapichino et al., 2014). Plant height, which may be considered as an indicator of vigour was highest in the plots withthe transparent PE film and lowest in the plots with the Mater-Bi T15C biodegradable film, revealing that mulching film, which generate different soil temperature and micro-environmental conditions, is important in conferring plant vigour. The first frutis to harvest were from plantscultivated on the transparent PE film (59.1 DAT), whereas, the last ones to harvest were from plants grown on the bare soil (75.6 DAT) (Table 3). However, plants grown on the Black PE, Mater-Bi T12A and Mater-Bi T15B films displayed good results in terms ofearly fruiting (65.2, 66.8, and 65.7 DAT, respectively). On the experimental plots with the conventional transparent PE film total yield per plant and marketable yield per plant were found over 3.9 and 3.5 kg, respectively (Table 3). The above mentioned yield parameters, were significantly lower in the plots mulched with black PE (3.5 and 3.3 kg, respectively), Mater-Bi T12A (3.4 and 3.2 kg, respectively) and Mater-Bi T15B (3.5 and 3.2 kg, respectively), which in turn showed higher values than plots mulched with Mater-Bi T12B (3.1 and 2.8 kg, respectively), Mater-Bi T15A (3.2 and 2.8 kg, respectively) and Mater-Bi T15C (3.0 and 2.9 kg, respectively). The lowest total yield per plant and marketable yield per plant were found on the bare soils (2.7 and 2.4 kg, respectively).Overall, plants grown on the mulched soils revealed a higher number of marketable fruits than those cultivated on the bare soil (Table 3). Data collected on average fruit weight supported the trend established for total and marketable yield per plant (Table 3).Our results are in accord with those obtained by Adamczewska-Sowińska et al. (2016), who reported that mulch appeared to favor eggplant yields. Furthermore, our findings are also consistent with those displayed by Miles et al. (2012), who revealed that increased soil temperature under the mulch treatmentfavored tomato yield. Other studies have reported that yield to be similar for biodegradable mulching films and traditional plastic films (transparent and black PE foils) in different crops such as *Lactuca sativa* (Brault et al., 2002), *Cucumis melo* (Filippi et al., 2011; Iapichino et al., 2014; Shogren and Hochmuth, 2004), *Solanum lycopersicon* (Cirujeda et al., 2012; Cowan et al., 2014; Martin-Closas et al., 2008; Moreno and Moreno, 2008), *Cucumis sativus*

(Wortman et al., 2016) and *Cucurbita pepo* (Ghimire et al., 2018).

When considering the fruit quality traits, no significant differences were found among treatments for fruit dry matter (average value of 5.9%) and firmness (average value of -47.9 N) (Table 4). However, we found that TSS values of fruits from plants grown on the plots with traditional plastic films (transparent and black PE films), Mater-Bi T12A and Mater-Bi T15B biodegradable films were significantly higher (5.0, 5.1, 5.0 and 5.0 °Brix, respectively) than fruits from plants cultivated on the Mater-Bi T12B, Mater-Bi T15A and Mater-Bi T15C biodegradable foils(4.6, 4.7 and 4.5 °Brix, respectively), which in turn were higher than fruits from plots cultivated on the bare soils (4.2 °Brix) (Table 4). Our outcomes are in accord with those obtained by Iapichino et al. (2014), who found higher TTS values in melon fruits from plots cultivated on black PE mulching and slightly lower in the melon fruits from plots cultivated on transparent and black biodegradable mulch materials which in turn did significantly differ from those of the bare soil. Cowan et al. (2014) also revealed higher TSS in tomato fruits grown on PE and biodegradable mulching films compared with bare ground. Furthermore, Ghimire et al. (2018) showed higher TSS in *Cucurbita pepo* fruits cultivated on the PE foil or on the biodegradable mulching films than fruits grown on the bare ground. In this respect, our results on plant vigour and TSS are in accord with those of Ghimire et al. (2018) who hypothesized that a lower TSS in the fruits grown on the control plots (bare soil) was because of less plant vigor, and lower nutrient and water uptake.Fruit CA content from plants cultivated on the transparent and black PE films,Mater-Bi T12A and Mater-Bi T15B biodegradable mulching foils were significantly higher versus fruits CA content from plants grown on the Mater-Bi T12B, Mater-Bi T15A, Mater-Bi T15C and bare soil (Table 4).

Table 4. Effects of mulching treatments on fruit dry matter, firmness, TSS and CA of 'Birgah' eggplant.

	Fruit dry matter (%)	Firmness (N)	TSS (°Brix)	CA (mg 100 g^{-1} of dry weight)
Bare soil	6.0 a	-45.7 a	4.2 c	813.5 b
Transparent PE	5.8 a	-42.6 a	5.0 a	1346.3 a
Black PE	5.7 a	-50.2 a	5.1 a	1286.8 a
Mater-Bi T12A	5.6 a	-51.3 a	5.0 a	1256.2 a
Mater-Bi T12B	6.0 a	-47.5 a	4.6 b	800.6 b
Mater-Bi T15A	5.7 a	-46.9 a	4.7 b	952.6 b
Mater-Bi T15B	5.9 a	-50.6 a	5.0 a	1289.7 a
Mater-Bi T15C	6.2 a	-48.7 a	4.5 b	875.9 b

Data within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey HSD Test.

CA is the main monomeric phenolic compound in eggplant fruits (Mennella et al., 2010). The higher CA concentration may be a marker of stress, probably due to the high temperature in the soil-mulch film gap, as stress conditions induce phenolics accumulation (Dixon and Paiva, 1995; Moglia et al., 2008).Mulching treatments influenced PSE over sampling time(Figure 2). PSE was higherforMater-Bi T12B, Mater-Bi

T15A and Mater-Bi T15C films (95.0, 100.0 and 100.0%, respectively)than PSE for Mater-Bi T12A and Mater-Bi T15B and reached 63.3 and 63.3%, respectively by the end of the season.Whereas, PSE for traditional plastic films (transparent and black PE films) attained 6.7 and 7.7%, respectively by the end of the cultivation period (Figure 2). Our results are consistent with those revealed by Iapichino et al. (2014), who showed that the above ground part of the biodegradable mulching film started to show initial signs of deterioration after approximately 30 days from installation and by the end of the season it is was completely torn.

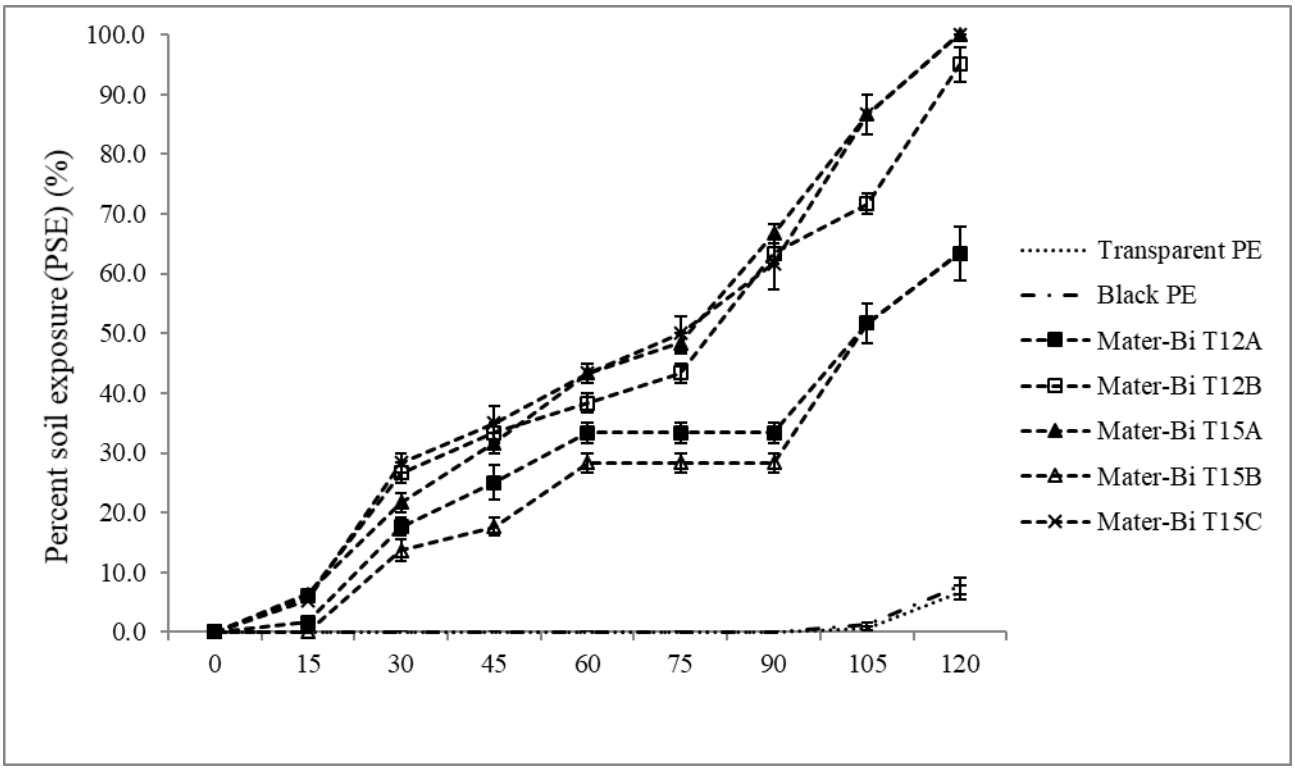

Figure 2. Percent soil exposure (PSE) for each mulching treatment during the production cycle of 'Birgah' eggplant at the experimental farm of the Department of Agricultural, Alimentary and Forest Sciences of Palermo (SAAF) in the northern coast of Sicily (Italy). Bars indicate the standard error of the mean.

4. Conclusions

It is very well known that mulches are functional in varying the hydrothermal regime of the soil and provide favorable soil biodegradable mulching films (Mater-Bi T12A and Mater-Bi T15B) reflected similar plant performance than black PE foil. They significantly enhanced vigour traits, early production, yield parameters and overall fruit quality. Consequently, Mater-Bi T12A and Mater-Bi T15B biodegradable mulching films environment for plant growth. In the present study, transparent PE film was more explicit for changing soil environment than black PE foil. Nevertheless, two out of five provided data which permitted us to form a very positive view on their potential extensive application and eventual replacement of black PE film. Thus, their use should be taken in consideration for open field eggplant production in Mediterranean environments.

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DOUGH RHEOLOGY, COOKING QUALITY AND SENSORY PROPERTIES OF WHEAT (*TRITICUM AESTIVUM*) –SWEET POTATO (*IPOMEA BATATAS*) BASED NOODLES FORTIFIED WITH SOYA BEAN (*GLYCINE MAX)* FLOUR

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ABSTRACT

Noodles were formulated using blends of wheat and sweet potato flours as base materials, then fortified with soybean flour. The formulations were in ratio 100:0:0, 95:5:0, 90:5:5, 85:10:5, 75:15:10, 65:20:15, and 55:25:20 for wheat, sweet potato and soya bean flours, respectively. Dough rheology, cooking quality, proximate composition, minerals, and sensory properties were evaluated using standard methods. While dough resistance and hydration increased with increase in the addition of sweet potato and soya bean flours, strength and extensibility decreased. Dough prepared with wheat, sweet potato and soya bean flour in ratio 75:15:10, 65:20:15 and 55:25:20 exhibited poor dough rheology. The addition of sweet potato and soya bean flours to wheat flour for noodle decreased optimal cooking time from 10.5 min to 8.3 min., however, cooking loss, cooking yield and swelling index increased. Moisture (9.21-10.15%), protein (9.21-10.15%), fat (9.21-10.15%), fibre (9.21-10.15%), ash (9.21-10.15%) and carbohydrate (9.21-10.15%) contents of the noodles varied significantly ($p<0.05$). Phosphorus, potassium and magnesium were the major minerals in the noodles while iron, calcium and sodium were relatively low. Sensory evaluation results revealed that the 100% wheat noodle was most preferred by the taste panel members. However, noodles prepared in the ratio of 90:5:5 seems to compare favourably well with the control in most of the sensory parameters measured. Acceptable noodles with improved protein and mineral content can thus be produced by the incorporation of sweet potato and soya bean flour into wheat flour up to the level of 5% each.

1. Introduction

Noodles are long thin extruded food products produced mainly from a mixture of flour, water and egg, usually cooked in soup and boiling water (Sanni et al., 2004). They are widely consumed as staples and accepted worldwide due to their associated convenience, accessibility and affordability (Abiodun et al., 2017). In 2013, World Instant Noodles Association reported that Nigeria had become the largest noodle market in recent time. This may be attributed to some of the properties of noodles like savoury taste, nutrition, convenience, safety, shelf-life stability and reasonable price (Gulia et al., 2014). The use of wheat flour as the main conventional raw material in the production of noodles is not unconnected with its vital gluten content. This makes it stand out among other cereal crops. Wheat flour is also a good source of digestible carbohydrate. However, there are some nutritional limitations associated with noodles

produced from solely wheat-based flour. First of all, wheat is inherently limited in protein, particularly in essential amino acids like lysine. Secondly, some essential nutrients such as dietary fibre, vitamins and minerals are lost during wheat flour refinement. This invariably indicates that noodles mainly produced from wheat flour leaves much to be desired in terms of nutritional quality. This is of even greater concern considering the fact that noodles are more widely consumed by children, whose need for quality nourishment cannot be overemphasised. Researches to improve the nutritional quality of noodles are therefore necessary. This will also provide an opportunity to study the suitability of non-traditional raw-materials (Del Nobile et al., 2005, Karim et al., 2016), and which are locally available. In Nigeria, efforts are being made to reduce the cost of importing wheat by substitution of wheat flour with flours from other roots and tubers. These include sweet potatoes (Collins and Pangloli, 1997) and cassava (Sanni et al., 2004). Sweet potatoes are a good source of dietary fibre, carotene and other essential nutrients. Literatures exist on the substitution of wheat flour with sweet potato flour in noodle production (Collado and Corke, 1996; Collado, et al., 2001). Research efforts have also been made to improve the protein content of many pasta products through fortification with legumes (Collins and Pangloli, 1997; Gallegos-Infante et al., 2010). The higher level of lysine in legumes confers on them the potential for use in fortifying pasta products. Other benefits of such added ingredients include the creation of variety, as noodles of different sensory attributes such as colours and flavours can be produced (Collins and Pangloli, 1997). However, depending on the level of incorporation, the quality of the product may be affected. For example, fortification of pasta with chick pea flour above 30% was found to be unsuitable (Wood, 2009). Similarly, negative changes in cooking quality and sensory attributes have been reported at higher level (above 10%) of substitution (Zhao et al., 2005, Torres, et al., 2007). The addition of sweet potato to wheat based noodles fortified with defatted soya bean flour has also been researched and reported to have increased the Beta carotene content of the noodles (Collins and Pangloli, 1997) but cooking loss of approximately 1.5% was recorded. Nevertheless, the use of whole soya bean flour in the fortification of wheat-sweet potato based noodles has not been reported. This present study was therefore designed to investigate dough rheology, cooking quality and sensory properties of wheat-sweet potato based noodles fortified with soya bean flour.

2. Materials and Methods

2.1. Materials

The sweet potato tubers were obtained from the Teaching and Research farm of the University of Ilorin. Wheat flour, soya bean seeds and other ingredients were purchased from a local market in Ilorin, Nigeria. All reagents used were laboratory-grade.

2.2. Methods

2.2.1. Production of sweet potato flour

Sweet potato flour was produced according to the method of Adeleke and Odedeji (2010). Sweet potato roots were manually washed, peeled, re-washed, sliced (2 mm thickness) and soaked in 0.2% sodium metabisulphite for 5 min. The slices were oven (D-37520, Thermo Fisher Scientific, Germany) dried at 60°C for 48 h. Dried slices were thereafter milled (Waring blender, 8010S, Torrington, USA) into flour, sieved (with a 350 μm pore size seive), packaged in high density polyethylene bags of 1.00 cm thickness and kept under room condition of 35±2°C for subsequent noodle production.

2.2.2. Production of soya bean flour

Soya bean flour was prepared following the method of Ihekoronye and Ngoddy (1985).

Soya bean seeds were sorted to remove extraneous matter. The cleaned seeds were boiled for 30 min to aid decortication. Boiled seeds were manually dehulled, drained and oven (D-37520, Thermo Fisher Scientific, Germany) dried at 60°C for 48 h. Moisture content of the flour was approximately 10%. Dried seeds were milled, cooled and sieved (350 µm) to obtain soya bean flour. The product was packaged in high density polyeyhlene bags of 1.00 cm thickness and kept under room condition of 35±2°C until needed for noodle production.

2.2.3. Noodles preparation

Noodles were prepared using the method of Khalid et al. (2012) with few modifications - the adjustment made to the revolving blade cutter in front of the die was such that the length of the noodle produced was 3 cm, and the extent of drying was to target 12% moisture content, whereas the authors (i.e., Khalid et al., 2012) had targeted 1.5 cm and < 10%, respectively. Wheat flour, sweet potato flour and soya bean flour blends were prepared in different ratios (Table 1), each with the addition of 2.5% of table salt. Required amount of water was then added in the mixing chamber of the extruder (P3 Monferrina, Italy) for 10 min to distribute the water uniformly throughout the flour blends. The flour blends were extruded through an adjustable die (No. 225). The speed of the revolving sharp blade cutter in front of the die was adjusted so that the length of the pasta finished at 3.0 cm and a thickness of 1 mm for each sample was attained. The pasta was oven (D-37520, Thermo Fisher Scientific, Germany) dried at 75°C for 3 h until the moisture content was approximately 12%. The dried products were packaged in a low density polyethylene bag, sealed and stored under room condition of 35±2°C for subsequent analyses.

Table 1. Wheat, sweet potato and soya bean blends used in noodle formulation

Keys	Wheat	Sweet potato	Soya bean
WPSN (100:0:0)	100	0	0
WPSN (95:5:0)	95	5	5
WPSN (90:5:5)	90	5	5
WPSN (85:10:5)	85	10	5
WPSN (75:15:10)	75	15	10
WPSN (65:20:15)	65	20	15
WPSN (55:25:20)	55	25	20

WPSN: Wheat-sweet potato-soy noodles. Numbers in brackets represent the ratio of the flours, respectively.

2.2.4. Chemical analyses

The noodles made from blends of wheat, sweet potato and soya bean flours were subjected to proximate and mineral analysis using AOAC methods (i.e., 2000 and 1990, respectively). Carbohydrates were calculated by difference. calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na) and iron (Fe) were determined from their ashes by Atomic Absorption Spectrophotometry using Acqua regia method (ARM). All analyses were determined in triplicates.

2.2.5. Functional properties

The dough rheology and gluten strength were determined using Alveograph machine as described by Maktouf et al. (2016). The cooking quality of the noodles (optimum cooking time, cooking loss, cooking yield and swelling index) were determined according to the approved method of Maktouf et al. (2016). The hydration level of the pasta mix was determined according to Manthey et al. (2004).

Determination of the aforementioned functional properties was in triplicates.

2.2.6. Sensory evaluation

Sensory evaluation of the noodles was carried out in the sensory laboratory of the Department of Home Economics and Food Science, University of Ilorin, Nigeria. Thirty trained panelists including some of the staff and students from the University evaluated the noodles for colour, taste, texture, aroma and overall acceptability using a 9-point hedonic scale.

2.2.7. Statistical analysis

Data generated from the analyses above were subjected to Analysis of Variance (ANOVA) and means obtained were separated using the Fisher Least Significant Difference (LSD) test at ($p<0.05$). Statistical Package for Social Sciences (SPSS, version 16.0) was used for the statistical analyses.

3. Results and Discussions

3.1. Rheological and hydration properties of wheat-sweet potato based noodles fortified with soya bean flour

The rheological properties of the noodles formulated with wheat, sweet potato and soya bean flour in the ratio 75:15:10, 65:20:15 and 55:25:20, respectively, were so poor that they could not be read from the alveograph. Therefore, after dough preparation, analyses were carried out only on dough produced from formulations in the ratio 100:0:0, 95:5:0, 90:5:5 and 85:10:5 of wheat:sweet potato:soya bean flours. Dough prepared from flour formulation containing 90%, 5% and 5% of wheat, sweet potato and soya bean flours, respectively (i.e., WPSN 90:5:5) showed higher resistance (106.3 mm) to shear than noodles from other formulations. The resistance of the dough to shear increased with increase in the amount of sweet potato and soya bean flours added (Table 2). Visco-elastic properties of high gluten dough as measured by extensibility are important in determining the use of wheat in food applications. Expectedly, dough prepared from whole wheat flour (WPSN 100:0:0) showed the highest ability to extend (Table 2). The extensibility of the dough decreased with increase in the addition of sweet potato and soya bean flours. The highest extensibility observed for dough prepared from whole wheat flour may be attributed to its higher level of gluten. The elastic nature of the dough therefore is expected to reduce with increase in the levels of sweet potato and soya bean flours. Similarly, dough prepared from whole wheat flour showed the highest (320 J) strength compared to other doughs (Table 2). The lowest strength was recorded for dough prepared from 85% wheat, 10% sweet potato and 5% soya bean flours. The result from this study agrees with previous findings (Manthey et al*.,* 2004). These authors reported that non-wheat ingredients lead to discontinuity within the gluten matrix and result in weaker dough. The higher strength value observed for dough prepared from whole wheat flour could be attributed to its higher extensibility which was conferred by its higher gluten content. This observation suggests that higher work and energy will be required to blow whole wheat dough. The ratio of resistance (P) to extensibility (L) as well as the strength of dough, which are important parameters in determining the suitability of flour for noodle production, varied among the doughs prepared. All the formulated blends met the standard required for noodle production. The hydration level of the dough was not very much different among the flour blends (Table 2). Water requirement of noodle may differ depending on the type of ingredient used. An increase in the addition of sweet potato flour and soya bean flour increased the water requirements (approx. 1.2 times) of the dough before leveling out. The slight increase in water requirements may be associated with the ability of proteins in soya bean flour to imbibe water during the mixing

process. Furthermore, a high fibre content of the dough (Table 3) may also have accounted for the increase in hydration level. Similarly high hydration level has been reported for noodles fortified with soya bean and chicken pea flours compared to noodles prepared from whole wheat flour (Khalid et al., 2012).

Table 2. Rheological and hydration properties of wheat-sweet potato-soya bean noodles

Wheat flour	Resistance (P) mm	Extensibility (L) mm	Strength (W) J	P/L	Hydration level
WPSN (100:0:0)	81.60^{b}±0.00	100.10^{a}±0.00	320.00^{a}±0.00	0.82^{c}±0.00	30.00^{a}±0.10
WPSN (95:5:0)	86.10^{b}±0.10	86.20^{b}±0.00	310.00^{a}±0.00	1.00^{b}±0.10	31.00^{a}±0.00
WPSN (90:5:5)	106.30^{a}±0.00	65.10^{c}±0.00	300.00^{a}±0.00	1.63^{a}±0.00	35.00^{a}±0.00
WPSN (85:10:5)	97.40^{a}±0.00	70.30^{c}±0.10	280.00^{b}±0.00	1.39^{a}±0.10	35.00^{a}±0.00
WPSN (75:15:10)	PDR	PDR	PDR	PDR	ND
WPSN (65:20:15)	PDR	PDR	PDR	PDR	ND
WPSN (55:25:20)	PDR	PDR	PDR	PDR	ND

WPSN: Wheat-sweet potato-soy noodles. Numbers in brackets represent the ratio of the flours, respectively; Data are reported as mean ± standard deviation. Mean values carrying different letters within the same column are significantly different ($p<0.05$); PDR: Poor dough rheology; ND: Not determined

3.2. Cooking quality of cooked wheat-sweet potato-soya bean noodles

The addition of sweet potato and soya bean flours to wheat flour for noodle decreased optimal cooking time from 10.5 min to 8.3 min (Fig. 1). Similar reduction in optimum cooking time has been reported for durum spaghetti with base quinoa, broad beans and chicken pea flours compared to whole wheat flour (Chillo et al., 2008). This reduction in cooking time was accompanied by an increase in cooking loss (Fig. 1). Whole wheat flour showed the lowest cooking loss (13.6%) and lowest swelling index (170.5%) compared to other noodles (Fig. 2.). The relatively higher cooking loss observed for other noodles compared to noodles prepared from whole wheat flour may be associated with the dilution effect on the gluten strength, and the possible weakening of the overall structure of the noodles. This seems plausible because according to Guehuen and Barbot (1988), legumes contain salt-soluble globulins and water-soluble albumin. These protein fractions thus may contribute to the weakening of the gluten structure (Petitot et al., 2010). Similarly, higher cooking losses were reported for pasta fortified with split pea and faba bean flours (Petitot et al., 2010), spaghetti containing buckwheat, amaranth and lupin flours (Rayas-Duarte et al., 1996, Torres et al., 2007) and spaghetti containing green, yellow pea, lentil and chickpea (Zhao et al., 2005). Furthermore, cooking loss may also be due to amylose leaching and solubilization of some of the salt soluble proteins (Petitot et al., 2010). High cooking loss is undesirable because it means that there was a high starch content in the cooking medium and that the noodles had a low cooking tolerance (Chakraborty et al., 2003). Similarly, the swelling index of the noodles increased with increase in the addition of sweet potato and soya bean flours (Fig. 2). According to Chillo et al. (2008), physical disruption of the gluten matrix due to the presence of fibres may have facilitated the penetration of water to the core of pasta. Therefore, the incorporation of sweet potato flour to wheat flour, which contains reasonably higher fibre content, may have contributed to the increased swelling index of the noodles. Cooking yield of the noodles was highest (287.9%) for noodles with 85% wheat, 10% sweet potato and 5% soy flours (WPSN 85:10:5) (Fig. 2) and lowest (195.0%) for noodles prepared from whole

wheat flour (WPSN 100:0:0). This can be attributed to the increased water binding capacity of the noodles brought about by increased inclusion of sweet potato flour. Mahmoud et al. (2012) reported a similar correlation between the water binding capacity and cooking yield of noodles produced from wheat flour fortified with different protein products from lupine.

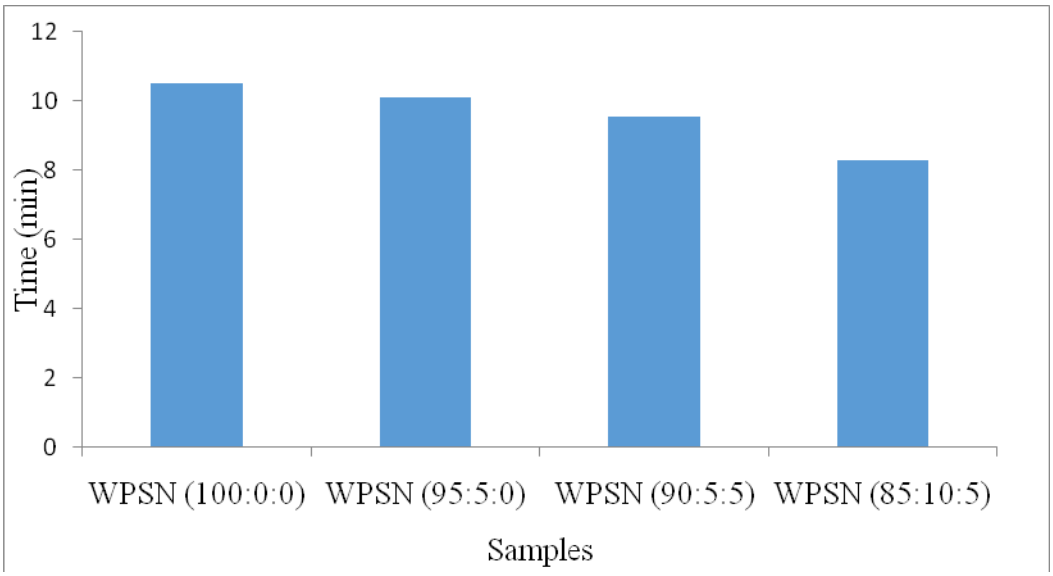

Fig. 1. Cooking time of wheat-sweet potato-soya bean noodles
WPSN: Wheat-sweet potato-soy noodles. Figures in brackets represent the ratio of the flours, respectively

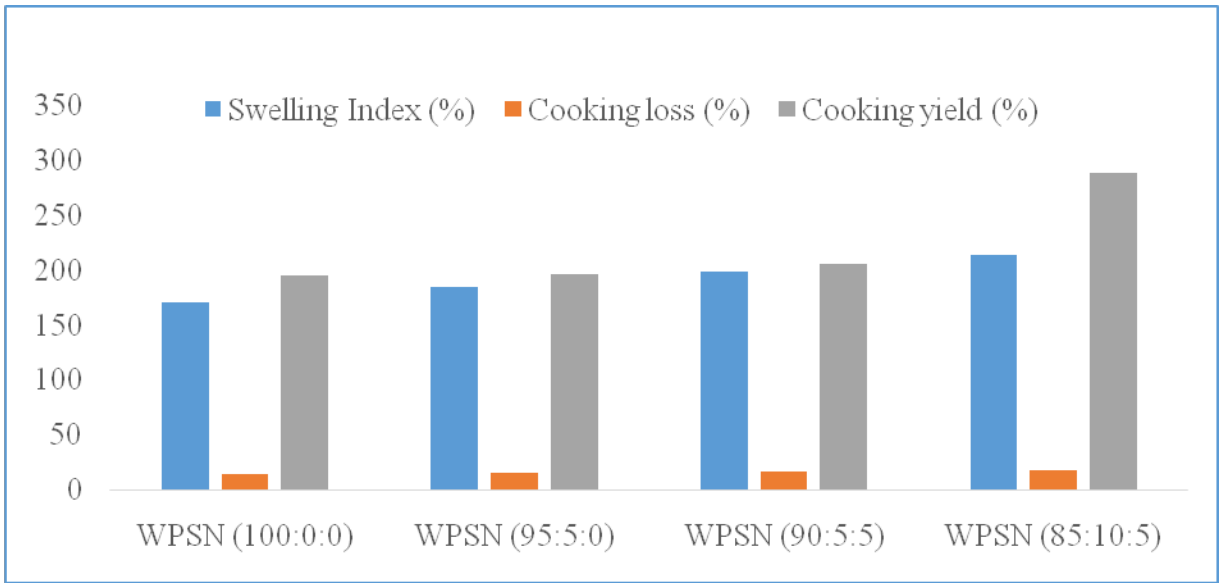

Fig. 2. Cooking yield, cooking loss and swelling index of cooked wheat-sweet potato-soya bean noodles
WPSN: Wheat-sweet potato-soy noodles. Figures in brackets represent the ratio of the flours, respectively.

3.3. Proximate composition of wheat-sweet potato-soy noodles

Protein and carbohydrate are the major components in the formulated noodles (Table 3). With the exception of noodles prepared from whole wheat flour, the protein contents of other noodles were almost similar (approx. 9%). Expectedly, the protein content of the noodles increased with increase in the addition of soya bean flour. The increase in protein content was associated with the higher protein content in soya bean flour. Similarly, the carbohydrate contents of the noodles were not very different (approx. 75%). Ash (1.1-1.4%) and fat (2.6-2.8%) contents of the noodles were very low and varied not significantly ($p > 0.05$) among the noodles. Values obtained for ash and fat contents are within those reported for pasta fortified with split pea and faba bean flour (Petitot et al., 2010). The moisture contents (approx. 11%) of the noodles in this study are slightly higher than values previously reported for pasta products (Gallegos-Infante, et al., 2010) but less than the 13% reported by Zaneta et al. (2007) as the moisture content of noodles produced with various supplements.

Table 3. Proximate composition of wheat-sweet potato-soya bean noodles (%)

Noodles	Moisture	Ash	Protein	Fat	Fibre	Carbohydrate
WPSN (100:0:0)	10.1^{a}±0.0	1.1^{c}±0.1	8.9^{b}±0.1	2.6^{b}±0.1	1.5^{c}±0.4	75.8^{a}±0.1
WPSN (95:5:0)	11.0^{a}±0.0	1.3^{b}±0.2	9.0^{b}±0.1	2.6^{b}±0.1	1.6^{b}±0.2	75.0^{a}±0.1
WPSN (90:5:5)	10.5^{a}±0.0	1.4^{a}±0.2	9.3^{a}±0.1	2.7^{a}±0.2	1.7^{a}±0.2	74.5^{a}±0.1
WPSN (85:10:5)	11.2^{a}±0.0	1.4^{a}±0.2	9.5^{a}±0.1	2.8^{a}±0.1	1.7^{a}±0.1	73.4^{a}±0.1

WPSN: Wheat-sweet potato-soy noodles. Numbers in brackets represent the ratio of the flours, respectively; Data are reported as mean ± standard deviation; Mean values carrying different letters within the same column are significantly different ($p<0.05$)

3.4. Mineral composition of wheat-sweet potato-soya bean noodles

Phosphorus, potassium and magnesium are the major minerals in the noodles (Table 4). Iron, calcium and sodium were relatively low. All the noodles showed increase in the mineral composition with increase in the addition of sweet potato and soya bean flour. The increase in minerals following the addition of these flours (sweet potato and soya bean) may be associated with higher mineral profile of soya bean flour and possibly also from the sweet potato flour used in the preparation of the noodles.

Table 4. Mineral composition of wheat-sweet potato-soya bean noodles (mg/100 g)

Noodles	Ca	P	K	Mg	Fe	Na
WPSN (100:0:0)	36.2^{c}±1.2	149.3^{d}±0.1	195.0^{c}±0.0	170.5^{d}±0.1	3.7^{c}±1.2	31.2^{b}±0.1
WPSN (95:5:0)	38.4^{c}±1.0	179.0^{c}±0.1	196.3^{c}±1.2	184.2^{c}±0.0	4.0^{c}±1.1	40.0^{a}±0.1
WPSN (90:5:5)	41.9^{b}±1.5	182.4^{b}±0.1	205.2^{b}±0.1	198.2^{b}±0.3	4.6^{b}±0.7	41.0^{a}±0.1
WPSN (85:10:5)	86.5^{a}±1.0	267.3^{a}±0.1	287.9^{a}±1.1	213.1^{a}±0.2	5.6^{a}±0.1	41.3^{a}±0.1

WPSN: Wheat-sweet potato-soy noodles. Numbers in brackets represent the ratio of the flours, respectively; Data are reported as mean ± standard deviation; Mean values carrying different letters within the same column are significantly different ($p<0.05$)

3.5. Sensory evaluation of wheat-sweet potato-soya bean noodles

The mean sensory scores of the noodles prepared from wheat, sweet potato and soya bean flours is presented in Table 5. Expectedly, noodles prepared from whole wheat flour had the highest rating for texture, aroma and overall acceptability when compared to other noodles. Noodles prepared in ratio 90:5:5 had ratings comparable to the control in terms of taste, aroma and overall acceptability. The relatively higher colour rating observed for noodles prepared from 85% wheat, 10% sweet potato and 5% soya bean flours may be associated with its relatively higher sweet potato flour. This seems plausible since sweet potato has been reported to contain carotene which may have influenced the colour of the noodles (Collins and Pangloli, 1997).

Table 5: Sensory scores of cooked wheat-sweet potato-soya bean noodles

Noodles	Colour	Taste	Texture	Aroma	Over all Acceptability
WPSN (100:0:0)	7.4^{a}±0.1	7.4^{a}±0.3	7.3^{a}±0.3	7.7^{a}±0.1	7.6^{a}±0.3
WPSN (95:5:0)	6.9^{b}±0.2	7.7^{a}±0.1	6.9^{b}±0.1	7.3^{a}±0.2	7.2^{a}±0.4
WPSN (90:5:5)	6.5^{b}±0.2	7.3^{a}±0.2	6.4^{b}±0.1	7.2^{a}±0.1	7.0^{a}±0.1
WPSN (85:10:5)	7.3^{a}±0.1	6.6^{b}±0.1	6.9^{b}±0.2	7.2^{a}±0.2	6.2^{b}±0.3

WPSN: Wheat-sweet potato-soy noodles. Numbers in brackets represent the ratio of the flours, respectively; Data are reported as mean ± standard deviation; Mean values carrying different letters within the same column are significantly different ($p<0.05$)

4. Conclusion

Acceptable noodles were produced by fortifying wheat-sweet potato flours with soy flour. Noodles containing 90% wheat flour, 5% sweet potato and 5% soy flour compared favourably well with the control in terms of nutritional, functional and sensory properties. Therefore, the use of sweet potato and soya bean flour in the improvement of noodle quality should not exceed these amounts. Development of such functional food will not only improve the nutritional status but also meet the daily requirements of the general population. Further studies should assess the shelf stability of the product.

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EFFECT MICROENCAPSULATION OF *Saccharomyces boulardii* ON VIABILITY OF YEAST IN VITRO AND ICE CREAM

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ABSTRACT

The effect of microencapsulation by alginate and alginate-chitosan on the survival of *Saccharomyces boulardii* ATCC MYA-796 in simulated gastric and intestinal juices was investigated. The survival of yeast microencapsulated cells treated in ice cream production. The percentage survival of alginate and alginate-chitosan encapsulated cells treated in simulated gastric juice after 240 was 80 % and 90 % respectively, simulated intestinal juice was 80% and 85% respectively. The viability of *Saccharomyces boulardii* (Log CUF/g) in ice cream after storage time (at freezing temperatures 21 day) was 7.11, 6 and 5.25 for alginate-chitosan microcapsules treatment, alginate treatment and free cell treatment respectively. The pH values range of ice cream in three treatments at the initial storage time was 4.70-4.77. After 21 days, the pH decreased in all treatments.

1.Introduction

Probiotics have been defined as live microbes, which transit the gastro-intestinal tract, and in doing so benefit the health of the consumer. Generally, probiotic microorganisms have a therapeutic effect for improved immune system (by microbial metabolites, cell wall components and DNA) (FAO/WHO, 2002). As well as the treatment of diarrhoea (antitoxin effects) by *Saccharomyces boulardii* and *Saccharomyces cerevisiae* are the most common yeast strains that have desirable properties used in probiotic products (Nousia et al., 2011; Arslan et al., 2016). *S. boulardii* is unique probiotic and bio-therapeutic yeast, known to survive in gastric acidity and it is not adversely affected or inhibited by antibiotics or does not alter or adversely affect the normal microflora in the bowl. *S. boulardii* has been utilized worldwide as a probiotic supplement to support gastrointestinal health. In recent years, by incorporating *S. boulardii* various dairy foods such as yoghurt, ultra high temperature treated (UHT) milk, acidophilus yeast milk, ice cream etc. (Hattingh, and Viljoen, 2001; Karaolis et al., 2013, Niamah, 2017). Ice cream is a product with peculiar textural and organoleptic features and is highly appreciated by a very broad spectrum of consumers. Ice cream's structure and colloidal design, together with its low-temperature storage, renders it a very promising carrier for the stabilization and in vivo delivery of bioactive compounds and beneficial microorganisms. (Soukoulis et al., 2014), However, temperature change, also referred to as "cold shock", during freezing and melting may cause damage such as reduction or even complete loss of metabolic activity (Mohammadi et al., 2011). A method for

encapsulation and stabilization of probiotic yeast as *S. boulardii* or bacterial strains (*Lactobacillus* spp. and *Bifidobacterium* spp.) in polymeric or biopolymeric fibers has been developed, biopolymers are natural polymers (chitosan, alginate) that are abundantly available and extractable from natural sources and these biopolymers offer a wide range of unique applications (Abd and Niamah, 2012; Al-Manhel et al. 2018). In order to achieve the claimed beneficial effects of probiotic bacteria, these specific microorganisms must be viable, active, and abundant in the product up to the expiry date (cell counts range from 10^6 to 10^9 CFU/g) (Casarotti and Penna, 2015). Microencapsulation is a process by which bioactive materials are coated with other protective materials or their mixtures (Huq et al., 2013). Ice cream has nutritional significance, but possesses no therapeutic properties. The growing interest of consumers in therapeutic products has led to the incorporation of probiotic cultures into ice cream to result in a dietetic ice cream. Some studies have demonstrated that it is possible to produce probiotic ice cream using different microorganism (Ahmadi et al., 2012).This study was undertaken to select a suitable for *S. boulardii* microencapsulation and checked for their viability, survival of the encapsulated probiotics under the stimulated gastric conditions and study was to manufacture a probiotic ice cream containing *S. boulardii* and to determine how long these yeasts would remain viable during frozen storage of the ice cream.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Sodium alginate and chitosan were purchased from (BDH, UK). Whole cow milk obtained from Animals station of Agriculture College / University of Basrah.

2.1.2. Yeast cell culture

S. boulardii ATCC MYA-796™ was obtained from Department of Food Science / College of Agriculture university of Basrah and grow in YPD media (content: 0.3% yeast extract, 0.5% peptone and 1% glucose) at 30°C for 48 h.

2.2. Methods

2.2.1. Yeast inoculum preparation

33×10^9 CFU/ mL of *S. boulardii* was inoculated in 50 ml of YPD media and incubated at 30ºC at 150 rpm in a shaking incubator for 48 h. The cells were harvested by spinning them at 5,000 rpm for 10 min. The cultures were then washed twice with sterile saline solution (0.9%) and used in the microencapsulation process.

2.2.2. Microencapsulation of yeast cells

A 3.0% of alginate solution was autoclave sterilized at 121ºC for 20 min and stored at 4ºC. After washing, the cells were suspended in 5 mL of sterile` distilled water and mixed with 20 mL of 2% (w/v) sodium alginate for at least 30 min. The cell suspension was placed in a sterile syringe and injected through a 0.11 mm needle into sterile 0.05 M $CaCl_2$. The 0.11 mm needle was used to produce beads with a diameter of 1-2 mm. Moreover, stirring with a magnetic stirrer was applied during dropping to produce spherical beads, and the distance between the needle and the surface of the solution was controlled to approximately 1 to 2 cm to avoid formation of flat beads. After 30 min gelification in $CaCl_2$, the beads were rinsed with, and then kept in, sterile water at 4°C (Krasaekoopt et al., 2004).

2.2.3. Preparation of alginate-chitosan microcapsules entrapping yeast cells

Chitosan solution (0.5% w/v) was prepared by the addition of chitosan to acetic acid solution (0.1M) with stirring until dissolution (~1 h). The calcium alginate microcapsules were immersed in 100 ml of chitosan solution and shaken at 100 rpm for 40 min on an orbital shaker for coating and then filtration. The chitosan-coated beads were washed and kept in sterile water at 4ºC (Krasaekoopt et al., 2004).

2.2.4. Preparation of simulated gastric and intestinal juices and inoculation of cells

The simulated juices were prepared according to Brinques et al. (2011). Simulated gastric juices were prepared by dissolving pepsin (Riedel-DeHaen Hannover) in sterile sodium chloride solution (0.5% w/v) to a final concentration of 3.0 g/L and adjusting the pH to 1.5 with hydrochloric acid. Simulated intestinal juices were prepared by suspending pancreatin (BDH) in sterile sodium chloride solution (0.5%, w/v) to a final concentration of 1 g/L with 4.5% bile salts (Oxoid, UK) and adjusting the pH to 8.0 with sterile NaOH (0.1 M). Both solutions were filtered for sterilization through a 0.22 μm membrane. *S. boulardii* inoculated to the simulated gastro-intestinal juice individually in three different forms, non-encapsulated, encapsulated with calcium alginate and encapsulated with alginate and chitosan. Then one gram of freshly encapsulated yeast samples or 1 mL of cell suspensions (free cells) were gently mixed with 10 mL of sterile simulated gastric juice or sterile simulated intestinal juice and incubated at 37 °C for 60,120, 180 and 240 min. Surviving yeast were enumerated by pour plate counts in YPG agar aerobically incubated at 30°C for 48 h. the survival percentage of yeast was calculated by the following formula:

Survival of yeast cells % = Final Log (CFU/ mL)/Control Log (CFU/ mL) × 100 (1)

2.2.5. Preparation of ice cream

Ice cream mix was prepared by mixing ingredients and then heated to 80°C for 30 sec. Mixes were cooled to 5°C and aged overnight at the same temperature. After ageing, the mix was heat treated to 80°C for 30 sec. and cooled to 37°C. Yeast probiotic (*S. boulardii*) were inoculated into ice cream mix at the rate of 5% (33×10^9 CFU/ mL) and incubated at 30°C for 5 h. The ice cream was filled in 50 ml food grade paper cups, covered with food grade lids and stored at -18°C (Pandiyan, 2010). The probiotic ice cream was prepared using the different treatments as shown below: Treatment **I**: Ice cream prepared with free cell of *S. bolardii* Treatment **II** : Ice cream prepared with sodium alginate microcapsules entrapping yeast cells. Treatment **III**: Ice cream prepared with alginate-chitosan microcapsules entrapping yeast cells.

2.2.6. Yeast viability

Frozen fermented ice cream was thawed and then diluted. The viability of free and microencapsulated yeast cells was enumerated by pour plate count method. To determine the probiotic viability count, the entrapped probiotics were released from the microcapsules. One gram of the ice cream samples transfers to 9 mL of sterile peptone water and then homogenized for 15sec. in a vortex mixer. The success of the encapsulating formula was tested. Thus, one-gram sample of microcapsules was diluted in sterile peptone water and then an aliquot of 100 mL was discharged into potato dextrose agar plates and incubated at 30ºC for 24–72 h. The population of *S. boulardii* was then quantified and expressed as the number of colony-forming units (CFU) per gram (CFU/g) Samples were taken at 0, 1, 7, 14 and 21days of storage (Zamora-vega et al., 2012). The pH of the samples was measured with a pH meter (SD-300, Germany) (Niamah et al., 2016).

3.Results and discussions

3.1. The survival *S. boulardii* in simulated gastric juice

The survival of the free cells and encapsulated *S. boulardii* in simulated gastric juice after being kept for 240 min is shown in Figure 1. Food stays in the stomach for 2 to 4 hours, while the liquids can only stay about 20 minutes. pH of gastric juice simulates affected the survival of both the free and encapsulated cells of *S. boulardii*. The encapsulated *S. boulardii* survived well in simulated gastric conditions compared to the free yeast cells and the encapsulated by alginate-chitosan was best than with alginate. The percentage survival of encapsulated *S. boulardii* in gastric juice after 3 h was 89% and 92%, for alginate

microcapsules method and alginate-chitosan microcapsules method while the percentage was 80% free yeast cells. After 240 min. the difference between alginate and alginate-chitosan microcapsules was more clearly in results. Whereas the yeast in the alginate-only microcapsules loss of percentage survival to 82%, the chitosan-mixed alginate microcapsules was 90%.

Alginate mixture remains stable structurally in acidity environments when the pH decreases as the viscosity of alginate solutions increase and the carboxylate groups in the alginate solutions backbone become protonated lead to form hydrogen bonds, which increased bindings yeast cells (Lee and Mooney, 2012). Encapsulation, either by alginate or by alginate/chitosan provide the best protection in the case of yeast cells encapsulated compared with non-encapsulated cells (free cells). This can be attributed to the polysaccharides of chitosan acting as a buffer, reducing the activity of the acid. It does protective effect to yeast cells.

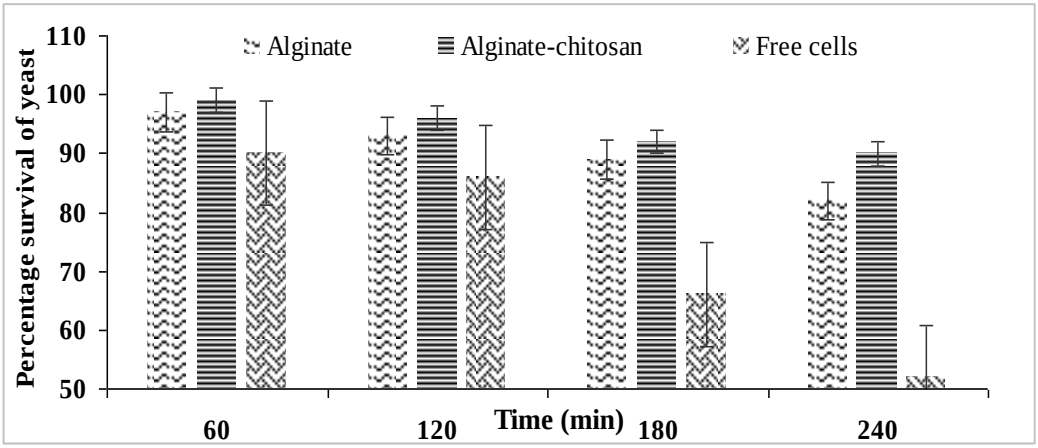

Figure 1. The percentage survival of yeast in simulated gastric

3.2.The survival *S. boulardii* in simulated intestinal juice

Bile salt tolerance is one of the basic characteristics of the probiotic to survive in the small intestine. Survival of encapsulated and free cells of *S. boulardii* after exposure to 4.5% bile salt for 240 min. This reflects the time of the stay of food in the small intestine is shown in Figure 2. The percentage survival of encapsulated *S. boulardii* was 80% and 85% after 240 min for alginate microcapsules method and alginate-chitosan microcapsules method while percentage survival of S. boulardii free cells was 67%. It was noted that the survival of alginate encapsulated and alginate-chitosan encapsulated *S. boulardii* reduced to somewhat compared with *S. boulardii* free cells. Mixed the alginate microcapsulation with chitosan more improve the viability of *S. boulardii* (Cook et al., 2011). The results of the study agreed with the (Dikit et al, 2015) who studied the effect of encapsulation on the survival of *Lactobacillus plantarum*. pH-base in the small intestine affecting the survival of *S. boulardii* cells. Microcapsulation of *S. boulardii* cells does to prevent the effect of the acid and bile salts. Chitosan polysaccharide chitosan is positively charged, and therefore, it is not suitable for encapsulated yeast when it is used by itself. A mixture of chitosan with other biological substances usually soluble in solvents such as alginate. Alginate is a negatively charged polysaccharide and best substance for growth and microcapsulation of probiotic microorganisms. Crosslinked three-dimensional made of chitosan and alginate provides much

better for microcapsulation process (Baysal et al., 2013).

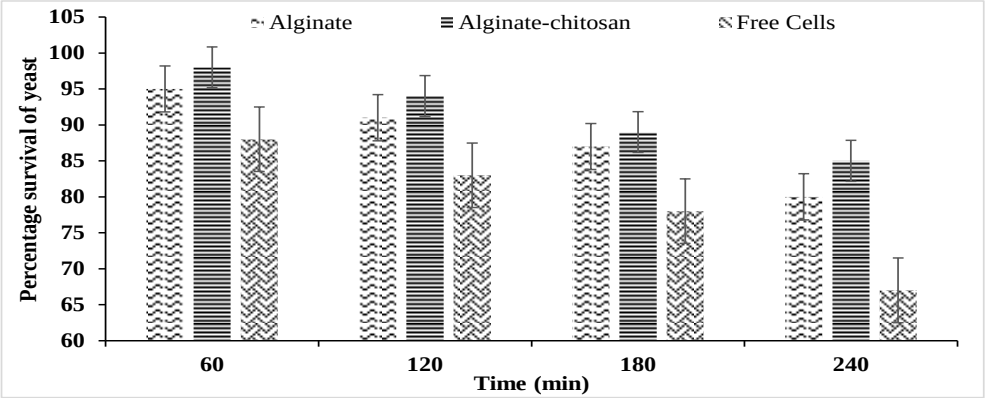

Figure 2. The percentage survival of yeast in intestinal environment

3.3. The viability of *S. boulardii* in ice cream

S. boulardii grew to high numbers in ice cream mix. Even the high solids level of the ice cream mix did not prevent growth of either free cells or microcapsule when a high percentage (4%) of inoculum was used. Viable cell count of *S. boulardii* (free cells) decreased from 8.55 to 5.25 log CFU/g due to encapsulation process; Changes in viable counts of S. boulardii in ice cream mix due to freezing and during frozen storage at -18°C are presented in Figure 3. Results showed that there was an approximately 3.3 Log cycle decrease in the count of probiotic cells in the free form immediately after freezing for 3 week compared with the encapsulated probiotic cells decreased 2.51 and 1.51 Log cycle in Treatment **II** and Treatment **III** respectively. This indicates that freezing had destructive effects on probiotic cells most probably due to the freezing injury of cells. During the freezing process, the cells of probiotics can be lethally injured by damage to their cell walls or membranes caused by thawing and the mechanical stresses (generated by mixing and incorporating oxygen into the mixture, during the manufacture of ice creams) of ice crystals forming in the external medium or inside the cells, by cold injuries and temperature decrease shock to the cells, by condensation of solutes in the extracellular/intracellular medium, or by dehydration of the cells. All these factors may result in even lower viability (Akin et al. 2007; Mohammadi et al., 2011). According to several findings, encapsulation is a useful alternative to increase the survival rate of probiotic bacteria in ice cream and fermented frozen dairy desserts. This result agrees with Ahmadi et al. (2012) has found that the viable counts of free probiotics decreased from ~9.55 to ~7.3 Log CFU/g after 60 days of frozen storage while that of encapsulated cells merely decreased less than 1 Log cycle. Encapsulation with alginate microbeads protected the probiotic cells against injuries in the freezing stage as well as, during frozen storage. Homayouni et al. (2008) reported that the survival of *L. casei* and *B. lactis* were monitored during the product's storage for 180 days at -20˚C. The viable cell number of *L. casei* and *B. lactis* in the free state in prepared ice cream mixture was 5.1×10^9 and 4.1×10^9 CFU/mL at day one and after 180 days storage at -20˚C, these numbers were decreased to 4.2×10^6 and 1.1×10^7 CFU/mL respectively. When encapsulated the mentioned probiotic bacteria in calcium alginate beads, the probiotic survival raised at a rate of 30% during the same period of storage at the same temperature. Akin et al. (2007) and Heydari et al. (2012) reported a similar observation. Encapsulation within alginate microbeads and alginate-chitosan protected the probiotic cells

against injuries in the freezing stage (Figure 3) as well as, during frozen storage. Viable number of encapsulated *L. acidophilus* was merely decreased less than 1 Log cycle after 60 days of frozen storage.

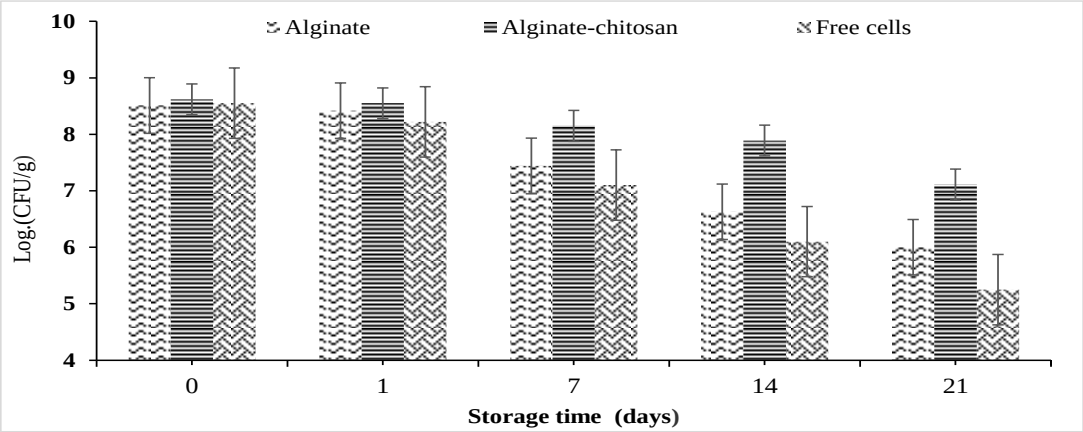

Figure 3. Viability of free and encapsulated *S. boulardii* (Log CFU/g) in ice cream during storage periods at freezing temperature.

3.4. pH of ice cream

Table 1. Show that the pH values of three ice cream treatments after process was between 4.70-4.77. The values decreased after storage for up to 4.20 in alginate-chitosan microcapsules treatment, 4.45 in alginate microcapsules treatment and 4.45 in free cell treatment. The reduced pH because of the ability of yeast to ferment sugars in the ice-cream mixture. *S. boulardii* either by acid hydrolysis or by producing enzymes that cleave the sucrose into glucose and fructose and glucose utilization rate was much faster than the fructose and sucrose (Kurtzman et al., 2011). *S. boulardii* is acetate produce from sugar utilization and anther carbohydrates (Anjum et al., 2010) . The high viability cells of *S. boulardii* in alginate-chitosan microcapsules treatment led to increased acidity and reduced pH compared with other treatments.

.

Table. 3 pH of ice cream treatment

Cell	pH				
	Time (day)				
	0	1	7	14	21
Alginate microcapsules	4.77 ± 0.03	4.72 ± 0.04	4.62 ± 0.01	4.53 ± 0.02	4.45 ± 0.01
Alginate-chitosan microcapsules	4.70 ± 0.03	4.65 ± 0.01	4.55 ± 0.02	4.43 ± 0.00	4.20 ± 0.05
Free cell	4.75 ± 0.05	4.70 ± 0.01	4.63 ± 0.01	4.60 ± 0.10	4.54 ± 0.06

±: Standard division (SD)

4. Conclusions

Microcapsulation during the emulsion technology was an effective way to increase the survival of probiotics yeasts as they pass during digestive tract conditions of warm-blooded animals. Adding chitosan when using encapsulation techniques can improve the survival of *S. boulardii* in simulated gastric

juice and intestinal juice. Probiotic ice cream can be manufactured using encapsulated *S. boulardii* to ferment ice cream mix. Coating alginate microcapsules with chitosan further improve the viability of *S. boulardii* during storage time of ice cream production. The probiotic yeast *S. boulardii* can be well incorporated into ice cream based foods to develop functional and therapeutic foods.

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QUALITY ASSESSMENT OF NIGERIAN MULTIFLORA HONEY MARKETED IN OGBOMOSO REGION

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ABSTRACT

The demand for high quality honey by consumers in developing countries calls for quality assessment of various honey samples displayed for sales to ascertain their originality. This study focused on assessing some quality attributes of honey samples sold in Ogbomoso region, comprising five local government areas. A total of 20 honey samples were obtained directly from major sellers of honey in different open markets in the region. Samples were analyzed for pH, diastatic activity, acidity, ash, total phenols, hydroxymethylfurfural (HMF), moisture content and total reducing sugars, and compared with honey standards specified by Codex Alimentarius Commission. The HMF content of different honey samples ranged from 14 to 99 mg/kg. Sixty percent of honey samples collected had values of HMF above the maximum limit of 40 mg/kg. Diastasic activity ranged from 0.00 to 27.8 schade unit, ash content ranged from 0.32 to 1.02%, total reducing sugar content ranged from 10.30 to 65.35% and were significantly ($p<0.05$) different. The pH and acidity of most honey samples were within the recommended values. Total phenols and total soluble solids of analysed honey samples ranged from 4.00 to 68.50 GAE/100 g and 70 to 78 °Brix, respectively. The moisture contents of the samples were significantly ($p<0.05$) different from each other. The results obtained showed that some honey samples were within the stipulated standard recommended by Codex Alimentarius Commission while others did not meet the recommended safe limit.

1. Introduction

Honey is a viscous, supersaturated solution of sugar produced by bees and mostly from the nectar of flowers. It is basically from the nectars of flower in which all the water content is evaporated (Haynes and Callanghan, 2011). The bees use it to feed their larvae and for their subsistence during winter. Honey is highly nutritious and has lots of medicinal uses and health benefits. It contains variety of antioxidants which include flavonoids and phenolic acids (Vaughn, 2011; Sampath et al., 2010). The specific composition, colour and flavour of any batch of honey depend largely on the flowers consumed by the bees that produced the honey (Vaughn, 2011). It is a natural sweetener which can be eaten as food or used in different types of cooking. The sugar in honey does not need to be digested but assimilated directly by the body which is a quick source of energy

(Bogdanov et al., 2008). Because of the high level of fructose, honey is 25 % sweeter than table sugar. It is medicinal as it is used in treating cough, curing of sore throat due to its antimicrobial contents. In general, light or coloured honeys are mild in flavour while darker honeys are usually more robust in flavour. Industrial users of honey such as bread bakers, food processors and beverage makers will often buy honey by colour (National Honey Board, 2007). The absence of foreign material is the main criterion used in the determination of the honey quality. Bits of wax, brood, dirt, pollen, dead bees, ashes, etc can contaminate honey during extraction or processing. Honey can be sold by farmers at market outlets within their immediate reach (Babarinde et al., 2010). In many parts of the world, people buy and consume honey due to its numerous advantages and the value it adds to life without considering whether they are actually consuming the one of good quality or the adulterated ones. Adulterated honey can be detrimental to consumers' health, depending on the type and level of the inherent toxic materials. Lack of policies governing quality of food products such as honey in developing countries has affected the ingenuity of honey quality displayed for sales. Ogbomoso region comprises five local government areas (Ogbomoso North, Ogbomoso South, Surulere, Oriire and Ogo Oluwa); and these local government areas are headed by Ogbomoso Metropolis which is their historical root. Ogbomoso (Lat. 8° 08′ 00″ N, Long. 4° 16′ 00″ E) is one of the major cities in south western Nigeria where honey is sold at different markets. It once hosted the office of the Beekeepers Association of Nigeria and is currently endowed with quite a number of modern and traditional beekeepers (Babarinde et al., 2011). Despite the abundance of different grades or shades of honey in the region, work has not been done to evaluate the physic-chemical diversity of the samples sold in the markets. It is essential to collect samples of honey from different markets in Ogbomoso region and carry out analysis on them so as to ascertain their authenticity and understand the variations in the physico-chemical profiles of the samples available to consumers. Therefore this study was carried out with the aim of determining the level of adulteration in different Ogbomoso honey samples in order to provide information to consumers on what to look for while purchasing honey.

2. Materials and methods

2.1. Materials

2.2.1. Collection of Honey samples

Twenty honey samples (1 liter each of three replicates) were collected from different sales point in Ogbomoso North, Ogbomoso South, Surulere, Ogo Oluwa and Orire Local Government Areas of Ogbomoso (Table 1). Samples were stored in glass containers at ambient temperature in a cool and dry place until needed for analysis.

2.2. Reagents and Standard

Reagents used (p-toludine, barbituric acid, Folin-Ciocalteau and sodium carbonate) were of pure quality and laboratory grade. They were obtained from Sigma Aldrich Co., St. Louis, MO, USA. Standard Gallic Acid solution was prepared from 1 mg/ml stock Gallic Acid.

2.3. Analyses

The pH was measured using a pH meter (Jenway Digital®) by dissolving 10 g honey in 75 ml of distilled water. The pH meter was first calibrated at 4.0 and 7.0 buffers. The pH of honey samples was determined according to AOAC method 962.19 (AOAC, 1990). Hydroxymethylfulfural was determined according to Food and Drugs Administration and Laboratory Services (1982). Ten gram of honey was weighed and dissolved in 20 ml of distilled water. Fifty (50) ml transferred to volumetric flask and made up to the mark. 2.0 ml of the honey solution was pipette into each of two test tubes. 5.0 ml of p-toludine solution was added to each of the tubes. One millilitre of water was added to one test tube

and 1 ml of the barbituric acid solution to the other. Both test tubes were mixed by shaking. The whole procedure was done without pausing and took 1–2 min. The absorbance of the sample was determined against the blank (the test tube to which barbituric acid was not added) at 550 nm using a 1 cm cell. This was done immediately the maximum colour intensity was reached. Diastase activity was determined according to the method described by AOAC Method 958.09 (AOAC, 1990). The titratable acidity was determined by dissolving 10 g of the sample in 100 ml distilled water and was titrated to phenolphthalein end point with 0.1 N NaOH. The result was calculated as milliequivalents per kilogram (AOAC Official Method 962.19) (AOAC, 1990).

Total phenol in honey was determined according to Food and Drugs Administration and Laboratory Services (1982). One millilitre of the above extract was oxidized with 1 ml of Folin Ciocalteau reagent diluted with reverse osmosis de-ionized water and incubated for 6 min in an incubator. Immediately after incubation, the reaction was neutralized with 3 ml of 70 g/l Na_2CO_3 (v/v). Standard Gallic Acid solutions of range 0.1 mg/ml–0.5 mg/ml were prepared from 1 mg/ml stock Gallic Acid solution. The standard solutions were treated like sample and incubated for same period of time. The absorbance of the resulting blue colour solutions for sample and standard solutions were read at a wavelength of 750 nm on a spectronic 21DUV spectrophotometer. The moisture content of the sample was determined by weighing 5 g of sample into a pre-weighed aluminum drying dish. The sample was dried to constant weight in an oven at 105°C for 4 hours under vacuum (Food and Drugs Administration and Laboratory Services, 1982). The ash contents were determined according to the method described by Williams et al. (2009). Two grams of the sample was ashed by incineration in a furnace at 600°C to a constant weight. Reducing sugars, fructose and glucose were determined using Lane-Eynon titration method as described by FDALS (Food and Drugs Administration and Laboratory Services, 1982).

Total solid was determined using Abbe refractometer at 20° and the reading was recorded as described by AOAC method 925.23 (AOAC, 1990). The specific gravity of honey was determined by dividing the weight of a pycnometer (50 ml) filled with honey to the weight of the same pycnometer filled with distilled water AOAC method 970.56 (AOAC, 1990).

Viscosity is determined by homogenizing honey and passing it through a digital viscometer (Brookfield digital viscometer USA Model DV-E 02346-1031), AOAC method 925.10 (AOAC, 1990).

2.4. Statistical analysis

The experiment was set up in complete randomized design. Data were subjected to analysis of variance (ANOVA) and significant means were separated using Duncan Multiple Range Test at 5% probability level using SPSS version 16 (SPSS, 2006).

3. Results and discussions

3.1. pH

The pH of all the samples analyzed ranged from 2.60 to 6.20 (Table 2) and were significantly ($p<0.05$) different. The results obtained were within the recommended range of Codex Alimentarius Commission (1998) for pH of honey; however, one of the samples had a pH above the recommended value. This could be as a result of fermentation due to inappropriate method of harvesting and processing (Babarinde et al., 2011). The pH range obtained differed slightly from 3.03 and 5.98 reported by Abdulkhaliq and Swaileh (2017) for Palestinal multifloral honey. There is wide variation between the pH values (4.3-6.0) reported by Adebiyi et al. (2004) for some Nigerian honeys and the samples obtained in this study. Baroni et al. (2009) reported 3.14 and 5.05 as pH range of honey collected in south and north Cordoba, Argentina. In

another study in India, Saxena *et al.* (2010) reported pH values that ranged from 3.9 to 4.4. The pH of honey does not directly reflect the total acid content alone; it however indicates the buffering action of the inorganic cation constituents and the organic acids present (Rashma et al., 2016). Difference in pH may be due to variation in acids and minerals present in honey (Kamal et al., 2002).

3. 2. Hydroxymethylfurfural (HMF)

The HMF contents of different honey samples ranged from 14 to 99 mg/kg (Table 2). The result showed significant differences among HMF contents of the honey samples, with some samples having higher HMF content levels than the Codex Alimentarius Commission (1998) recommended values of 40 mg/kg. Samples from Jagun, Owode, Sabo, Oja Titun, Iresaadu and Takie Markets, and LAUTECH Farm showed a lower diastastic values. Diastase level and HMF are important parameters in honey analysis. The higher values of HMF indicate excessive exposure to heat during the honey harvesting or process and longer period of storage of honey samples. It also serves as an indicator of adulteration with invert sugars. HMF is a breakdown product of fructose (one of the main sugars in honey) formed slowly during storage and very quickly when honey is heated. The amount of HMF present in honey is therefore used as a guide to storage length and the amount of heating which has taken place. Tosi et al. (2002) reported that thermal treatment can increase HMF content of honey. Sixty percent of honey samples had values of HMF above the maximum limit of 40 mg/kg specified by Fallico et al. (2004). Overheating of honey sample during processing or storage for very long period could lead to conversion of sugars to HMF (Saxena et al., 2010). Babarinde et al. (2011) reported higher HMF values were obtained in honey harvested traditionally, which are characteristically done by usage of naked flame to kill the aggressive bees prior to harvest. The higher values of HMF obtained in some samples could be due to inappropriate method of harvesting or adulteration. Higher level of HMF content in honey obtained in this study could also be due to the fact that they were obtained from tropical country. Codex Alimentarius Commission (1998) declared that HMF content of honey from countries or regions with tropical ambient temperatures shall not be more than 80 mg/kg. Higher HMF in honey can also be an indicator of adulteration with invert sugars (cane sugar or sucrose is inverted by heating with a food acid, and this process creates HMF).

3.3 Diastatic activity

The diastatic value of honey ranged from 0.00 to 27.8 schade unit with some samples having lower value than the minimum value of 8 shade units established by European commission. The lower values can be attributed to the effect of heat applied during harvesting. This confirms the finding of Wintersteen et al. (2005) who reported deactivation of natural enzymes by heating and Oddo et al. (1995) who reported that high temperature lowers enzymes' activity. Babarinde et al. (2011) reported diastase ratio ranging from 19.1 to 21.8 schade units (Table 2) for honey harvested using traditional and modern methods. Honey diastase activity is a quality factor, influenced by honey storage and heating and thus an indicator of honey freshness or overheating. Although there is a large natural variation of diastase, the standard of a minimum value of 8 has been proven to be useful (Balasubramanyam, 1999).

3.4. Titratable acidity

The titratable acidity values of all samples ranged from 0.03 to 0.10% (Table 2). The values obtained were within Argmark specifications with a maximum limit of 0.3% for honey acidity (Williams et al., 2009). Variation in the acidity values of the honey samples was due to different sources of nectar. The acidic nature of honey is due to the action of osmophilic yeast and sugar tolerant bacterial which readily act on honey sugars. Acids contained in multiflora honeys are acetic,

propionic, butyric and mallic acids (Balasubramanyam and Chadrasekara, 2011).

3.5. Total phenols

The results of total phenols are shown in Table 2. It ranged from 4.00 to 68.50 GAE/100 g and were significantly different ($p<0.05$) from each other. Phenolic compounds are products of the secondary metabolism of plants.

These compounds are reported to exhibit anticarcinogenic,antiinflammatory, antiantherogenic, antithrombotic immune modulating and analgesic activities, among others and exert these functions as antioxidants (Reshma et al., 2016).

3.6. Moisture content

According to USDA (2007), honey is graded into 3 different grades; A, B and C. Grade A 17.2%, grade B 18.6% and grade C 20%. The moisture contents of the honey samples were significantly ($p<0.05$) different from each other (Table 3). The moisture content of honey plays a critical role in its quality. Honey is very hydroscopic which means that it will absorb moisture from the air. The moisture content of honey is important because all unpasteurized honey contains wild yeast. Due to the high sugar concentration, this yeast will pose little risk in low moisture honey because osmosis will draw sufficient water from yeast to force them into dormancy. In honey that has a higher proportion of water, the yeast may survive and cause fermentation to begin in storage (Diacu and Tantaveanu, 2007). A larger percentage of the honey samples fell below the USDA standard, while samples obtained from Jagun, Sabo, Takie, Oja Oba and Ikose Market and LAUTECH Farm were within the grade A and B recommended by (USDA, 2007).

3.7. Ash content

The value of the ash content ranged from 0.32 to 1.02% (Table 3) which is higher than the values reported by Adebiyi et al. (2004) for some Nigerian honey. Most of the ash contents of the analyzed samples fell within the standard values of less than 0.6% specified by Codex Alimentarius Commission (1998), while other samples had higher values. The ash contents showed a significant difference for all the samples of honey analyzed at 5% probability level. According to Abu-Tarbousch et al. (1993), the ash content in honey is generally small and depends on nectar composition of predominant plant in their formation, the soil types in which the original nectar- bearing plant was located also influence the type and quantity of minerals present in the ash. Differences in variation of ash contents can be due to different geographical locations, different environmental conditions of producing regions and technological aspects involved in apicultural practices and processing of honey (Latorre et al., 2000).

3.8. Total reducing sugar content

Total reducing sugar content of honey samples ranged from 10.30 to 65.33% (Table 3). Total reducing sugars include monosaccharide units in form of laevulose, ketose sugar, and dextrose, aldose sugar. The total reducing sugar of honey samples differ from the Indian standard specification for extracted honey which prescribed the minimum level of 65% for total reducing sugar. Khatija and Ramanujan (1993) reported 72.66% to 75.30% of total reducing sugar from honey from Hyderabad. The fructose and glucose contents ranged from 4.27% to 33.87% and 4.43 to 32.17%, respectively (Table 3). Some samples differed significantly from others. Glucose result also showed a significant difference in all the samples except samples from Oja igbo, Oja oba, Owode, Iresaapa, Caretaker, Oja Titun and Iresaadu Market. Sugars in honey are the main factors in determining the tendency of honey to crystallize. Generally, the higher the glucose, the faster honey crystallizes, and the higher the fructose, the slower it crystallizes (Babarinde et al., 2011). Most of the honey samples had higher values of

fructose, thus indicating they are less susceptible to early crystallization. Factors such as molecular weight of honey, acidity and moisture content can also influence crystallization in honey (Gairola et al., 2013).

3.7. Total soluble solids (TSS)

The TSS level of the honey samples ranged from 70 to 78 °Brix (Table 3). Sample obtained from Jagun Market had the lowest brix and sample from California Market had the highest brix. The values of the brix were significantly different from one another. The values were within the range reported by Babarinde et al. (2011) of freshly harvested multiflora Nigeria honey.

3.8. Specific gravity

The specific gravity of honey samples ranged from 1.22 kg/L in honey obtained from Sanuaje Market to 1.55 kg/L in honey obtained from Caretaker Market (Table 4). The values were significantly ($p< 0.05$) different except for values from Arada and Ikose Market, which were not significantly different at 5% probability level. Specific gravity of honey is dependent mainly on water content and floral source Babarinde *et al.* (2011). Also according to the USDA (2009) recommendation, the specific gravity of honey is between 1.36 kg/L - 1.45 kg/L. The higher specific gravity of honey obtained from Caretaker Market could be as a result of either direct addition of water or exposure of the honey to air due to the hydroscopic nature of honey.

3.9. Viscosity

The values of viscosity obtained from honey samples in Ikoyi and Jagun Market ranged between 453.53 cP and 2788.30 cP, respectively (Table 4). The samples were significantly $p<0.05$ different in values. Temperature and moisture contents are the main determinant of viscosity. Viscosity of honey decreases rapidly as the temperature rises (Sharma, 2005). The higher values of viscosity found in honey samples obtained in Jagun, Otamakun, Aroowomole, Iresa- dudu, LAUTECH Farm, Sabo, Takie, Oja Titun, Caretaker, Sanuaje and Oja-Oba Markets might be due to various types of exposure to heat.

Table 1. Description of the locations of honey samples collected from Ogbomoso, south western Nigeria

Sample code	Location of sample collection	Local Government Area
HS1	Arada	Ogbomoso South
HS2	Oja-igbo	Ogbomoso North
HS3	Jagun	Ogbomoso North
HS4	Owode	Ogbomoso North
HS5	Sabo	Ogbomoso North
HS6	Oja- Titun	Ogbomoso North
HS7	Takie	Ogbomoso North
HS8	Ladoke Akintola University of Technology (LAUTECH)	Ogbomoso North
HS9	Iresapupa	Surulere
HS10	Iresadudu	Surulere
HS11	Ikoyi	Oriire
HS12	Arowomole	Ogbomoso South
HS13	California	Ogbomoso South
HS14	Lagbedu-Orile	Ogo-Oluwa
HS15	Sunsun	Ogbomoso South
HS16	Care-taker	Ogbomoso South

HS17	Sanuaje	Ogbomoso South
HS18	Oja-Oba	Ogbomoso South
HS19	Ikose	Ogbomoso North
HS20	Otamakun	Ogo-Oluwa

Table 2. Chemical Attributes of Nigerian honey samples sold in Ogbomoso

*Sample	HMF content (mg/kg)	Diastase Activity (Schade unit)	Acidity (%)	pH	Total phenols (GAE/l00 g)
HS1	16.0±0.00^{h}	22.30±0.00^{b}	0.03±0.00^{g}	4.70±0.00^{d}	65.5±0.00^{d}
HS2	14.0±0. 00^{h}	27.80±0.00^{a}	0.05±0.00^{e}	4.30±0.00^{h}	67.5±0.00^{b}
HS3	99.0±0.00^{a}	0.00±0.00^{i}	0.06±0.00^{d}	4.00±0.00^{k}	4.00±0.00^{p}
HS4	83.3±0.40^{c}	2.20±0.00^{h}	0.04±0.00^{f}	2.60±0.00^{n}	64.00±0.00^{d}
HS5	49.0±0.00^{ef}	4.50±0.00^{g}	0.05±0.00^{e}	4.10±0.00^{j}	12.50±0.00^{l}
HS6	73.0±0.00^{d}	2.20±0.0^{h}	0.06±0.00^{d}	4.30±0.00^{h}	10.50±0.00^{m}
HS7	58.0±0.00^{e}	6.70±0.00^{f}	0.09±0.00^{b}	4.50±0.00^{f}	18.00±0.00^{k}
HS8	96.0±0.00^{b}	0.00±0.00^{i}	0.07±0.00^{c}	6.20±0.00^{a}	6.50±0.00^{n}
HS9	62.0±0.00^{e}	11.10±0.00^{e}	0.06±0.00^{d}	4.20±0.00^{i}	24.50±0.00^{i}
HS10	86.0±0.00^{c}	2.20±0.00^{h}	0.05±0.00^{e}	4.20±0.00^{i}	5.00±0.00^{o}
HS11	36.0±0.00^{f}	16.70±0.00^{c}	0.06±0.00^{d}	6.10±0.00^{b}	28.00±0.00^{h}
HS12	62.0±0.00^{e}	11.10±0.00^{e}	0.06±0.00^{d}	4.60±0.00^{e}	21.00±0.00^{j}
HS13	24.0±0.00^{g}	27.80±0.00^{a}	0.07±0.00^{c}	4.60±0.00^{e}	66.00±0.00^{c}
HS14	36.0±0.00^{f}	16.70±0.00^{c}	0.06±0.00^{d}	3.80±0.00^{m}	37.00±0.00^{g}
HS15	24.0±0.00^{g}	22.30±0.00^{b}	0.03±0.00^{g}	4.30±0.00^{h}	61.00±0.00^{f}
HS16	62.0±0.00^{e}	13.40±0.00^{d}	0.07±0.00^{c}	4.40±0.00^{g}	22.50±0.00^{i}
HS17	73.0±0.00^{d}	11.10±0.00^{e}	0.06±0.00^{d}	4.40±0.00^{g}	22.50±0.00^{i}
HS18	44.0±0.00^{f}	22.30±0.00^{b}	0.07±0.00^{c}	3.90±0.00^{l}	62.00±0.00^{e}
HS19	24.0±0.00^{g}	27.80±0.00^{a}	0.09±0.00^{b}	5.50±0.00^{c}	68.50±0.00^{a}
HS20	96.0±0.00^{b}	0.00±0.00^{i}	0.10±0.00^{a}	4.30±0.00^{h}	5.00±0.00^{o}

Mean values within the same column with different superscripts are significantly ($p<0.05$) different at 5% probability level using Duncan multiple range test.

*Code: HS1. Arada HS2 Oja-igbo HS3. Jagun HS4. Owode HS5. Sabo HS6. Oja- Titun HS7. Takie HS8. LAUTECH Farm HS9. Iresapupa HS10. Iresadudu HS11. Ikoyi HS12. Arowomole HS13. Califonia HS14. Lagbedu-Orile HS15. Sunsun HS16.Care-taker HS17. Sanuaje HS18. Oja-Oba HS19. Ikose HS20. Otamokun

Table 3. Functional attributes of Nigerian honey samples sold in Ogbomoso

Sample	Moisture (%)	Total reducing sugars (%)	Glucose (%)	Fructose (%)	Ash (%)	Brix
HS1	15.76±0.00^{f}	62.83±0.06^{ab}	29.07±0.06^{e}	33.53±0.06^{a}	0.99±0.00^{b}	76.00±0.00^{b}
HS2	15.02±0.00^{h}	65.33±0.02^{a}	33.23±0.20^{a}	33.87±0.06^{a}	1.22±0.00^{a}	77.00±0.00^{ab}
HS3	18.91±0.00^{a}	11.27±0.15^{g}	4.04±0.21^{m}	6.70±0.00^{m}	0.85±0.00^{b}	70.33±0.58^{e}

HS4	17.02±0.00^{c}	34.23±0.12^{e}	29.57±0.25^{d}	14.37±0.12^{l}	0.52±0.00^{e}	73.00±0.00^{cd}
HS5	18.91±0.00^{a}	38.50±0.10^{e}	18.57±0.06^{j}	17.57±0.21^{j}	0.84±0.00^{b}	74.00±0.00^{c}
HS6	6.02±0.00^{l}	29.40±6.32^{f}	17.13±0.12^{j}	15.70±0.17^{k}	0.52±0.00^{e}	72.00±0.00^{d}
HS7	18.91±0.00^{a}	46.53±0.15^{d}	24.13±0.15g	21.57±0.15^{i}	0.84±0.00^{b}	75.00±0.00^{bc}
HS8	17.21±0.00^{b}	10.30±0.10^{g}	5.17±0.12^{lm}	4.73±0.06^{n}	0.52±0.00^{e}	72.00±0.00^{d}
HS9	10.99±0.00^{k}	50.37±0.21^{d}	23.57±0.21^{h}	25.77±0.12^{h}	0.55±0.00^{e}	73.67±0.58^{d}
HS10	11.06±0.00^{k}	36.07±0.12^{e}	16.73±0.6^{k}	19.30±0.10^{i}	0.39±0.00^{fg}	75.00±0.00^{bc}
HS11	12.9±0.00^{j}	56.57±0.15^{bc}	26.87± 0.12^{f}	29.27±0.06^{f}	0.42± 0.00^{f}	76.00± 0.00^{b}
HS12	14.96±0.00^{h}	49.07± 0.15^{d}	22.47±0.06^{h}	25.70± 0.10^{h}	0.59± 0.00^{d}	76.00± 0.00^{b}
HS13	13.79±0.00^{i}	64.77± 0.21^{ab}	32.17±0.15^{a}	32.37± 0.25^{b}	0.41±0.00^{f}	78.00±0.00^{a}
HS14	16.21± 0.00^{d}	55.93±1.88^{bc}	26.40± 0.10^{f}	27.70 ±0.10^{g}	0.32± 0.00^{g}	75.00±0.00^{bc}
HS15	15.35± 0.00^{g}	60.23±0.15^{b}	28.60±0.10^{e}	30.17± 0.06^{de}	0.35± 0.00^{g}	71.00±0.00^{e}
HS16	16.02±0.00^{e}	55.47±0.02^{c}	23.67±0.12^{h}	21.63±0.12^{i}	0.72±0.00^{c}	75.00±0.00^{bc}
HS17	17.09±0.00^{c}	38.57±0.21^{e}	19.17±0.15^{i}	18.37±0.06^{j}	0.50± 0.00^{e}	72.00±0.00^{d}
HS18	17.21±0.00^{b}	61.50±0.26^{ab}	29.90±0.10^{c}	30.73± 0.06^{d}	0.61±0.00^{d}	75.00±0.00^{bc}
HS19	18.92±0.00^{a}	65.10± 0.20^{a}	31.53± 0.06^{b}	31.67± 0.55^{c}	0.84± 0.00^{b}	76.00±0.01^{b}
HS20	13.91±0.00^{m}	11.00±0.17^{g}	6.27± 0.06^{l}	4.27±0.15^{n}	0.79± 1.00^{c}	72.00± 0.00^{d}

Mean values within the same column with different alphabet are significantly ($p < 0.05$) different at 5% probability level using Duncan multiple range test.

Table 4. Physical attributes of Nigerian honey samples sold in Ogbomoso

Sample	Specific Gravity (kg/L)	Viscosity (cP)
HS1	1.26±0.00^{l}	786.53±0.40^{p}
HS2	1.25± 0.00^{l}	987.23±0.70^{m}
HS3	1.33±0.00^{j}	2788.30±0.70^{a}
HS4	1.40±0.00^{h}	1207.10±0.75^{k}
HS5	1.42±0.00^{g}	1076.0±0.70^{l}
HS6	1.47±0. 00^{d}	1211.23±0.65^{j}
HS7	1.45±0.00^{e}	1408.43±0.45^{h}
HS8	1.29±0.00^{k}	1661.33±0.35^{e}
HS9	1.24±0.00^{m}	664.37±0.35^{q}
HS10	1.44±0.00^{f}	1632.97±0.45^{f}
HS11	1.53±0.00^{b}	453.53±0.35^{t}
HS12	1.49±0.00^{c}	1922.97±0.45^{c}
HS13	1.44±0.00^{f}	622.07±0.21^{r}
HS14	1.35±0.00^{i}	575.50±0.40^{s}
HS15	1.13±0.00^{o}	875.13±0.42^{o}

HS16	1.55 ± 0.00^{a}	1885.33 ± 0.25^{d}
HS17	1.22 ± 0.00^{n}	1492.30 ± 0.26^{g}
HS18	1.26 ± 0.00^{l}	1305.10 ± 0.36^{i}
HS19	1.33 ± 0.00^{j}	921.23 ± 0.35^{n}
HS20	1.31 ± 0.00^{j}	2596.00 ± 0.30^{b}

Code: HS1. Arada HS2 Oja-igbo HS3. Jagun HS4. Owode HS5. Sabo HS6. Oja- Titun HS7. Takie HS8. LAUTECH Farm HS9. Iresapupa HS10. Iresadudu HS11. Ikoyi HS12. Arowomole HS13. Califonia HS14. Lagbedu-Orile HS15. Sunsun HS16.Care-taker HS17. Sanuaje HS18. Oja-Oba HS19. Ikose HS20. Otamokun

4. Conclusions

The results obtained from this analysis revealed the chemical and functional attributes of the twenty honey samples obtained from markets in Ogbomoso region. In most of the samples, the parameters analyzed were within the limits established by Codex standard, honey regulations and USDA standard for honey. The moisture content of most of the honey samples fell below the standard with the exception of seven samples. The agreement of the acidity values of all the samples with standard indicates that no fermentation occurred in all the honey samples. This is an indication of good quality for the samples collected in this study. Some of the honey samples were in agreement with stipulated standard while a little other failed to meet the standard.

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ASSESSMENT OF THE PROTEOLYSIS AND MELTING OF MOZZARELLA CHEESE MADE WITH FROZEN BUFFALO MILK CURD

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ABSTRACT

The objective of this study was to evaluate the effect of the freezing time of fermented buffalo curd on the melting properties and on the proteolysis of mozzarella cheese stored under refrigeration. The treatments consisted of different frozen storage times of the fermented curd (0, 40, 80, 120 and 160 days), which were thaw on each time. Subsequently, the curd was used to produce mozzarella cheese, which was stored in a refrigerated environment, for the times of 10, 20 and 30 days. The cheese samples were subjected to analyses of their melting properties and ripening index by the chemical and electrophoretic methods. No effect of freezing the fermented curd on the ability of the cheese to melt was found, but the refrigerated storage time significantly influenced the increase in this parameter. Proteolysis was more intense in cheeses prepared from frozen curd, with the rate depending on the duration of the frozen storage. It was concluded that the use of frozen buffalo milk curd in the manufacture of mozzarella cheese could be a viable alternative to overcome seasonal shortages of buffalo milk.

1. Introduction

Buffalo milk presents higher fat, protein and calcium contents than cow's milk, giving higher industrial yields, and is therefore widely used in the manufacture of mozzarella cheese (Sindhu and Arora, 2011). However, buffalo milk shows seasonal production, making the supply of milk to make the products difficult at certain times of the year, especially in summer (Czerwenka *et al*., 2010; Gonçalves *et al*., 2016).

The use of freezing as a preservation method is well established for many food, but little attention has been given to the conservation of the buffalo curd for the subsequent manufacture of cheeses (Hussain *et al*., 2012).

Research has been carried out to evaluate the freezing of curd from the milk of other species, such as ewes (Picon *et al*., 2010) and goats (Picon *et al*., 2013). However, there is a void in the knowledge about frozen buffalo curd. The preservation of this product is essential to complement the lack of cheeses (mozzarella) during the

summer, when an increase in demand is observed.

The freezing of the fermented curd for mozzarella production and the freezing of the mozzarella at the end of the production process arise as viable alternatives to minimize those factors.

The mozzarella, originated in Italy, is a cheese with mass in filaments, produced with buffalo whole milk, exclusively (Ahmed *et al*., 2011). In Brazil, mozzarella is the cheese elaborated from filing of acidified curd (intermediary product, obtained from milk coagulation through rennet and/or other proper coagulating enzymes), complemented or not by the action of specific lactic bacteria, produced only with pasteurized milk, unlike the Italian production, that uses raw milk.

Freezing the food assists on the maintenance of its nutritional, sensorial and technologic features (Kuo and Gunasekaran, 2003). There are few studies on the effects of freezing on the physicochemical features of the mozzarella produced from fermented curd (Hussain *et al*., 2012). In the mozzarella industry, this technique has been avoided due to rheological changes (texture changes, protein denaturation and destruction of cell membranes) caused by the cold (Van Hekken *et al*., 2005; Kuo and Gunasekaran, 2009).

Thus the use of frozen curd could be a good alternative to solve the problem of the seasonality and regional difficulties of buffalo milk production, although studies are required to determine the effects of freezing the buffalo curd on proteolysis and melting capability of the mozzarella cheese. Due to limiting factors on the buffalo milk production, the freezing of the curd would present itself as a financially viable technique, as long as the final product maintains its features.

Meltability is associated with a change in phase when the cheese is heated, the fat changing from the solid to the liquid phase. A lot of factors can influence meltability in mozzarella cheese, such as the moisture, fat and salt contents, starter cultures, free water and proteolysis (Kindstedt *et al*., 2001).

Proteolysis is the degradation of protein (mainly casein (CN)) by endogenous enzymes (plasmin) and exogenous (microbial and from the curd), in the case of milk, resulting in the production of peptides and free amino acids (Walstra *et al*., 2006). Primary proteolysis is caused mainly by the proteolytic action of rennet or residual coagulant on the α_{s1}-casein (α_{s1}-CN) and, to a lesser extent, on the β-casein, producing peptides of high and medium molecular weight that can be evaluated by polyacrylamide gel electrophoresis in the presence of urea (urea-PAGE) or sodium dodecyl sulfate (SDS-PAGE) and by isoelectric focusing. On the other hand, secondary proteolysis is caused by macrobiotic peptidases and can be identified by means of reversed phase high performance liquid chromatography (RP-HPLC) (Fox, 2000; Walstra *et al*., 2001).

Studies on the proteolysis and meltability of cheeses produced from frozen buffalo curd could explain how the technology affected the cheese. Thus the aim of this study was to evaluate the effect of the time of frozen storage of the buffalo milk curd on the proteolysis and meltability of mozzarella cheese stored under refrigeration.

2. Materials and methods

2.1. Production of samples

The mozzarella cheeses were processed between September, 2012 and May, 2013. A total volume of 50 L of milk was used for each formulation with the fat content standardized to 4.0% (v/v) after preparing the formulations using the mechanical milk skimmer (GR, Goiânia, GO, Brazil), totalizing about 10 kg of cheese ate the end of the production process. The milk was pasteurized at 65 ºC $\pm$ 1 ºC/30 min and cooled to 35 ºC $\pm$ 2 ºC and then inoculated with 1.0 g of a freeze-dried mesophilic lactic

culture (DVS-R704 *Chr. Hansen*, Boge Allé, DK, Denmark) constituted of the species *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*. When the temperature reached 35 ± 1 ºC, 10 mL of 50% (w/v) calcium chloride (Coalhopar, Coalhos Bio Paraná LTDA, Alto Piquiri, PR, Brazil) and 20.0 mL of rennet (liquid rennet HA-LA®, Brazil - *Chr Hansen* – strength 1:3,000) were added. After coagulation of the mass in about 20 min, it was cut into approximately 1 cm sided cubes and stirred for 30 min until a cooked, firm curd was obtained. The whey was then drained off from the curd, and the latter allowed to ferment at room temperature (25 ºC ± 3 ºC) for 18 h, and then divided into five batches (treatments) as follows: (T1 - control, fresh); and the others were frozen at -20 ºC for 40 d (T2), 80 d (T3), 120 d (T4) and 160 d (T5). After the periods of freezing for each treatment the curds were melted on the refrigeration temperatures (5 ºC ± 2 ºC, cold air) and the curd was then sliced and submitted to the stringing process in water at 80 ºC ± 5 ºC and packed into appropriate moulds of 500 g. The cheeses were then salted in a 20% (w/v) NaCl brine for 1 h, before drying at 6 ºC ± 2 ºC for 12 h and finally vaccum packed (BS 320, R. Baião, Vila Casal Ubá, MG, Brazil) in heat-shrinkable polyethylene packaging, where they were kept at refrigeration temperature (5 ºC ± 2 ºC) (cold air) for 10, 20 and 30 days until the analysis. All the processes of curd freezing and cheese productions were conducted with three samples.

2.2. Chemical composition and physical-chemical description of the cheeses

The water percentage was determined by gravimetric method in stove at 105 ºC; ashes in oven at 550 ºC; fat content in dry extract (FDE) through the Gerber method and total Nitrogen through the Kjeldahl method. The pH was determined by a pHmeter (HI 221, Hanna Instruments, Woonsocket, Rhode Island, USA) and titratable acidity with solution of 0.1 M NaOH. All analysis were conducted in three samples of three cheese repetition to achieve the average values, according to methodologies described by the Association of Official Analytical Chemists (AOAC, 2012).

2.3. Meltability

The meltability was tested in triplicate using (26 mm diameter and 7 mm depth) cheese discs using an adaptation of Schreiber's method for cheese (Pizaia *et al*., 2003). The discs were heated at 105 ºC ± 2 ºC for 7 min, and the increase in disk diameter measured at six points and averaged. A value of 1.0 indicated no change in disk diameter and melt values increased by 1.0 for every 1.0 cm increase in diameter (Van Hekken *et al*., 2005). The meltability was determined according to Eq. (1):

$$\%M = \frac{A_F - A_i}{A_i \times 100} \quad (1)$$

Where:

M = Meltability;
A_F = final area (calculated from final average diameter);
A_i = initial area (calculated from initial average diameter).

2.4. Proteolysis

Cheese proteolysis was evaluated both chemically and electrophoretically. The chemical proteolysis was determined from the total nitrogen by the Kjeldahl method, non-protein nitrogen and non-casein nitrogen (AOAC, 2012). Proteolysis was estimated from the CN / true protein ratio, expressed as nitrogen (N)-protein equivalent using eqs. (2)-(4):

$$TP = (TN - NPN) \times 6{,}38 \quad (1)$$

$$CN = (TN - NCN) \times 6{,}38 \quad (2)$$

$$\text{Proteolysis} = \frac{CN}{TP} \quad (3)$$

Where:

TP = true protein;
TN = total nitrogen;
NPN = non-protein nitrogen;
CN = casein;
NCN = non-casein nitrogen.

The proteolysis extent index and proteolysis depth index were evaluated according to Pizaia *et al*. (2003) using eqs. (5)-(6):

$$\%PEI = \frac{NCN}{TN \times 100} \quad (4)$$

$$\%PDI = \frac{NPN}{TN \times 100} \quad (5)$$

Where:

PEI = proteolysis extent index;
PDI = proteolysis depth index.

SDS-PAGE was used as the technique to evaluate the protein and proteolysis. The concentration of the polyacrylamide gel was 4.9% (w/v) polyacrylamide (Sigma, St. Louis, MO, USA) in 0.125 M Tris-HCl buffer (Vetec, Duque de Caxias, RJ, Brazil), pH 6.8 and that of the separation gel consisted of 15.4% (w/v) polyacrylamide in 0.38 M Tris-HCl buffer, pH 8.8 plus 0.1% (w/v) SDS (Cromoline, Diadema, SP, Brazil) (Laemmli, 1970). The freeze dried cheese samples (2 $mg \cdot mL^{-1}$) were dissolved in 0.125 M Tris-HCl buffer, pH 6.8 in the presence of 0.1% (w/v) SDS and 5% (v/v) β-mercaptoethanol (Sigma, St. Louis, MO, USA). The samples were heated at 100 °C ± 1 ºC/3 min and 20 µL aliquots applied to the mini gels. Electrophoresis was carried out at 4 °C ± 2 ºC for 90 min at 250 V, 30 mA and 15 W (Apelex PS 304 Minipac II, France). The molecular mass standards used were: myosin (200.0 kDa), β-galactosidase (116.2 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.5 kDa) and aprotinin (6.5 kDa) (Bio-Rad, Hercules, CA, USA).

After the run, the proteins were fixed in 12% (w/v) trichloroacetic acid (TCA) (Sigma, St. Louis, MO, USA) solution for 30 min, and then stained using a 0.1% (w/v) Coomassie brilliant blue R250 (Vetec, Duque de Caxias, RJ, Brazil) dissolved in a mixture of 50% (v/v) ethanol (Vetec, Duque de Caxias, RJ, Brazil) and 12% (w/v) TCA, for 120 min. The gels were destained in a 30% (v/v) solution of ethanol with 7.5% (v/v) acetic acid (Vetec, Duque de Caxias, RJ, Brazil).

The gels were scanned and subsequently submitted to a second staining with silver nitrate (Sigma, St. Louis, MO, USA) (Bloom and Beier, 1987). After washing the gels three times with distilled water for 20 min each, they were immersed in 0.02% (w/v) sodium thiosulfate (Sigma, St. Louis, MO, USA) for 1 min and then in 0.1% (w/v) silver nitrate 0.1% for 30 min, with constant stirring and protection from the light. The proteins were visualized using a 12% (w/v) calcium carbonate (Sigma, St. Louis, MO, USA) solution, 40 µL sodium thiosulfate (Sigma, St. Louis, MO, USA) 2% (w/v) and 200 µL of formaldehyde (Vetec, Duque de Caxias, RJ, Brazil). The reaction was stopped with a 1% acetic acid solution (v/v) before scanning. The gels were photographed and digitalised.

2.5. Statistical analysis

A completely randomized (5 x 3) factorial experimental design was used with 5 treatments (frozen curd: 0, 40, 80, 120 and 160 days frozen) and 3 cheese refrigerated storage times (10, 20 and 30 days). The fresh curd was the control.

The R Development Core Team (2010) statistical program was used together with regression analyses. The variable sources were time frozen, refrigerated storage time

and the interactions between them, with *P*-value = 5%.

3. Results and discussion

Among the studied variables, an interaction ($P \leq 0,05$) between the refrigeration times of the cheeses and the freezing times of the fermented curd was observed only for titratable acidity. Significant differences ($P \leq 0,05$) in the chemical composition (Table 1) and the physicochemical features (figs. (1)-(2)) freezing times of the fermented curd were observed, comparing the freezing times of the fermented curd.

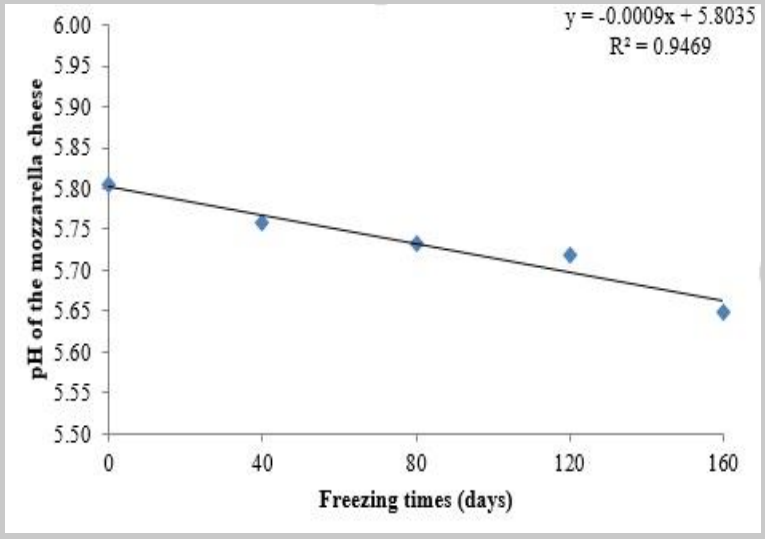

Figure 1. Behavior of the effect of the fermented curd freezing times on the pH of the mozzarella

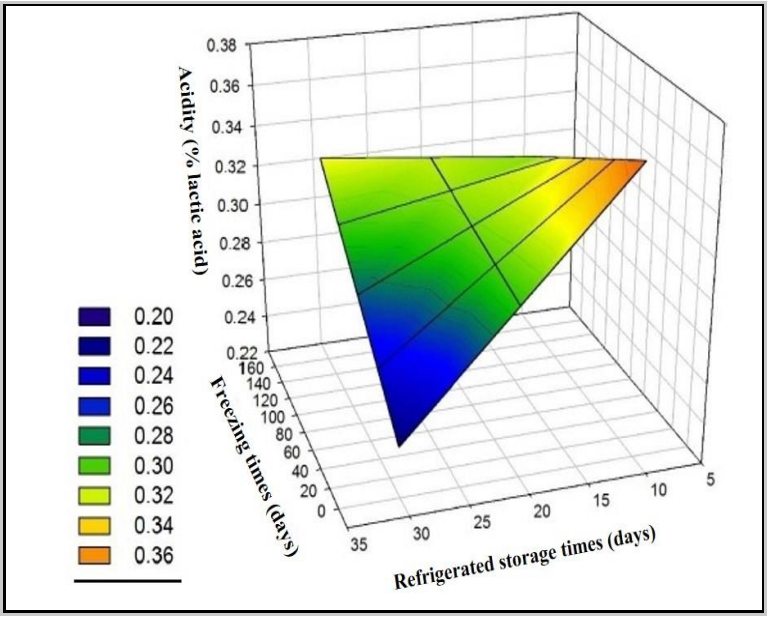

Figure 2. Response surface of the effects of the fermented curd freezing times on the acidity of the mozzarella cheeses stored in different times of refrigeration.

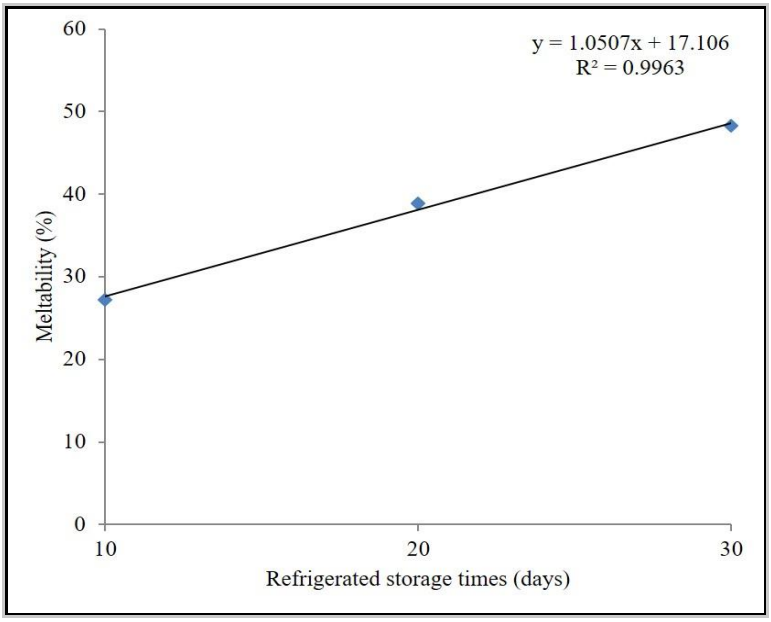

Figure 3. Behavior of the meltability of the buffalo mozzarella cheese made from frozen curd and storage at different times under refrigeration.

Quadratic effect was observed between the treatment for the water percentage, ashes and FDE and increasing linear effect for proteins (Table 1).

Table 1. Mean values, adjusted regression equations, coefficients of variation and determination (R^2) for chemical composition of the mozzarella cheese in different times of freezing of the fermented curd.

Parameters (%)	**Freezing times (days)**					**[3]CV (%)**	**Equations**	**[4]R^2**
	0	**40**	**80**	**120**	**160**			
Humidity	55.65	53.66	52.52	55.10	55.35	4.30	$\hat{Y}=0.0004C^2-0.06C+55.46$	0.75
Ashes	5.36	6.33	6.85	6.64	6.30	7.85	$\hat{Y}=0.0002C^2+0.03C+5.40$	0.96
[1]FDE	63.58	57.79	51.01	52.81	56.90	8.27	$\hat{Y}=0.0013C^2-0.25C+64.14$	0.95
Protein	19.47	19.80	20.10	21.32	21.83	7.80	$\hat{Y}=0.004C+19.95$	0.81
[2]EST	44.45	46.42	47.54	44.87	44.65	5.03	$\hat{Y}=0.0004C^2+0.06C+44.65$	0.76

[1] = Fat content in dry extract, [2] = Total dry extract, [3] = Variation coeficiente, [4] = Determination coeficiente, $\hat{Y}$ = asnwer variable, C = treatments (formulations)

Table 2. Average of proteolysis and extent index and depth index of the proteolysis of the buffalo mozzarella cheese made from frozen frozen curd and storage at different times under refrigeration.

Measurements	Freezing times (days)					[3]TR (Days)	Equations
	0	40	80	120	160		
Proteolysis	1.00	0.99	0.99	1.00	0.99	10	$\hat{Y}$=0.994
	1.00	0.99	0.98	1.00	0.99	20	$\hat{Y}$=0.992
	0.99	0.99	0.99	1.00	1.00	30	$\hat{Y}$=0.994
[1]PEI	2.00	3.15	2.53	2.78	2.73	10	$\hat{Y}$=2.64
	2.63	2.31	3.57	1.89	2.98	20	$\hat{Y}$=2.68
	2.50	2.89	2.92	2.33	2.92	30	$\hat{Y}$=2.71
[2]PDI	2.24	1.89	1.89	2.05	2.02	10	$\hat{Y}$=2.02
	2.32	1.72	2.23	1.91	1.98	20	$\hat{Y}$=2.03
	1.72	2.44	1.84	2.10	3.11	30	$\hat{Y}$=2.24

[1] = proteolysis extent index, [2] = proteolysis depth index, [3] = refrigeration times, $\hat{Y}$ = asnwer variable.

The cheeses obtained from the frozen fermented curds displayed smaller percentages of moisture and FDE and larger content of ashes and protein. This can be explained by structural changes in the micelles of casein during the process of freezing, which may lead to a reduction in the capacity of water retention, a larger presence of minerals that cause strong protein-protein interactions, favoring the syneresis (Damodaran *et al*, 2010), changes in the lipoprotein membranes of the fat globules, facilitating the loss of this component on the filming process (Rudan *et al*., 1999), and hydrolyses of the proteins to amino acids and peptides (Ahmed *et al*., 2011).

There was a linear decrease on the pH of the mozzarella cheeses related to the freezing times of the fermented curd (Fig. 1), with slight decrease of the pH, ranging from 5.81 to 5.65, due to the freezing temperatures used, which may have allowed the degradation of the lactose to lactic acid through bacteria action. The freezing time of the fermented curd caused an acidity rise (Fig. 2).

The cheeses produced with fermented curd frozen for 40 days displayed increased acidity after 10 days of refrigerated storage (0.35% of lactic acid) when compared to the cheeses with fresh curd.

The meltability of the buffalo mozzarella cheese was not affected by the time the curd was frozen ($P > 0.05$), but the storage time of the cheese did affect it ($P \leq 0.05$), increasing this parameter (Fig. 3).

Cheese meltability improved with refrigerated storage time, increasing from 27.2% (10 days) to 38.9% (20 days) and finally 48.3% (30 days). This was probably a consequence of the weakening of the cheese protein matrix, mostly due to the proteolytic action of the rennet or residual coagulant (primary proteolysis), increasing cheese meltability, water retention and free oil but decreasing firmness and elasticity (Fox, 1989).

Right after processing, the mozzarella displays high molecular mass proteins,

which makes the increase of melting capability difficult. The refrigerated storage time facilitates the proteins hydrolysis, making them more soluble and with better melting capability (Kindstedt, 1993). This can be explained by the changes in the types of interactions between the water molecules and the protein matrix (Ray *et al*., 2016).

The loss of solubility of the casein can also be explained by the effects of the pH, in which results inferior to 6.0 cause that, with decrease of the melting capability. Thus, the cheeses pH values (Fig. 1), in a certain way, influenced on the increase of melting capability during the studied refrigeration period, for they enhance the Chymosin retention within the structure.

According to Laurienzo *et al*. (2007), after 21 days of storage the fresh mozzarella cheese showed changes in its microscopic structure, mostly in the protein matrix, with losses in the protein fiber characteristics, increasing their volume and thus increasing meltability.

The mozzarella cheese made from frozen curd presented the same behavior as that made from fresh curd. The meltablity increased with increase in the refrigerated storage time. These results indicate that the frozen curd could be used as a technological tool, since meltability is one of the determining factors of cheese quality and consumer acceptability.

The PEI and PDI were not affected ($P > 0.05$) by refrigeration and time frozen (Table 2). These inexpressive effects occurred due to the low temperature (-20 ºC $\pm$ 2 ºC) used during the fermented curd freezing periods, which hinders the proteolytic enzymes action, as well as the small period of refrigeration (10, 20 and 30 days) of the cheeses, which doesn't characterize them as highly matured cheeses and with significant proteolytic alterations.

The PEI is characterized by primary proteolysis originating peptides of high and average molecular mass (Fox, 1989), while the PDI covers Nitrogenous substances of low molecular mass (Gutierrez *et al*., 2004), Thus, these are indexes that do not totally reflect the transformations that the proteins underwent during the studied freezing and refrigerated storage periods.

Despite the increase in meltability, chemical proteolysis was not observed. It should be highlighted that by chemical methodology one can provide global information about the proteolytic activity of various agents. The effects of the freezing and the refrigeration of the fermented curd on the mozzarella cheese features must be studied by a larger diversity of analytic methods, because according to Ray *et al*. (2016) they don't depend on only one marker.

Thus to characterize proteolysis, one needs to isolate, identify and quantify all the substances formed. These characteristics can be evaluated by electrophoresis. From the electrophoretic profiles of the cheeses obtained from fresh (T1) and frozen curd (T2, T3, T4 and T5) during the refrigerated storage (10, 20 and 30 days) and stained using Blue Coomassie, the presence of alfa$_s$-casein (α_s-CN), beta-casein (β-CN), kappa-casein (κ-CN), para-kappa-casein (para-κ-CN) and peptides produced during cheese making and storage time was observed (Fig. 4).

The cheeses obtained from curd frozen for 80 days (T3), showed intensive peptide formation resulting from proteolysis during 30 days of refrigerated storage, and during this period, the greater the intensity of peptide formation, with bigger degradation of α_{s1}-CN. The residual coagulant (Chymosin) used during the cheeses production, also present in the curd, causes the casein degradation, specifically acting on the α_{s1}-CN and lesser on the β-CN during the cheeses storage (Feeney *et al*., 2002; Pino *et al*., 2009).

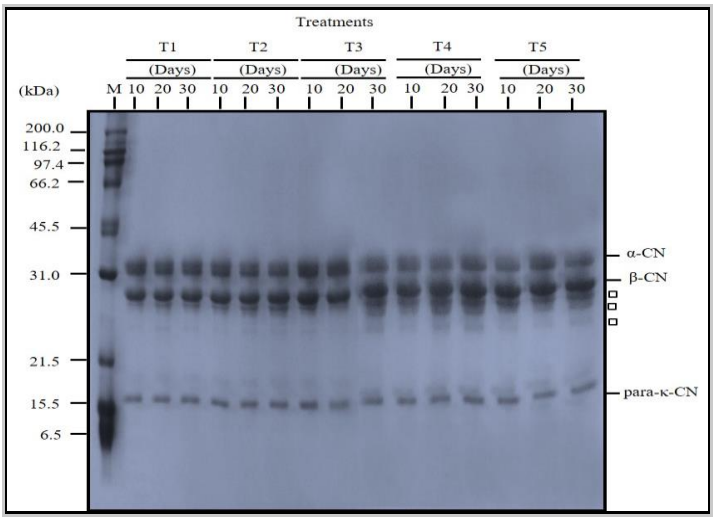

Figure 4. Electrophoretic profile (SDS-PAGE) of the buffalo mozzarella cheese made from frozen curd after revelation with Coomassie Blue (20 µg of protein/well). M - Standard molecular weigh; T1 - control, fresh; T2 - frozen curd for 40 days; T3 - frozen curd for 80 days; T4 - frozen curd for 120 days and T5 - frozen curd for 160 days. Cheese storage on 10, 20 and 30 days over 5°C ± 2 ºC of temperature; alfa$_s$-casein (α_s-CN); beta-casein (β-CN); kappa-casein (κ-CN); para-kappa-casein (para-κ-CN); □ - presence of peptides.

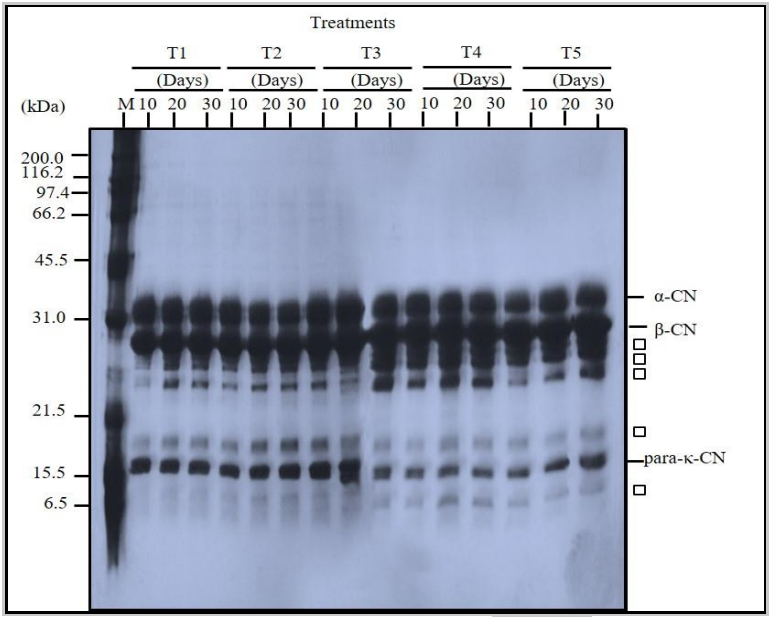

Figure 5. Electrophoretic profile (SDS-PAGE) of the buffalo mozzarella cheese made from frozen curd after revelation with silver nitrate (20 µg of protein/well). M - Standard molecular weigh; T1 - control, fresh; T2 - frozen curd for 40 days; T3 - frozen curd for 80 days; T4 - frozen curd for 120 days and T5 - frozen curd for 160 days. Cheese storage on 10, 20 and 30 days over 5°C ± 2 ºC of temperature. alfa$_s$-casein (α_s-CN); beta-casein (β-CN); kappa-casein (κ-CN); para-kappa-casein (para-κ-CN); γ_1-casein (γ_1-CN); γ_2-casein (γ_2-CN); γ_3-casein (γ_3-CN); β-casein f(69-209) (β-CN f(69-209)); □ - presence of peptides.

The para-κ-CN peptides were pronounced (nearly 15.5 kDa) in all treatments. The dominant action of rennet in cheese making is specific and hydrolyzes the κ-CN, breaking down the colloidal stability of the casein micelle (curd formation). Chymosin acts specifically on the phenylalanine$_{105}$-metionine$_{106}$ (Phe$_{105}$-Met$_{106}$) bond and two fragments are formed: para-κ-CN and a glucomacropeptide (GMP). Most of the GMP is eliminated in the whey, but the para-κ-CN remains in the casein micelle, and is incorporated into the cheese. When the aim is to obtain greater sensitivity in identifying the peptide fractions originating from protein degradation, silver nitrate is used (Fig. 5).

The silver nitrate technique provided more distinctness in detecting the peptides originating from proteolysis, with the emergence of new fractions not visualized in the gel subjected to staining with Coomassie blue, such as the peptides observed in 25 kDa, in the range between 21.5 kDa and 6.5 kDa, results of the Plasmin action, the curd and the microbial enzymes (Petrella *et al*., 2015).

The peptides could be generated by the action of the Plasmin that acts on the casein, specifically on the β-CN (Fox *et al*., 1994) and on the α_{s2}-CN (Le Bars and Gripon., 1989), cleaving the β-CN in the peptide bonds between the amino acids Lys28-Lys29, Lys105-His106 and Lys107-Glu108, with the formation of fragments of β-CN, f29-209 (γ1-CN), f106-209 (γ2-CN) and f108-209 (γ3-CN) (Petrella *et al*., 2015), visualized in this study.

In addition to better visualization, mostly in the case of T3 during the 30 days of refrigerated storage, it was shown that proteolysis was intensified by increased frozen storage of the curd and increased refrigerated storage of the cheese. From this period on, we observed the structural weakening of the para-κ-CN, result of the primary action of the hydrolysis of the α_{s1}-CN by the Chymosin on the peptide bonds between the amino acids Phe23-Phe24, with increase of the rupture resistence of the cheeses (Feeney *et al*., 2002) and enhance in the melting capability, especially with the increase of the storage time (Fig. 3).

Alichanidis *et al*. (1981) studied Teleme cheese made from frozen curd and stored at -20 ºC for 30, 60 and 180 days and evaluated α_s-CN and β-CN degradation during the maturation time using electrophoresis. It was observed that 83.75% of the α_s-CN was degraded in the cheese produced from frozen curd after 4 months, while in the control treatment it was only 74.25%. On the other hand, β-CN degradation was less intense, being 28.33% after 4 months in the cheese produced from frozen curd and 12.22% in the control treatment.

Petrella *et al*. (2015) studied the effects of proteolysis on the casein of buffalo mozzarella, aiming to find specific markers for each buffalo species. SDS-PAGE showed itself efficient on the separation and identification of the protein fractions and peptides resulting from the proteolic degradation of the casein, with samples displaying similar and different fractions to the molecular mass marker. The β-CN degradation by the Plasmin caused the formation of low molecular mass peptides (17.5 kDa), denominated β-CN f(69-209), also visualized on the present study.

In this study, electrophoresis was effective in identifying protein degradation in the mozzarella cheese made with frozen curd and stored under refrigeration, complementing the results obtained by the chemical methods studied.

The protein degradation rate is strongly associated with the access to the substrate by proteolytic enzymes, which depends on the structure of the cheese protein matrix (Park, 2001). The use of frozen curd in buffalo milk mozzarella cheese-making could be used to overcome the seasonality of buffalo milk, since even with the proteolysis identified by electrophoresis, it

did not show excessive melting, which would make it unfit for consumption. The period of up to 80 days of freezing of the fermented curd showed itself viable for the mozzarella cheese production, being considered the most efficient, for there were no physical or chemical alterations on the cheeses during the period studied, even though it causes small proteolytic alterations on the cheeses.

4. Conclusions

The freezing time of the fermented curd showed significant effects on the chemical composition and on the physicochemical features evaluated on the mozzarella cheeses, causing structural modification on the casein micelles, with alterations on the moisture, ashes, FDE and protein. There was an elevation of the acidity and a linear decrease on the pH of the samples, causing variation on the technologic characteristics of the cheeses.

The melting was influenced by the time of refrigeration, with protein hydrolysis, affected by the low pH values (< 6.0), enhancing the melting capability.

The low temperatures used on the fermented curd freezing and the small period on the refrigeration of the cheese did not cause significant changes on the proteolysis indexes, PEI and PDI.

SDS-PAGE was efficient to separate and identify the protein fractions and peptides resulting from the proteolysis during the times of freezing of the fermented curd and refrigeration of the produced cheeses, thus, the freezing of the fermented curds showed itself viable for the mozzarella cheese production.

5. References

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OPTIMISATION OF A READY TO USE 'NUTRITIOUS MIX' INCORPORATING INDIAN HERBS USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Indian medicinal herbs are the natural and healthy alternative source of medications possessing side effects for various ailments. There incorporation in food products can make it both nutritious and healthful. Nutritious mix was formulated as an instant food that can be reconstituted for consumption effortlessly. The purpose of the study was to optimise the amounts of Indian herbs as functional foods for incorporation in the powder to enhance its nutritional and functional properties. RSM (response surface methodology) and CCRD (central composite rotable design) were utilised for optimisation with three process variables (namely, amounts of apple powder, *Rauvolfia serpentina* and black cumin seeds) and potassium, sodium, fibre and overall acceptability as response variables. The response surface plots along with regression models were produced and regression coefficients and lack of fit tests were used to test the adequacy. The optimum levels that were attained for in range potassium (477.71 mg), minimum sodium (39.85 mg), maximum fibre (4.09 g) and maximum overall acceptability (87.61) were: 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder. Optimum recipe was nutritionally adequate and highly acceptable. Nutritious mix can provide beneficial roles to the people in maintaining their health without changing their regular diet patterns.

1. Introduction

Medicinal plants are regaining importance as a result of side effects caused by modern synthetic drugs. However, herbal medicines have sustained to be in demand among the developing countries as a result of being easily accessible, cost effective and culturally acceptable (Sewell and Rafieian-Kopaei, 2014). Herbal medicine remains to be the core of approximately 75 to 80 percent world's population, mostly among the developing world, for primary health care as a result of having enhanced cultural acceptance, being more compatible with the human's body, and possessing fewer side effects (Vidyarthi *et al.*, 2013). India is an immense repository of medicinal plants being traditionally utilised in the treatment of various ailments (Agrawal *et al.*, 2010). Hypertension is a leading public health problem worldwide. Chemical medicines for hypertension generally cause side effects

making the usage of medicinal herbs necessary (Pourjabali *et al.,* 2017). There are many scientifically studied and frequently used naturally occurring medicinal plants for the management of hypertension including Black cumin seeds, Rauwolfia and others (Agrawal *et al.,* 2010).

The roots, leaves, seeds, fruits and juice of *Rauvolfia serpentina* having medicinal benefits have drawn the attention of those practicing indigenous therapies. It has been used as a therapy for combating anxiety, epilepsy, excitement, gastrointestinal disorders, hypertension, insanity, mental agitation, traumas, schizophrenia, sedative insomnia (Malviya and Sason, 2016), body aches, burns and skin diseases (Poonam *et al.,* 2013). Current scientific researches on black cumin seeds ((*Nigella sativa* L.) and its oil have shown numerous bioactivities for the plant including anticarcinogenic, antihyperlipidemic, anti-inflammatory, antipyretic and analgesic, antiulcer, antibacterial and antifungal, antihypertensive, hepatoprotective and antioxidant activities as well that includes scavenging the reactive species of oxygen, preventing rheumatoid arthritis in rat models (Toma *et al.*, 2015).

In recent times, the rising health issues have resulted in the transference towards the optimal nutrition diet. Thus, food manufacturers are tended to produce such food products that can satisfy both consumer's appetite and desires for health promotion (Olaiya *et al.,* 2016). Nutraceuticals have come out to be an alternative source of the modern medicines and have shown positive results in decreasing the conventional medicines requirement along with reducing the possibilities of adverse effects (Sharma *et al.,* 2017). Currently, nutraceuticals and functional foods have gained the attention as potential alternative therapies in the hypertension treatment (Chen *et al.,* 2009). Thus, incorporation of medicinal herbs like *Rauvolfia serpentina* and black cumin seeds as nutraceuticals can come out to be a potential alternative source of medicines for hypertension management. Additionally, incorporation of heart healthy food like apple can help in enriching the food product's health benefits.

In spite of having highly beneficial roles in several diseases, incorporation of above mentioned herbs and apples in higher quantities can compromise the overall acceptance of various sensory attributes of developed food product. Therefore, there is a need for such techniques that can help in getting optimum solutions to produce a recipe which is adequate from nutritional point of view and is accepted organoleptically as well. Process optimisation is the one of current techniques being utilised in the formulation of optimum food products with increased nutritional properties. "Response surface methodology (RSM) is a powerful mathematical model with a collection of statistical techniques where in, interactions between numerous process variables can be recognized with fewer experimental trials. It is extensively used to study and optimize the operational variables for experiment designing, model developing and factors and conditions optimization (Karuppaiya *et al.*, 2010)".

Therefore, in the present study, a ready to use nutritious mix incorporating Indian herbs, appropriate for hypertensive people was formulated as instant food with the objective to get the statistically valid optimum combination of amount of apple powder, *Rauvolfia serpentina* and black cumin seeds powder as process variables for their incorporation and in range potassium, minimum sodium, maximum fibre and overall acceptability as response variables through CCRD of response surface methodology.

2. Materials and methods

The present work was done in Banasthali Vidyapith, Rajasthan, India, during the time span of July, 2014 to April, 2015. The raw ingredients and apple as functional food were purchased from Banasthali Vidyapith's local

market. Black cumin seeds of brand with a good repute were acquired from general store of Ghaziabad whereas *Rauvolfia serpentina* was obtained from a reputed ayurvedic pharmacy.

2.1. Formulation of nutritious mix

Rauvolfia serpentina powder (0.5 g), black cumin seeds powder (5 g), apple powder (10 g), tomato powder (5 g), whole wheat flour (15 g) and roasted bengal gram (15 g) were incorporated for the preparation of nutritious mix. Apples were cut into thin slices, blanched, oven dried at 60°C for 48 hours and then powdered. Black cumin seeds were cleaned and powdered. No specific treatment was given to root powder of *Rauvolfia serpentina* for the purpose of product development. Drying of tomatoes and tamarind was done at 60°C in an oven for 2 days and then were grinded. Roasted bengal gram was grinded to make powder. Black cumin seeds powder and wheat flour were roasted followed by addition of apple powder, *Rauvolfia serpentina* powder, tomato powder and tamarind powder proportionally to make nutritious mix. Auto seal sachets were used for storage of the prepared mix and these were kept in container being air tight. Sensory analysis of reconstituted thick drink was conducted. Reconstitution was done by addition of 100 ml butter milk (*Saras*, plain buttermilk) to the weighed quantity of 25 g nutritious mix and a pinch of powder of cumin seeds after roasting was mixed to it. The formulation of total product of 50g was done which was sufficient for a couple of servings.

2.2. Design of experiments

Developed food product was process optimised through RSM. RSM consists of statistical and mathematical techniques that are beneficial in development, improvement and optimisation procedure (Carley *et al.,* 2004). CCRD comprising of 3 independent variables (process variables) at 5 levels was utilised to define the optimum conditions in formulating nutritious mix as presented in table 1. Twenty experimental runs were generated as a result

Table 1. Levels of process factors to optimise nutritious mix

	Name	Units	-1 level	+1 level	-alpha	+alpha
A	Apple powder	G	5.0	15.0	1.591040	18.4090000
B	*Rauvolfia serpentina*	G	0.3	0.7	0.163641	0.836359
C	Black cumin seeds powder	G	3.0	10.0	0.613725	12.386300

when replication was carried at the center point (0) combination for six times. CCRD comprises of 3 points that are factorial points, centre points and star points and these let to estimate the curvature. The distance in-between the centre of design space and star point is ±α (Singh *et al.,* 2007). Depending upon the one-at-a-time preliminary experiments, the critical factors (process variables), amounts each of apple powder, *Rauvolfia serpentina* and black cumin seeds powder were selected for process optimisation. As per the central composite rotable design, the experiments number in totality is $(2)^n$ + 2n + central points, where n stands for sum total of variables. In present study, there are 3 variables in total for which the experiments' total number for every critical factor will be 20. The codes, -α, -1, 0, 1 and α were given for different 5 levels in every experiment; where $\alpha=2^{n/4}=2^{3/4}$ = 1.682. Thus, the codes were -1.682 (lowest), 0 (middle) and 1.682 (highest) for process variables. Each of

the critical factors was analysed for its effect on the dependent variables (response variables)– calculated potassium, calculated sodium, fibre and overall acceptability. 'Design-Expert software (9.0)' (Statease Inc., Minneapolis, MN, USA) came out to generate 20 sample combinations (table 2) by the use of design

matrix and combinations of variables in experimental runs. Each one of the sample combinations was produced in food preparation laboratory of Banasthali Vidyapith. The values for dependent variable, fibre were estimated through laboratory analysis whereas calculation of potassium and sodium content was done by the use of values provided in Nutritive Value of Indian Food (Gopalan *et al.,* 2007). The semi trained panelists were asked to score in between 1 to 100 depending upon liking of each combination for overall acceptability. The data sheet of the software was entered with all of these values. Order for carrying out the experiments was random.

Table 2. Experimental designs generated and observed responses of nutritious mix

	Generated			Estimated			
S. No.	**Apple powder (g)**	***Rauvolfia serpentina* (g)**	**Black cumin seeds powder (g)**	**Potassium (mg)**	**Sodium (mg)**	**Fibre (g)**	**Overall Acceptability**
1	10.00	0.50	12.39	535.35	51.17	3.97	86.53
2	10.00	0.50	6.50	431.35	44.32	2.80	88.73
3	5.00	0.30	10.00	492.52	39.90	3.74	85.40
4	10.00	0.84	6.50	431.35	44.32	3.42	86.60
5	10.00	0.50	6.50	431.35	44.32	3.01	85.80
6	10.00	0.50	0.61	327.33	37.80	2.01	86.80
7	15.00	0.30	10.00	493.78	57.02	3.92	88.00
8	18.41	0.50	6.50	432.39	58.70	2.94	88.60
9	10.00	0.50	6.50	431.35	44.32	3.52	87.53
10	15.00	0.30	3.00	370.18	49.08	2.04	89.53
11	10.00	0.50	6.50	431.35	44.32	2.98	88.66
12	10.00	0.50	6.50	431.35	44.32	3.28	86.06
13	5.00	0.70	10.00	492.52	39.90	4.32	87.33
14	5.00	0.70	3.00	368.91	31.96	2.04	88.06
15	10.00	0.16	6.50	431.35	44.32	2.81	86.60
16	1.59	0.50	6.50	430.28	29.92	3.02	88.13
17	15.00	0.70	3.00	470.18	49.08	3.16	85.80
18	15.00	0.70	10.00	493.78	57.02	4.97	87.60
19	10.00	0.50	6.50	431.35	44.32	2.96	87.13
20	5.00	0.30	3.00	368.91	31.96	2.08	88.46

2.3. Data analysis and optimization

The data obtained by performing experiments on different combinations were then dispensed for a second order polynomial regression analysis by the use of least square regression method and the analysis of the significant ($p<0.05$) effect of all the process variables on the responses was conducted. The second order polynomial equation given below can define the system behaviour:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x^2 + \sum\sum \beta_{ij} x_i x_j \quad (1)$$

Where Y stands for predicted response, β_0 for the interception coefficients, β_i for the linear term, β_{ii} for the quadratic term, β_{ij} for the interaction term and x_i and x_j are representatives of the levels coded for process variables. Goodness of fit and the significance of linear,

quadratic and interaction effects were calculated through the ANOVA of the regression equation. The independent variables for ANOVA were amounts of apple powder, *Rauvolfia serpentina* and black cumin seeds powder whereas potassium, sodium, fibre and overall acceptability were the dependent ones. Estimation of the validity attained of the models was the function of their coefficients of determination (R^2) values and the lack of fit analysis. A good model should be significant and lack of fit should be insignificant. The value of predicted R^2 should be in reasonable agreement with adjusted R^2. It can be described as the ratio of explained variation which was a degree of fit measure (Chan *et al.,* 2009). The coefficient of variation (CV) can be defined as the dimensionless numeral that measures the degree of variability relative to the mean. Various interactions of any two independent variables along with hold of the third variable's value at the midpoint are depicted through generation of response surfaces and contour plots. Accuracy in geometrical representation as well as useful information accuracy is provided about the system behaviour within the experimental design by the generated contour plots. The aim of optimisation process was to find the levels of process variables that would give potassium, sodium, fibre and overall acceptability as per the set goals. Design-Expert Software's (9.0) numerical optimisation technique was utilised for the concurrent optimisation of these responses. As evident from table 3, desired goals and responses were chosen for each factor in accordance to which the software generated certain optimum solutions. An optimum solution with the highest desirability was chosen as the optimised recipe. This optimised recipe was formulated in food preparation laboratory and further analysis of its nutritional properties was carried out.

Table 3. Optimisation criteria for different process variables and response variables of nutritious mix

Factors/responses	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Apple powder (g)	In range	5.00	15.00	1.00	1.00	3.00
Rauvolfia serpentina (g)	In range	0.30	0.70	1.00	1.00	3.00
Black cumin seeds powder (g)	In range	3.00	10.00	1.00	1.00	3.00
Potassium (mg)	In range	327.33	535.35	1.00	1.00	3.00
Sodium (mg)	Minimize	29.92	58.70	1.00	1.00	3.00
Fibre (g)	Maximize	2.01	4.97	1.00	1.00	3.00
Overall acceptability	Maximize	85.40	89.53	1.00	1.00	3.00

2.4. Sensory analysis

A selection of semi trained panel of 15 members was done using triangle test to conduct the sensory evaluation (Jellinek, 1985). The overall acceptability (dependent variable of process optimisation) of produced combinations of nutritious mix was evaluated through 100 point scale. This scale was utilised to acquire fitness of the model for overall acceptability in process optimisation.

2.5. Nutritional analysis

Nutritional evaluation was conducted of the optimised recipe only. Estimations of moisture and ash were done by standard AOAC (2002) procedures. Semiautomatic instrumentation technique was utilised for protein and fat

analysis where, assessment of protein was done through microkjeldahl method using Kel Plus (model no. KES06L, manufactured by Pelican, India) and fat was analysed through soxhlet method by the use of Socs Plus (model no SCS6, manufactured by Pelican, India). Carbohydrate was calculated using substraction method and estimation of crude fibre was done through acid alkali digestion method (AOAC, 2002). Iron through Wong's method and vitamin C and calcium using titrametric methods were estimated (NIN, 2003).

3. Results and discussion

3.1. Results

3.1.1. Optimisation of parameters for ANOVA

Selection of a suitable model for a response to compare the models on the basis of p-values was done by fit summary statistics. The model is said to be "significant if the p value comes to be <0.05". ANOVA is importantly used to evaluate whether the regression model and individual model coefficients are significant and the goodness of fit of regression model (Fentie *et al.,* 2014). The results of ANOVA for the independent variables' effect on potassium specified that, the two factor interaction design model (2FI) had a significant ($p<0.05$) effect on potassium (dependent variable). The effect of independent variables on sodium indicated that the quadratic model had significant ($p<0.05$) effect on sodium as a dependent variable. Effect of independent variables on fibre depicted that, the linear model had a significant ($p<0.05$) effect on fibre as a dependent variable. Lack of fit had non-significant ($p>0.05$) effect on the model, suggesting that model fits the data well. The model (2FI) had a non-significant effect on overall acceptability, which was a response variable, when observed with respect to the process variables. Lack of fit had non-significant ($p>0.05$) effect on the model for this response, depicting that the model fit the data well.

3.1.2. Optimisation of parameters for regression coefficients (R^2)

Table 4 represents the parameters acquired by fitting of potassium, sodium, fibre and overall acceptability data. It also presents regression coefficients of model's intercept, linear, quadratic and cross product terms. The coefficient of determination was utilised to evaluate if the model is fit and adeuate. The model with the higher order polynomial where the model is significant is said to be a suitable model. The nearer is the R^2 value towards the unity, the better is the empirical model said to fit the actual data (Zaibunnisa *et al.,* 2009). R^2 value for sodium was 1.00 which suggested that the model completely fits the actual data. Gan *et al.* (2007) recommended that to obtain good fit model, value of R^2 should be at least 80% (R^2 = 0.80). R^2 value for fibre was 0.84 suggesting a good fit of model. Evidence indicated that generated models were highly adequate if the value of R^2 was > 90% (R^2 >0.90) (Das *et al.,* 2012; Demirel and Kayan, 2012; Seth and Rajamanickam, 2012). R^2 value for potassium was 0.92 suggesting the model to be highly adequate. The model's R^2 value denotes the "proportion of variation in the model rather than random error". The regression model could explain 92% of variations in potassium content, 84% of variations in fibre content, 48% variations in overall acceptability and no variation in sodium content of nutritious mix (table 4). The results of being precise and reliable were depicted by lesser CV values of potassium, sodium and overall acceptability. The greater CV values of fibre revealed the results to be comparatively less precise and reliable.

Table 4. Regression coefficients of predicted quadratic polynomial models of nutritious mix (generated by design expert)

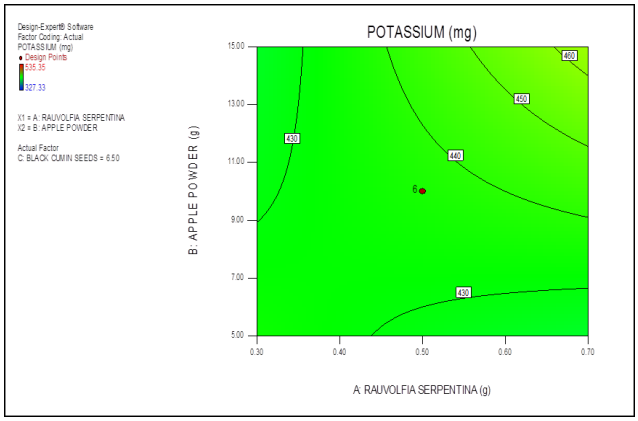
Coefficient	Potassium	Sodium	Fibre	Overall acceptability
Intercept	436.350	44.320	3.150	87.370
Linear				
A	7.320	-4.625E-015	0.270	-0.190
B	7.950	8.560	0.130	0.180
C	54.500	3.970	0.800	-0.290
Quadratic				
A^2		0.024		
B^2		0.021		
C^2		0.083		
Cross product				
AB	12.500	5.321E-015		-0.710
AC	-12.500	5.782E-015		0.710
BC	-12.500	6.647E-015		0.510
R^2	0.928	1.000	0.846	0.482
Adjusted R^2	0.894	0.999	0.817	0.243
CV%	3.790	0.150	10.71	1.150

3.1.3. Effect of process conditions for calculated potassium

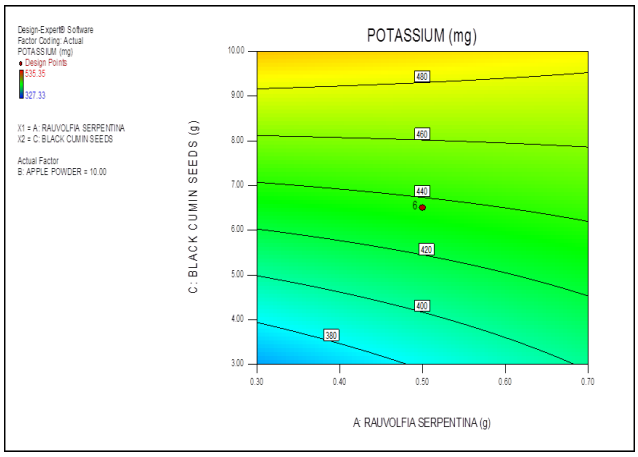
Table 2 depicts the observations for potassium along with the different combination of independent variables. The process variables' effect on potassium as a response of nutritious mix is described by the regression equation given as:

Potassium= 258.95181 + 27.68308 * *Rauvolfia serpentina* - 0.015678 * Apple powder + 31.64362 * Black cumin seeds powder + 12.50000 * *Rauvolfia serpentina* * Apple powder -17.85714 * *Rauvolfia serpentina* * Black cumin seeds powder - 0.71443 * Apple powder * Black cumin seeds powder

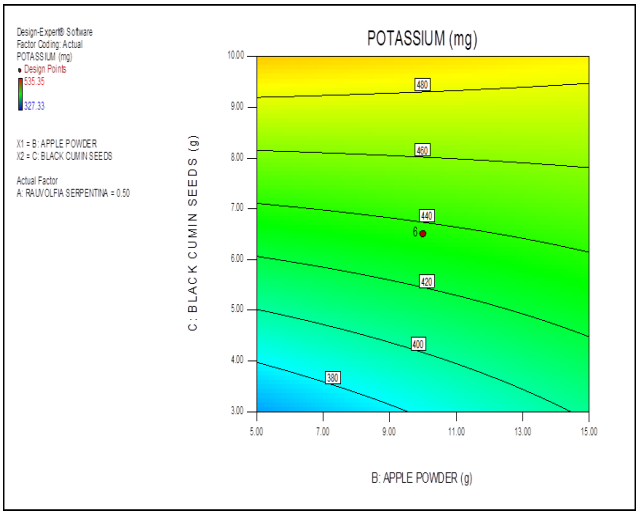
Linear curves with *Rauvolfia serpentina* and apple powder are evident from developed response surface (figure 1(a)). The observation was that linear term of *Rauvolfia serpentina* (p=0.125) and cross product of *Rauvolfia serpentina* with black cumin seeds powder (p=0.052) had non-significant effect on the potassium content of nutritious mixs. Centre points (6) are depicted through red colour in middle of each graph. Curvilinear plots were observed with *Rauvolfia serpentina* and black cumin seeds powder (figure 1(b)). It was depicted that linear term of black cumin seeds powder (p<0.000) had significant effect and cross product of apple powder with black cumin seeds powder (p=0.052) had non-significant effect on the potassium. The linear curves with apple powder and black cumin seeds powder (figure 1(c)) were developed. The linear term of apple powder (p=0.099) and cross product of *Rauvolfia serpentina* with apple powder (p=0.052) had non-significant effect on the potassium of nutritious mix.



(a)

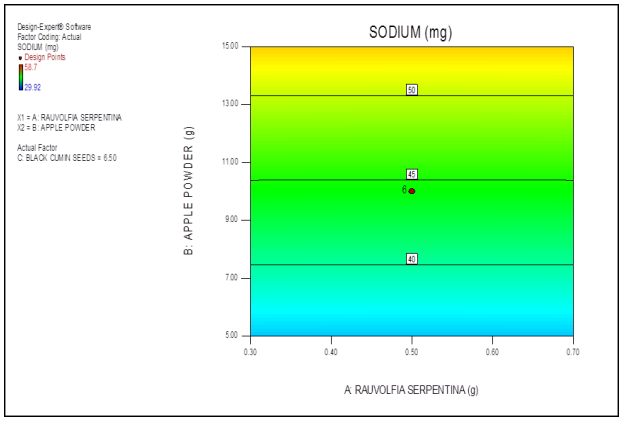


(b)

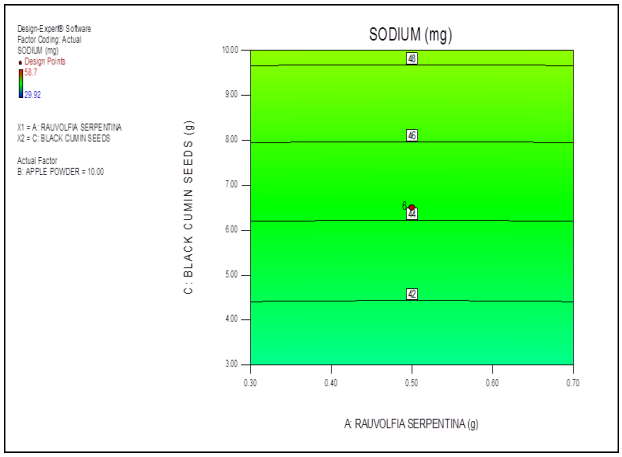


(c)

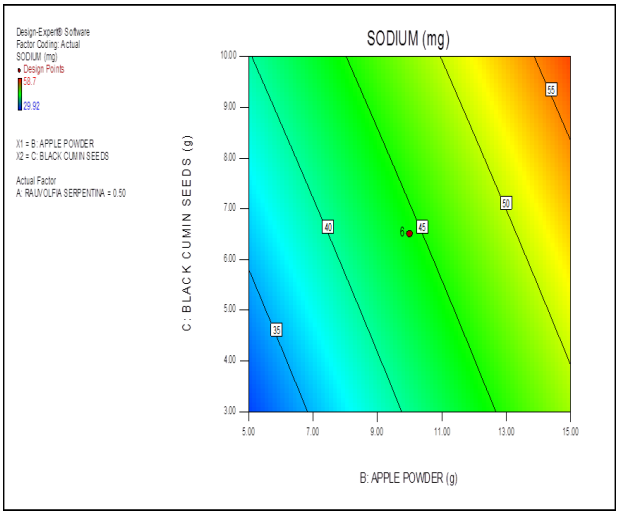
Figure 1. Interactive effect of *Rauvolfia serpentina* and apple powder (a), *Rauvolfia serpentina* and black cumin seeds powder (b) and apple powder and black cumin seeds powder (c) on potassium content of nutritious mix



(a)



(b)



(c)

Figure 2. Interactive effect of *Rauvolfia serpentina* and apple powder (a), *Rauvolfia serpentina* and black cumin seeds powder (b) and apple powder and black cumin seeds powder (c) on sodium content of nutritious mix

3.1.4. Effect of process condition for calculated sodium

Table 2 represents the observations for sodium as a response variable with different combination of independent variables. The independent variables' effect on response, sodium of nutritious mix in terms of actual level of variables is described by the regression equation given as:

Sodium= 20.34274 - 0.60775 * *Rauvolfia serpentina* + 1.69508 * Apple powder + 1.04716 * Black cumin seeds powder - 1.50553E-0.15 * *Rauvolfia serpentina* * Apple powder - 1.67980E-015 * *Rauvolfia serpentina* * Black cumin seeds powder + 4.09840E-017 * Apple powder * Black cumin seeds powder + 0.60775 * *Rauvolfia serpentina*^{2} + 8.30971E-004 * Apple powder^2 + 6.74662E-003 * Black cumin seeds powder^2

The response surface developed in figure 2(a) shows linear curves with *Rauvolfia serpentina* and apple powder. The observation was that the linear term of *Rauvolfia serpentina* (p=1.000), cross product of *Rauvolfia serpentina* and black cumin seeds powder (p=1.000) and quadratic term of *Rauvolfia serpentina* (p=0.18) had non-significant effect on sodium (dependent variable). Curvilinear plots were observed with *Rauvolfia serpentina* and black cumin seeds powder (figure 2(b)). Black cumin seeds powder shows significant influence (p<0.000) in terms of linear model, whereas it shows non-significant effect in terms of cross product with apple powder (p=1.000) and quadratic term of black cumin seeds (0.000) shows significant effect on sodium. Linear curves were developed with apple powder and black cumin seeds powder (figure 2(c)). As observed, the linear term of apple powder (p=<0.000) had significant influence whereas cross product of apple powder and *Rauvolfia serpentina* (p=1.000) had non-significant influence and quadratic term of apple powder (p=0.254) had non-significant influence on the response, sodium.

3.1.5. Effect of process condition for fibre

Table 2 depicts the observations for fibre as a response variable with different combination of independent variables. The effect of the independent variables on response, fibre of nutritious mix in actual level terms of variable is described by regression equation given as:

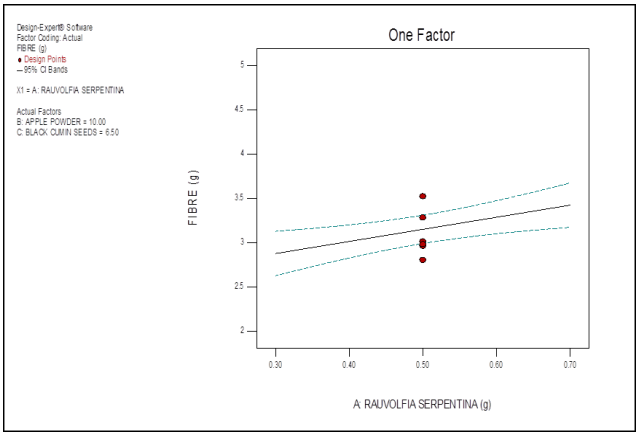
Fibre= 0.86560 + 1.12247 * *Rauvolfia serpentina* + 0.012182 * Apple powder + 0.23267 * Black cumin seeds powder

Linear plots with *Rauvolfia serpentina* (figure 3(a)), apple powder (figure 3(b)) and black cumin seeds powder (figure 3(c)) are shown on the response plots. The interactive effect of amount of *Rauvolfia serpentina* on independent variable, fibre in figure 3(a) indicates maximum fibre content (3.3 g) obtained at 0.70 g of *Rauvolfia serpentina* and minimum fibre content (2.8 g) attained at 0.3 g of *Rauvolfia serpentina*. The interactive effect of apple powder with fibre in figure 3(b) indicates maximum fibre content (3.2 g) observed at 15.0 g of apple powder and minimum fibre content (3.0 g) obtained at 5.0 g of apple powder. The interactive effect of black cumin seeds powder on fibre (figure 3(c)) specifies the maximum fibre content (3.8 g) observed at 10.0 g black cumin seeds powder and minimum fibre content (2.4 g) was observed at 3.0 g black cumin seeds powder. ***3.1.6. Effect of process condition for overall acceptability***

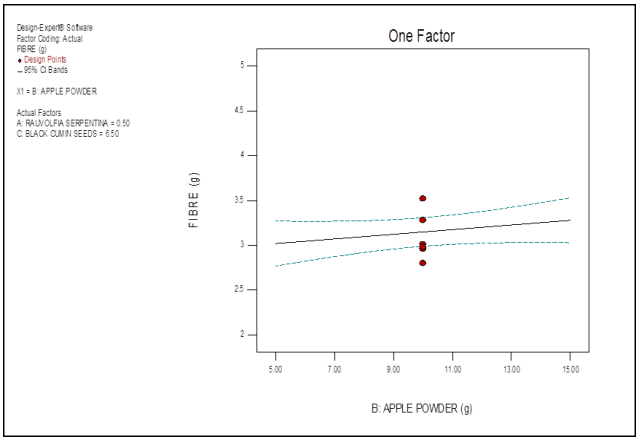
Organoleptic characteristics have a significant importance in modifying, improving, developing and accepting the innovative food products (Yadav *et al.,* 2007). Overall acceptability is a significant factor having direct relation to the likeability of any developed novel food product. Table 2 represents the results observed for overall acceptability with different combination of independent variables. The effect of the independent variables on overall acceptability of nutritious mix in terms of actual level of variables is described by regression equation given as:

Overall acceptability= 89.65441 - 0.44655 * *Rauvolfia serpentina* + 0.20143 * Apple powder -0.87850 * Black cumin seeds powder - 0.70750 * *Rauvolfia serpentina* * Apple powder + 1.01071 * *Rauvolfia serpentina* * Black cumin seeds powder + 0.029000 * Apple powder * Black cumin seeds powder

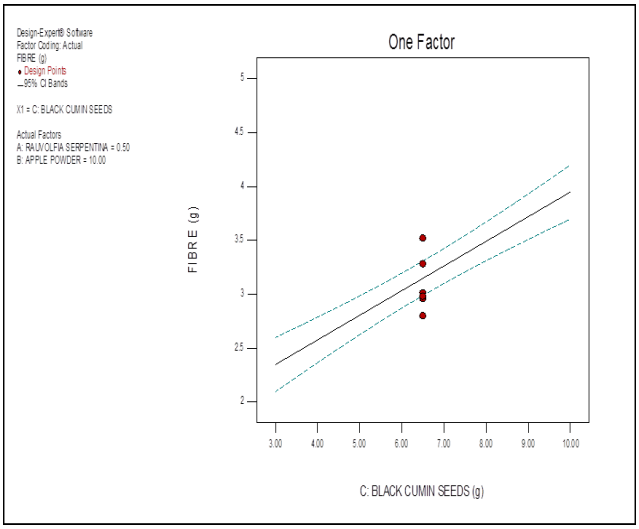
Curvilinear plots were observed with *Rauvolfia serpentina* and apple powder (figure 4(a)). *Rauvolfia serpentina* in its linear term (p=0.494) and in its cross product term with black cumin seeds powder (p=0.067) had non-significant effect on the response, overall acceptability. The response surface developed in figure 4(b) shows linear curves with *Rauvolfia serpentina* and black cumin seeds powder. Linear term of black cumin seeds powder (p=0.302) and cross product term of *Rauvolfia serpentina* and apple powder (p=0.671) had non-significant on the overall acceptability. Linear curves were developed with apple powder and black cumin seeds powder (figure 4(c)). Linear term of apple powder (p=0.516) and its cross product with black cumin seeds powder (p=0.175) as observed had non-significant influence on the response, overall acceptability.



(a)

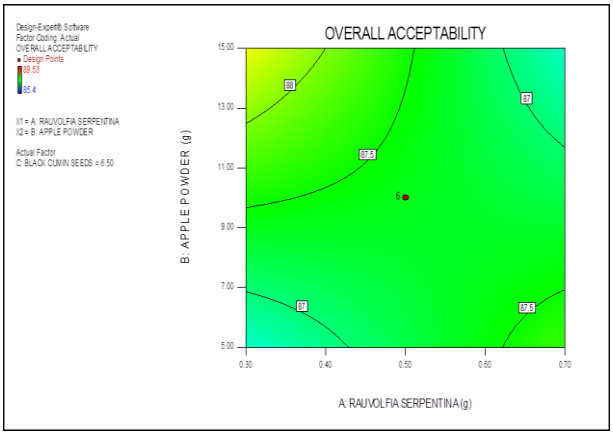


(b)

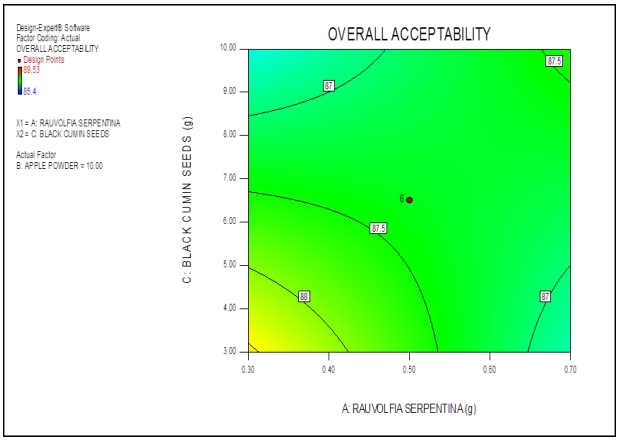


(c)

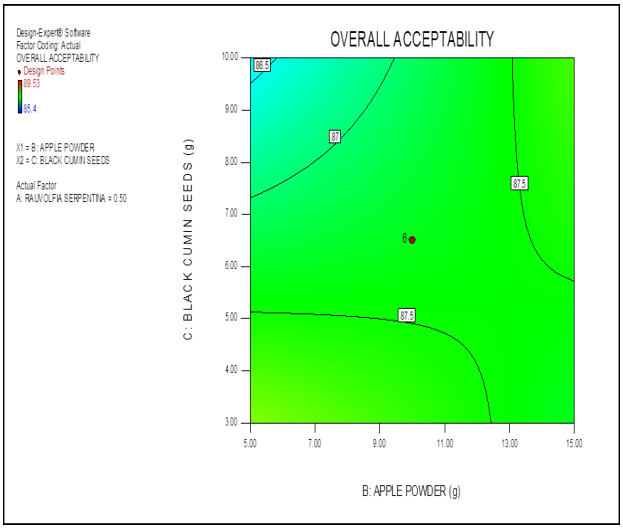
Figure 3. Interactive effect of *Rauvolfia serpentina* (a), apple powder (b) and black cumin seeds powder (c) on fibre content of nutritious mix



(a)



(b)



(c)

Figure 4. Interactive effect of *Rauvolfia serpentina* and apple powder (a), *Rauvolfia serpentina* and black cumin seeds powder (b) and apple powder and black cumin seeds powder (c) on overall acceptability of nutritious mix

3.1.7. Optimisation of process parameters

The above mentioned results signify the fact that the quality of nutritious mix does not depend on the particular key factor. The properties of the nutritious mix were determined by significant role of all process variables leading to the next step that was to get the best combination of process variables having the ability of producing the expected properties of end product. Thus, numerical optimisation of the process parameters was done to obtain best combination of nutritious mix. Simultaneous optimisation of the multiple response variables took place through the Design Expert (9.0). Table 3 depicts the chosen desired goals for each factor and response. Thirty solutions of independent variables with the predicted responses were generated through the software. The range between 0.569-0.627 was obtained for desirability of optimum solutions. Four optimum solutions were attained depending upon the highest desirability. The optimum recipes consisted (i) 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder with 477.71 mg potassium, 39.85 mg sodium, 4.09 g fibre and 87.61 overall acceptability score; (ii) 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 9.96 g black cumin seeds powder with 477.15 mg potassium, 39.81 mg sodium, 4.08 g fibre and 87.61 score of overall acceptability; (iii) 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 9.92 black cumin seeds powder with 476.49 mg potassium, 39.76 mg sodium, 4.07 g fibre and 87.61 score of overall acceptability, (iv) 5.04 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder with 477.78 mg potassium, 39.92 mg sodium, 4.09 g fibre and 87.61 overall acceptability score in about 51 g of products. The range of processes which might possibly be contemplated as the optimum range for best quality food product in terms of potassium, sodium, fibre and overall acceptability was provided through these optimum solutions. These were suitable conditions to formulate nutritious mix providing nutritional adequacy without compromising the organoleptic characteristics. Formulation of optimised and enhanced nutritious mix was done using the best solution chosen. Solution 1 with the maximum desirability value of 0.627 in a range of 0.569-0.627, along with in range potassium, minimum

sodium, maximum fibre and maximum overall acceptability was chosen for subsequent laboratory estimation.

3.1.8. Nutritional analysis

The nutrients estimation was done as per 100 g quantity. The optimum recipe was adequate in terms of nutrition having 72.46 g carbohydrate, 5.99 g moisture, 0.94 g ash, 15.63 g protein, 3.29 g fat, 1.69 g crude fibre, 9.03 mg iron, 236.29 mg calcium and 1.09 mg vitamin C.

3.2. Discussion

Rapid urbanisation, industrial development and consequential variations in lifestyles of individuals have resulted in progressive formulations of instant dry mixes and ready-to-eat convenient food products (Balasubramanian *et al.,* 2014). These products are gaining popularity as a result of ease of consumption and increased shelf life (Bunkar *et al.,* 2014) along with reducing the time for preparation by eradicating numerous steps of cooking (Balasuramanian *et al.,* 2014). Several researches have been carried out for the development of instant foods including soy-fortified instant *upma* mix (Yadav and Sharma, 2008), *halwa* dry mix (Yadav *et al.,* 2007), pearl millet based *upma* dry mix (Balasubramanian *et al.,* 2014) and instant wheat porridge (*dalia*) mix (Khan *et al.,* 2014).

With convenience, there also comes an increased demand of consumers for value added products with health advantages (Gadhiya *et al.,* 2015). The improvements in the understanding of association between nutrition and health lead to the functional foods development which is a practical and new approach for the achievement of optimum status by promoting the state of being healthy and thus probably decreasing the diseases' risk (Siró *et al.,* 2008). Such products that are claimed to be healthy and have functional and/or heath properties are gaining priority in researches in production of novel foods (de Sousa *et al.,* 2011). Nutraceutical potential of medicinal plants makes them beneficial to be used in medicine and for therapeutic purposes (Harsha and Aarti, 2015). Various herbs possess many therapeutic properties including antioxidative, antihypertensive, anti-inflammatory, antidiabetic, antimicrobial, etc (Oraon *et al.,* 2017). Thus incorporation of these herbs as functional foods can provide several health benefits to the consumers. Several researches related to development of food products incorporating herbs and other functional foods have been conducted including herbal juice development from traditional Indian plants using *Citrus limetta* as base (Harsha and Aarti, 2015) powdered food developed with addition of *Spirulina* (Santos *et al.,* 2016), development of an apple snack rich in flavonoid (Betoret *et al.,* 2012), development of blended papaya- Aloe vera ready to serve beverage (Boghani *et al.,* 2012) to enhance the nutritional properties of the food products. In the present study *Rauvolfia serpentina,* black cumin seeds and apples were incorporated as functional ingredients to enhance the nutritional properties of the food product.

Rauvolfia serpentina and black cumin seeds were found to significantly increase the amount of fibre, whereas apple powder and black cumin seeds significantly decreased the amount of sodium in nutritious mix. This recipe has low sodium content that can be an additional benefit as a result of direct relation of sodium consumption with hypertension in humans (Malviya and Sason, 2016). Apples provide a good source of carbohydrates and vitamins and have less contribution in calories along with no contribution in fat, sodium or cholesterol (Harris *et al.,* 2007). Black cumin seeds constitutes of proteins, minerals, vitamins, enzymes, carbohydrates and fats having about overall fat contained in the form of omega-3 and omega-6 fatty acids in rich amount. They also contain vitamins A, B_1, B_2, B_3 and C as well as calcium, iron, magnesium, zinc and selenium (Hussain and Hussain, 2016). Thus,

the incorporation of apples, *Rauvolfia serpentina* and black cumin seeds in nutritious mix have together contributed in obtaining the goal of low sodium and high fibre content.

Optimisation of ingredients in the food formulation is essential for the development of a product. There are number of techniques that are available to draw the best levels of input variables that in turn optimise their responses (Nadeem *et al.,* 2012). RSM is the one which is stated to be an effective measure for optimising a process when the independent variables are hypothesised to possess a dominant or accumulative effect on the desired responses (Martínez *et al.,* 2004). The observations of effect of independent variables on fibre in this study represented the significant effect of linear model on the response. The results for effect of independent variables on sodium showed that the quadratic model had significant effect on response. The 2FI model indicates the non-significant process variables' effect on the response, overall acceptability. Similar studies were conducted, (i) process optimisation for formulating cowpea incorporated instant *kheer* mix by the use of RSM was conducted in which amounts of cowpea and malted wheat flour and cowpea soaking time and were the process variables and protein, crude fiber and overall acceptability were the responses. Results revealed that the models had non-significant effect on the response, crude fibre and overall acceptability (Gupta *et al.,* 2014); (ii) optimisation of multigrain premix (MGP) to develop high protein and dietary fibre biscuits through RSM was conducted in which levels of MGP and wheat flour concentration were the process variables and protein, soluble, insoluble fibres, biscuit dough hardness, breaking strength and overall acceptability were the response variables. Results revealed that the incorporation of MGP significantly increases the soluble and insoluble fibres content of biscuits (Kumar *et al.,* 2015a); (iii) process optimisation of vegetable cereal mix using RSM was conducted in which amounts of Trigonella *foenum-graecum* and *Gymnema sylvestre* and soaking time of *Trigonella foenum-graecum* were the independent variables and fat, fibre, carbohydrate and overall acceptability were the responses. Results for effect of process variables on the response showed that 2FI model had a significant effect on fibre (dependent variable) whereas 2FI model had non-significant effect of process variables on the response, overall acceptability (Gupta *et al.,* 2016). There are some other similar studies in which food product development of various premixes was conducted using RSM to enhance their nutritional characteristics like optimisation of instant dalia dessert pre-mix formulation by the use of RSM (Jha *et al.*, 2015) and production of multigrain premixes-its effect on rheological, textural, and micro- structural characteristics of dough and quality of biscuits (Kumar *et al.*, 2015b).

The nutritional analysis of optimum recipe resulted in recipe being nutritionally adequate and was rich in iron and good source of calcium. Bhadana *et al.* (2016) carried out a study on product development and nutrients evaluation of value added product incorporated with spirulina powder, soya flour and rice flour. for nutritional analysis revealed that the sample The results containing spirulina powder 20 g, soya flour and rice flour had moisture content of 2.48 percent per 50 g and 1.10 percent per 50 g fat content that were similar to the present study. The instant foods are beneficial in saving very important resources such as time and energy (Lohekar and Arya, 2014) and the value addition of functional ingredients in optimum levels can enhance the nutritional properties of these foods without compromising their acceptability. The nutritious mix owing to low sodium can be of benefit to hypertensive patients as rise in blood pressure is a common disorder in India (Raghupathy *et al.,* 2014) affecting all age groups.

4. Conclusions

RSM came out to be a successful tool to derive the best combination of different processes (amount of apple powder, *Rauvolfia serpentina* and black cumin seeds) for formulation of nutritious mix. Out of 30 suggested combinations, 4 combinations had highest desirability value (0.627) in comparison to others. Recipe having 5.00 g apple powder, 0.70 g *Rauvolfia serpentina* and 10.00 g black cumin seeds powder with 477.71 mg potassium, 39.85 mg sodium, 4.09 g fibre and 87.61 overall acceptability score was selected optimum recipe and subjected for further nutritional analysis. Optimum recipe had adequate potassium, low sodium, high fibre and high overall acceptability. It was rich in iron and good source of calcium. Nutritious mix is the instant food which is convenient to be used, affordable and of nutritional importance as well. Incorporation of Indian medicinal herbs into it makes it highly beneficial for various ailments like hypertension. Thus, it can be effortlessly utilised by the consumers as food add-on devoid of any variation in their regular diets.

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NUTRITIONAL VALUE AND COLOR CHANGES OF TOMATO ACCESSIONS AFTER THERMAL PROCESSING

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ABSTRACT
With the development of food industry, several bioactive compounds of tomato were explored due to their positive impact on human health. Nutrition and taste components were not in the focus of tomato breeding in the last decades. Inclusion of landraces with a hypothesized higher nutritional value into the production could be reasonable. Their role in modern processing industry is unclear. The aim of the present study is to fill this gap in the case of four tomato accessions.
In our two-year experiment four accessions were compared with 'Kecskeméti 549' variety, in terms of nutritional parameters (BRIX, pH, lycopene, ascorbic acid, FRAP, TPC) and color (C*, h^o); changes over hot and cold break treatments were also monitored. The results of the raw samples of accessions were slightly higher or equal to those of the control variety. The effect of heat treatments on nutritional parameters were significant in most cases. The changes of BRIX, C*, h^o and TPC results were consequential over the two experimental years. Based on the data of the two years, hot break gave better results in the samples of variety 'Kecskeméti 549' and accession RCAT030278, while cold break had more favorable effect on accessions RCAT030587, RCAT057829, and RCAT079310.

1.Introduction

Tomato (*Solanum lycopersicum* L.) is one of the World's most important types of vegetable, with an estimated total production of 170.8 million tons in 2016 (FAOSTAT 2017). Tomato is important not only because it is one of the most commonly consumed vegetable crops, but also because of their high health and nutritional contributions to human body (Campbell *et al*., 2004, Burton-Freeman *et al*., 2012). Tomato is an important source of micronutrients, certain minerals and carboxylic acids (ascorbic, citric, malic, fumaric and oxalic acids). Tomato and its products are rich in food components that have antioxidant effect and considered to be a source of carotenoids -in particular lycopene-, and phenolic compounds, but low in fat and calories, as well as being cholesterol-free (Canene–Adams *et al*., 2004, Frusciante *et al*., 2007).

Tomato processing industry has made tremendous advances developing many forms of tomato-based foods, such as puree, sauces, soups, pastes, juices and canned

tomatoes. An additional portion of the harvested fruit is peeled and processed into products such as whole peeled and diced tomato. Many variations in the quality of the paste can occur, depending on factors such as the cultivar of tomatoes used, the finisher screen size and, most importantly, the break temperature (Abushita *et al*., 2000, Kalt, 2005, Anton and Barrett, 2012). Two methods are used commercially to produce tomato juice: hot break and cold break (Kelebek *et al*., 2017). "Hot" typically refers to a chopping temperature of 85 to 90 °C, which causes inactivation of enzymes important to aroma and viscosity. Pectinmethylesterase (PME) and polygalacturonase (PG) break down chains of pectin in tomato tissue. By inactivating these enzymes in the hot break process, a more viscous product can be achieved (Fito *et al*., 1983), which is usually desirable. Conversely, lipoxygenase is the initiating enzyme involved in the creation of important fresh aroma compounds through the breakdown of unsaturated fatty acids, and when it is inactivated in the hot break, fewer aroma compounds are present. "Cold" refers to a temperature below 70 °C to increase enzyme activity. A characteristic of the cold break process is a decrease in viscosity. Advantage of cold breaking over hot breaking is that the final product is said to have a more natural color and a fresher flavor (Gould, 1992), but in contrast, Fonseca and Luh (1976) found that aroma and flavor were rated better for hot break tomato juice. The hot break method is used to produce the majority of tomato products to maintain a high viscosity. However, an increase in the use of the cold break method should produce tomato products with a fresher aroma (Goodman *et al*., 2002).

Two important quality attributes of processing tomatoes are pH and titratable acidity. Tomato typically has sufficient acidity to maintain a pH below 4.6 and, accordingly, is not classified as a low acid food. Because of this, tomato generally do not require drastic thermal treatments. However, in the case of low acid foods, this intervention is necessary to destruct spoilage microorganisms and to ensure food safety (Anton and Barrett, 2012).

The health promoting benefits of tomato and tomato products have been attributed mostly to the significant amount of lycopene the food commodity contains. There are studies which have shown that compounds having antioxidative effects, such as lycopene or β-carotene may be increased as a result of food processing (Chen *et al*., 2000, Chang *et al*., 2006, Boubidi *et al*., 2013). More recently, studies have also described the effect of thermal processing or high-pressure treatment on some antioxidants in tomato juice (Gahler *et al*., 2003, Hsu, 2008). However, a scant few studies have considered the joint effect of different industrial treatments commonly used in the food industry not only on carotenoids or some antioxidants, but also on other bioactive compounds present in tomato (Kelebek *et al*., 2017).

Researches have shown that thermal processing elevated total antioxidant activity and bioaccessible lycopene content in tomato and produced no significant changes in the total phenolics and total flavonoids content, although loss of ascorbic acid was observed (DeWanto *et al*., 2002, Gahler *et al*., 2003). During processing, ascorbic acid is destroyed mainly due to oxidation reactions and the heat applied in the presence of air (Leoni, 2002).

The important quality aspects of tomato products are color, flavor, and consistency (Hayes *et al*., 1998, Ordóñez-Santos *et al*., 2008). The pigments of tomato juice degraded more rapidly with increasing temperature, therefore, one of the advantages of cold break over hot break is that the final

product had more natural color (Goodman *et al*., 2002).

Recently, fresh or processed fruits of tomato heirlooms and landraces could be found more frequently in the market. Tomato landraces are referred as premium products, but there is a lack of information about the quality parameters of fresh and processed fruits. The goal of our research was to determine the changes of quality parameters of four Hungarian tomato accessions according to different breaking temperatures.

The present study was designed to investigate how the content of several bioactive compounds and characters of tomato puree naturally occurring in tomatoes were affected by industrial processing. Three types of tomato puree commonly used in the food industry, namely raw tomato puree (RTP), „cold break" tomato puree (CTP) and "hot break" tomato puree (HTP), were employed. The bioactive compound analyses included lycopene, phenolic compounds, ascorbic acid, and hydrophilic antioxidant capacity (FRAP and TPC assays). Puree characteristics analysis included pH, soluble solids (Brix◦), hue (h^o), and chromaticity (C*).

The objective of the present study was to investigate the nutritional changes of four tomato accessions in order to identify the most advantageous processing methodology from a nutritional point of view. The hypothesis of the research was that accessions have higher nutritional value compared to the applied commercial variety and that the effect of processing methodology on nutritional parameters depends on the variety/accession.

2. Materials and methods

2.1. Materials

2.1.1. Samples

One tomato cultivar 'Kecskeméti 549' (K 549) as reference point, and four Hungarian gene bank accessions – RCAT030278 (Jászberény), RCAT030587 (Farmos), RCAT057829 (Dány), RCAT079310 (Szentlőrinckáta) – were grown on the Experimental and Educational Site of Szent István University (Soroksár, Hungary, 47°23'N 19°08'E, 115 m above sea level) in 2013 and 2014 in organic farming conditions. The selection of accessions were based on their origin (Middle-Hungarian Region). The commercial variety is a popular domestic one in Hungary. Both the variety and the accessions have determinate growing type. Their fruit is middle sized (80-100g), round and red colored. The propagation material of gene bank accessions was provided by Plant Diversity Center, Tápiószele. The investigated variety and the accessions were produced on open field. The conditions of cultivation were the same in both years, with sandy loam soil type, drip irrigation, maize as fore crop, and no additional nutrient supply or chemicals.

Weather conditions were different in the two investigated years. The vegetation period of 2013 was slightly arid, as well as that of the beginning of the second year. However, in 2014, the second half of the vegetation period was extremely rainy, with 1.5-fold precipitation of the average.

2.2. Methods

2.2.1. Instrumental measurements

Collection of samples was done in full ripening on the 22nd of August 2013 and 18th of August 2014 with 1500g of fruit harvested from each variety or accession. After washing and chopping, fresh tomato samples were broken by laboratory homogenizer at high speed for 5 minutes with no dilution. Homogenates were heated to 50±2°C, and to 92±2°C in case of 'cold break' (CTP), and of 'hot break' (HTP), respectively on induction cooker with set temperature. Hot tomato sauce was added to the pulp when it was crushed. The seeds were removed from the samples by a presser.

Water soluble solids content of tomato samples was determined by an Atago DBX-55 (ATAGO USA Inc., Bellevue, WA, USA) refractometer, according to Codex Alimentarius 558/93. A drop of homogenate was put onto the prism of the device, which expressed the result in Brix°.

pH measurement was carried out according to MSZ 17590 by a Testo 206 type (Testo SE & Co. KGaA, Lenzkirch, Germany) digital pH measurement tool. Before the measurement, the device was calibrated by buffer solutions of pH 4 and 7.

Regarding color measurements, L* (white to black or light to dark), a* (green to red), and b* (yellow to blue) measurements were taken by a Konica Minolta CR 410 (Konica Minolta Inc., Tokyo, Japan) Chroma Meter. The Chroma Meter was calibrated by a white tile and a standard tile of a color similar to that of the sample. L*, a*, and b* values were determined by averaging the results of 3 independent readings per sample. From L*, a*, and b* values, Chroma and hue values were calculated.The lycopene content was measured by the method of Fish *et al*. (2002). After exploring the lycopene content by the mixture of acetone, methanol and hexane, its quantity was measured spectrophotometrically by a Hitachi U-2900 (Hitachi High- Technologies Co., Kyoto, Japan) spectrophotometer. The results are expressed in mg/kg. Phenolic content (TPC) was measured according to Singleton and Rossi (1965). The samples were kept for two hours before the absorbance was measured on 765 nm against 500 µl distilled water as blank by a Hitachi U-2900 (Hitachi High-Technologies Co., Kyoto, Japan) spectrophotometer. The results are expressed in mg/l gallic acid equivalent.

Ferric Reducing Antioxidant Power (FRAP) of samples were determined by the methodology of Benzie and Strain (1996). The extinction value of the sample is compared on 593 nm with those of a sample of known Fe^{2+} concentration. For the measurements, a Hitachi U-2900 (Hitachi High- Technologies Co., Kyoto, Japan) spectrophotometer was used. The results were determined by the calibration curve and are expressed in mg/100g ascorbic acid equivalent. Ascorbic acid (AA) content was determined by reverse-phase HPLC using an RP-18 column at 22 °C with a flow speed of 1 L/min (device type: SHIMADZU LC-10AD, Shimadzu, Kyoto, Japan, controller: CBM-20A). A pH 4.75 buffer made of EDTA and phosphoric acid was used for isocratic elution. Absorbance at 254 nm was measured by UV detector. An extraction solution of 5% phosphoric acid and 0.01% sodium-EDTA and a cellulose membrane filter with 0.45 µm pore size were used for sample preparation prior to separation.The absorbance was measured on 254nm by a SPD-20A type UV-detector. The calibration curve was set up using crystallic ascorbic acid standard. The measurements were done in triplicates and are expressed in mg/100g dimension.

2.2.1. Statistical analysis

All quantitative analyses were expressed as mean value±SD for three replicates. Differences among treatments for each parameter (BRIX, FRAP, TPC, Lycopene, ascorbic acid as well as color parameters C* and h^o) studied in tomato puree were determined by using two-way MANOVA model with factors 'accession' ('K 549', RCAT030278, RCAT030587, RCAT057829, RCAT079310) and 'treatment' (RTP, CTP, HTP). Normality of residuals was accepted by d'Agostino's test ($p>0.05$). If significant overall difference was detected, a univariate follow-up ANOVA was run to examine the between-subjects 'accession' and 'treatment' effects parameter by parameter. Finally, Games-Howell's or Tukey's HSD post-hoc test was employed depending on whether the homogeneity of

variances was violated or not checked by Levene's test (at α=0.05).
Principal Component Analysis was conducted on the dataset after standardization, using Varimax rotation. Statistical analyses were run using IBM SPSS Statistics version 25.

3.Results and discussions

3.1. Statistical analysis

According to MANOVA, significant overall effect of both 'accession' and 'treatment' was found in both years (Wilk's $\lambda<0.01$; $p<0.001$ in all cases). Accession*treatment interaction was also highly significant ($p<0.001$) indicating the examined effects as accession-dependent ones.

Between-subjects effects of both 'accession' and 'treatment' (together with interaction) were also significant for all parameters ($F_{accession}(4;30)>3.7$; $p<0.05$; $F_{treatment}(2;30)>6.7$; $p<0.01$); $F_{interaction}(8;30)>3.1$; $p<0.05$) except only in case of ascorbic acid in year 2014 ($F(2;30)=0.995$; $p=0.46$).

3.2. Instrumental measurements

3.2.1. Soluble solids

Concerning RTP, the highest BRIX values were given by accessions RCAT030587 and RCAT030278 in 2013 (Table 1). With regards to HTP, accession RCAT079310 and variety 'K 549' scored the highest values. In case of CTP, accession RCAT057829 gave the highest soluble solids values. Codex Allimentarius Hungaricus defines the raw material of tomato concentrate in a minimum of 5%. This rule is fulfilled by every sample in 2013, except the raw sample of variety 'K 549'. Heat treatments resulted in a lower BRIX in the case of accessions RCAT030278 and RCAT030587, while a higher value was experienced in the case of the other accessions and the variety. The difference between the treatments were significant in every, but one case. In the samples of 'K 549', RCAT030587, and RCAT079310, HTP resulted in significantly higher results, than those of CTP. In the other cases, the decrease was minimal, however, due to the low standard deviation of the instrumental methodology, even low CTP-HTP differences of accession RCAT057829 have been detected as significant.

As a consequence of the humid season, the water soluble solids contents of the samples were lower in 2014 (Table 2). The lower temperature treatment contributed to a slightly lower BRIX values, while an increase was experienced after HTP, except that of accession RCAT079310. With the exception of the latter one, HTP resulted in significantly higher values compared to those of RTP and CTP. The threshold level of 5% was not fulfilled by any treatment of any sample.

Comparing the average results of the heat treatments, HTP gave the highest results in both years, the difference was significant in the case of CTP and HTP.

3.2.2. pH

pH values of investigated accessions were in a narrow interval of 4.09 and 4.62 in both years and treatments (Table 1 and 2.). The 2-602 directive of Codex Alimentarius Hungaricus 2-602 defines the minimum and maximum pH of concentrates and tomato juices as 4.2 and 4.5, respectively. pH values lower than 4.35 are ideal for canning. In 2013, none of the samples exceeded this value, while in 2014, certain raw and treated samples reached this critical value. In 2013, pH changed only to a lower extent (±0.1) according to the treatments, while the changes in 2014 were more radical. In both years, both thermal treatments resulted in a decrease of pH in the case of RCAT030278; the treatments had a reverse effect in the samples of RCAT030587 and RCAT057829.

3.2.3. Colorimetric results

According to MANOVA, significant overall effect of both 'accession' and 'treatment' was found in both years (Wilk's $\lambda<0.01$; $p<0.001$ in all cases). Accession*treatment interaction was also highly significant ($p<0.001$) indicating the examined effects as accession-dependent ones also in case of color parameters.

Between-subjects effects of both 'accession' and 'treatment' (together with interaction) were also significant for both parameters ($F_{accession}(4;30)>113$; $p<0.001$; $F_{treatment}(2;30)>108$; $p<0.001$); $F_{interaction}(8;30)>31$; $p<0.001$).

The mean and standard deviation values of color parameters together with the results of post hoc test are represented in Table 1 and 2. In both years, C* values showed a significant decrease in relation with the raw samples, which resulted in lower pulp color saturation. This unfavorable effect was the most serious in the case of RCAT079310 in both years. The impact of heat treatments on color saturation do not differ significantly. The color parameters of accessions showed higher changes after CTP or HTP, while the variety 'K 549' has rather stable values, and even shows a significant increase in 2014 in the case of HTP. C* values of raw samples differ significantly in both years, with the highest results of accession RCAT057829. Both CTP and HTP had an impact on the hue of the samples. CTP resulted in generally lower values, while HTP increased the h^o results of the samples. In 2013, the raw samples of RCAT030587, RCAT057829, and RCAT079310 (Table 6.), while in 2014 RCAT057829 and 'K 549' gave the lowest results. An extreme increase in h^o was experienced in the case of RCAT079310 in 2013, and of RCAT057829 in 2014. HTP effect was significantly different from the ones of CTP and RTP in 2013, and of CTP in 2014. A correlation between increased values of h^o and concentration of tomatoes was reported also by Kelebi *et al*. (2012).

3.2.4. Lycopene

In 2013, the highest lycopene content was measured in the case of the raw sample of RCAT079310, while the other samples were moving between 3-4 mg/100g (Table 1). With the exception of RCAT079310 and the HTP sample of RCAT057829, every treatments caused an increase in the lycopene content of accessions and the variety. In all cases, but RCAT030278, CTP resulted in higher lycopene levels in comparison with HTP. This difference was rather low, as it was significant only in the case of RCAT057829 and RCAT079310. The reverse change of RCAT030278 was not significant, either. CTP caused an outstanding increase, while HTP resulted in a drastic setback in the case of accession RCAT057829. In 2014, the results were slightly higher, possibly due to the differences in weather conditions (Table 2). The highest RTP values were given by RCAT057829 and 'K 549', respectively. The impact of heat treatments on the lycopene levels of individual samples was the same as that of the previous year, with the exception of variety 'K 549'. The treatments resulted in an increase, compared to the values of the raw samples. Similarly to the results of the previous year, CTP increased, while HTP decreased the lycopene level under to that of the raw sample. With the exception of variety 'K 549', the impact of treatments was in both years the same, therefore the variety dependence of this characteristic can be assumed.When comparing the treatments, CTP in 2013, while both heat treatments in 2014 significantly differ from RTP results. Based on our results, the measurable lycopene content is increasing, which is in agreement with other studies (Gartner *et al*., 1997, Alda *et. al*., 2009, Hwang *et al*., 2012).

Table 1. Nutritional value changes of the investigated raw samples (RTP) in 2013 due to cold (CTP) and hot (HTP) treatments. Different letters within a variety/accession mean significant differences between treatments (p<0,05)

2013	Treatment	BRIX^0	pH	C*	h^o	AA (mg/100g)	Lycopene (mg/100g)	FRAP (mg/100g AA)	TPC (mg/l GA)
'Kecskeméti 549'	RTP	4.87 ± 0.06 a	4.17	24.18 ± 0.10 b	48.70 ± 0.38 a	13.29 ± 1.80 a	3.51 ± 0.27 a	817.32 ± 26.40 a	378.43 ± 18.90 a
	CTP	5.67 ± 0.06 b	4.17	20.71 ± 0.31 a	47.60 ± 0.02 a	20.19 ± 0.33 b	4.85 ± 0.13 b	1719.84 ± 76.15 b	441.43 ± 38.84 ab
	HTP	6.67 ± 0.06 c	4.21	20.13 ± 0.14 a	52.46 ± 0.65 b	24.19 ± 2.16 b	4.28 ± 0.32 ab	2899.68 ± 182.66 c	550.10 ± 56.51 b
RCAT030278	RTP	6.93 ± 0.06 b	4.26	26.17 ± 0.36 c	52.44 ± 0.36 c	14.09 ± 0.67 b	4.15 ± 0.16 a	1081.70 ± 140.42 a	557.10 ± 41.33 a
	CTP	6.03 ± 0.06 a	4.24	19.83 ± 0.24 a	46.81 ± 0.16 a	10.43 ± 0.48 a	5.30 ± 0.15 b	1247.69 ± 27.46 a	468.10 ± 57.26 a
	HTP	6.00 ± 0.00 a	4.18	20.64 ± 0.23 b	50.62 ± 0.42 b	22.48 ± 1.57 c	5.39 ± 0.63 ab	1287.27 ± 104.45 a	506.10 ± 49.15 a
RCAT030587	RTP	6.83 ± 0.06 c	4.21	25.70 ± 0.13 c	44.17 ± 0.93 a	17.60 ± 0.17 b	4.16 ± 0.15 a	2268.93 ± 9.89 b	502.43 ± 42.74 a
	CTP	6.03 ± 0.06 a	4.21	19.45 ± 0.23 a	46.18 ± 0.30 ab	15.01 ± 0.38 a	5.67 ± 0.22 b	1129.52 ± 89.51 a	398.77 ± 8.96 a
	HTP	6.33 ± 0.06 b	4.26	21.52 ± 0.41 b	47.55 ± 0.78 b	20.22 ± 2.11 ab	5.33 ± 0.28 b	1052.02 ± 63.37 a	674.43 ± 35.35 b
RCAT057829	RTP	6.33 ± 0.06 a	4.13	27.30 ± 0.59 c	47.20 ± 1.65 a	14.38 ± 0.43 a	3.51 ± 0.05 a	1071.26 ± 53.18 b	317.10 ± 55.24 a
	CTP	6.57 ± 0.06 b	4.15	21.72 ± 0.37 a	42.53 ± 0.90 a	20.14 ± 0.56 b	5.77 ± 0.22 b	1160.30 ± 64.65 b	396.77 ± 12.09 a
	HTP	6.47 ± 0.06 ab	4.18	23.58 ± 0.16 b	42.29 ± 0.55 b	11.09 ± 2.31 a	3.09 ± 0.32 a	860.74 ± 19.44 a	462.10 ± 32.42 a
RCAT079310	RTP	5.87 ± 0.06 a	4.21	26.76 ± 0.28 c	40.55 ± 0.09 a	12.29 ± 0.42 a	7.97 ± 0.18 c	1114.21 ± 94.72 ab	326.77 ± 18.17 a
	CTP	6.43 ± 0.06 b	4.15	23.83 ± 0.24 b	42.99 ± 0.49 b	24.04 ± 0.62 b	6.48 ± 0.29 b	1076.20 ± 50.48 b	529.10 ± 31.76 b
	HTP	6.77 ± 0.06 c	4.09	12.72 ± 0.10 a	52.90 ± 1.33 c	13.87 ± 0.64 a	5.01 ± 0.15 a	911.31 ± 10.47 a	403.77 ± 31.13 a

Table 2. Nutritional value changes of the investigated raw samples (RTP) in 2014 due to cold (CTP) and hot (HTP) treatments. Different letters within a variety/accession mean significant differences between treatments (p<0,05)

2014	Treatment	BRIX^0	pH	C*	h^o	AA (mg/100g)	Lycopene (mg/100g)	FRAP (mg/100g AA)	TPC (mg/l GA)
'Kecskeméti 549'	RTP	3.73 ± 0.06 a	4.6	19.52 ± 0.07 b	44.00 ± 2.00 ab	5.12 ± 0.06 a	4.99 ± 0.13 b	194.01 ± 3.12 a	234.31 ± 11.31 a
	CTP	3.93 ± 0.06 b	4.36	18.78 ± 0.13 a	40.83 ± 0.28 a	5.49 ± 0.76 a	4.09 ± 0.23 a	430.54 ± 34.65 b	247.64 ± 12.48 a
	HTP	4.03 ± 0.06 b	4.3	20.48 ± 0.58 ab	49.77 ± 0.46 b	4.77 ± 1.00 a	6.70 ± 0.19 c	561.02 ± 31.83 c	285.97 ± 10.75 b
RCAT030278	RTP	3.97 ± 0.06 a	4.4	19.18 ± 0.23 b	44.88 ± 1.23 a	4.60 ± 0.34 a	4.19 ± 0.12 a	358.64 ± 0.68 b	258.47 ± 20.45 a
	CTP	4.03 ± 0.06 a	4.34	15.88 ± 0.29 a	43.55 ± 0.31 a	4.21 ± 0.35 a	5.10 ± 0.14 b	275.74 ± 15.56 a	261.81 ± 20.92 a
	HTP	4.43 ± 0.06 b	4.3	19.28 ± 0.57 b	51.73 ± 0.57 b	3.93 ± 0.08 a	7.82 ± 0.09 c	613.24 ± 16.05 c	312.92 ± 7.95 a
RCAT030587	RTP	4.30 ± 0.00 a	4.3	18.17 ± 0.14 b	45.15 ± 0.36 b	3.87 ± 0.09 a	4.11 ± 0.45 a	356.74 ± 41.75 a	261.25 ± 17.02 a
	CTP	4.33 ± 0.06 a	4.32	18.96 ± 0.36 b	40.29 ± 0.13 a	5.13 ± 0.77 a	5.19 ± 0.19 a	699.29 ± 15.83 b	297.64 ± 13.80 a
	HTP	4.97 ± 0.06 b	4.62	12.91 ± 0.43 a	52.73 ± 0.76 c	3.62 ± 0.03 a	4.61 ± 0.34 a	643.89 ± 28.32 b	251.81 ± 38.71 a
RCAT057829	RTP	4.23 ± 0.06 a	4.25	21.83 ± 0.09 c	35.17 ± 0.09 a	3.44 ± 0.86 a	5.57 ± 0.68 a	346.85 ± 18.04 a	244.86 ± 13.47 a
	CTP	4.30 ± 0.00 a	4.24	20.09 ± 0.47 b	40.44 ± 0.45 b	3.33 ± 0.91 a	9.03 ± 0.35 b	536.23 ± 9.45 b	292.36 ± 31.97 a
	HTP	4.97 ± 0.06 b	4.56	15.29 ± 0.27 a	49.76 ± 0.35 c	2.85 ± 0.06 a	5.00 ± 0.49 a	989.64 ± 20.14 c	289.03 ± 20.48 a
RCAT079310	RTP	4.63 ± 0.06 b	4.3	20.29 ± 0.51 c	50.01 ± 1.08 b	4.12 ± 0.71 a	3.59 ± 0.11 a	363.75 ± 12.75 a	236.53 ± 30.89 a
	CTP	4.33 ± 0.06 a	4.23	17.45 ± 0.07 b	44.28 ± 0.40 a	4.00 ± 0.74 a	5.10 ± 0.19 b	575.92 ± 7.36 b	252.92 ± 3.63 a
	HTP	4.57 ± 0.06 b	4.58	13.36 ± 0.06 a	55.10 ± 0.25 c	3.03 ± 0.77 a	4.29 ± 0.33 ab	950.35 ± 17.17 c	253.47 ± 14.17 a

3.2.5. Ascorbic acid

In 2013, the highest ascorbic acid content was measured in the raw sample of accession RCAT030587 (Table 1). Due to the cold break treatment, the values significantly increased in the case of the variety 'K 549', and the accessions RCAT057829 and RCAT079310. As the result of the cold break treatment, a significant decrease was recorded in the samples of accessions RCAT030587 and RCAT030278, compared to the raw samples. The hot break treatment increased the ascorbic acid content of three samples ('K 549', RCAT030278, RCAT030587), the increase was significant, except that of the last one.

In 2014, the ascorbic acid content of the samples was gradually lower (Table 2). In this year, the highest value was given by the raw sample of variety 'K 549'. No significant difference was found between the treatments. HTP resulted in a decrease in case of every sample, while CTP caused an increase in the samples of 'K 549' and RCAT030587. When comparing the results to those of the previous year, a particular similarity can be seen considering the variety 'K 549', and accessions RCAT057829, and RCAT079310.

Both treatments increased the ascorbic acid content of the samples in 2013 significantly. In 2014, the difference from RTP was not remarkable, however, it was significant between the treatments. HTP had a higher impact on ascorbic acid content in both years.

3.2.6. Antioxidant capacity (FRAP)

The highest FRAP values measured in the raw samples were given by the accession RCAT030587 in 2013 (Table 1). In the case of 'K 549' and RCAT030278 samples, both CTP and HTP took an increase in FRAP values; the difference was significant in the first case. CTP and HTP caused a significant decrease in the case of accession RCAT030587, while in the cases of RCAT057829 and RCAT079310, only HTP had significant effect.

In 2014, the antioxidant capacity of the raw samples were lower, than in the first year (Table 2). The humid weather was less stressful for the plants, than the hot and arid season of the previous year. A significant increase was experienced followed both the cold and hot treatments in the case of the variety 'K 549', the accessions RCAT030587, RCAT057829 and RCAT079310. Only the HTP took an increase in the FRAP value of accession RCAT030278; the CTP resulted in a significant decrease.

No significant difference was found between the treatment effects in 2013. In 2014, the increase of both heat treatments resulted in a significant increase in antioxidant capacity. CTP gave significantly lower FRAP values than HTP.

3.2.7. Total Phenolic Content (TPC)

Regarding polyphenol content of the raw samples, the accessions RCAT030587 and RCAT030278 gave the highest results in both years (Table 1 and 2). Great differences were found between the results of the two years, which can be explained by the seasonality of the experimental site. In 2013, the CTP samples of RCAT057829 and RCAT079310 showed a higher polyphenolic content, compared to the values of the raw sample. The hot break treatment gave significantly higher values in the case of 'K 549', RCAT030587, RCAT057829, and RCAT079310 samples, compared to RTP. With the exception of RCAT079310, all samples showed higher results after HTP, the difference was significant in most cases.

With the exception of RCAT030587, both CTP and HTP resulted in higher values in 2014, compared to the results of the raw samples. The HTP results differed significantly from CTP and RTP only in the

cases of 'K 549' and RCAT030278, the other differences were not significant. The differences between CTP and HTP were rather low, the tendencies of the previous year was repeated only by 'K 549' and RCAT030278 samples.

Concerning the comparison of treatments, HTP took a significant increase in TPC values in both years. Similarly, the cold treatment took an increase, but the difference was significant only in 2014.

3.3. Coherences of the nutritional values and seasonality

The dataset of the two years enables to draw more general conclusions, regardless to weather conditions. At the same time, the two years took obvious differences in nutritional parameters, which was experienced not caused by the effect of the treatments. The humid weather conditions of the second year caused a drastic decrease in the ascorbic acid and FRAP values of the samples, while the decrease was lower in the case of polyphenolic and BRIX values.

The influence of weather was rather low in the case of color parameters, pH, and lycopene values. The decease of BRIX value is explained by the amount of the precipitation, which increased the water content of the fruits, while the sugar content was diluted. Since a remarkable part of the water-soluble antioxidant capacity (FRAP) is given by ascorbic acid (Cano *et al.*, 2003), these two parameters change mutually. As a consequence of the humid weather of the second year, the daily sunlight showed a decrease, which had a disadvantageous effect on the ascorbic acid content of the raw samples (Davies and Hobson, 1981, Dumas *et al.*, 2003). With the decrease of the temperature, the heat stress was moderate, which can be a reason for the decrease of the polyphenolic content (Rivero *et al*., 2001). This change contributed to the setback of the FRAP values (George *et al*., 2004). The mutual changes of the color parameters and the lycopene can be explained by the fact, that lycopene is the main pigment of the tomato (D' Souse *et al*., 1992).

3.4. The effect of heat treatments on samples

3.4.1. Comparison of the two year

Both CTP and HTP had an effect on every nutritional parameters, which resulted in a remarkable change in the nutritional parameters of the raw samples. While the two-year dataset of the variety and the accessions did not give a consensual result concerning any parameters, but hue, it is suspected, that besides genetic background, the nutritional values of the raw material can influence the effect of the heat treatments.

Concerning color parameters, L*, a*, and b* values measured from homogenate showed a low standard deviation, which resulted in significant differences in several cases, regardless of the fact, that the value changes were low. The same is true for BRIX value contradictions of accessions RCAT030278 and RCAT057829, although the results are significant only in the latter case. Regarding lycopene, only the results of 'K 549' were contradictory, though it was significant only in the second year. The FRAP and ascorbic acid results, on which the weather has a strong impact, showed adverse tendencies in the case of accessions RCAT057829, RCAT079310, and RCAT030587 and RCAT057829, respectively, however, in these cases only the results of one year was significant. The same is true for the TPC values of accessions RCAT030587 and RCAT057829.

3.4.2. Processing industry criteria

Although the BRIX of the raw samples of the accessions were generally higher in both years, the change due to heat treatments were lower, than that of 'K 549'. In several cases,

heat treatments resulted in a decrease or water soluble solids.

In 2014, the hot break treatment drastically increased the pH of the samples, except those of the variety 'K 549' and accession RCAT030278. Therefore, it is presumed, that it rather refers to the weather conditions, than those of the genetic background.

3.5. Summary of the nutritional parameters

The heat treatments employed had an adverse effect on the nutritional parameters of the variety and the accessions. This can mainly explained by the different genetic background (Kalt, 2005). When summarizing the more favorable treatments by year and by sample (Table 3), it can be concluded, that for 'K 549' and accession RCAT030278, the hot break, while for accessions RCAT030587, RCAT057829, and RCAT079310, the cold break processing resulted in a puree with more favorable nutritional values.

Regarding BRIX, C*, h^o, and TPC the data of the two years were similar, while in the case of other parameters, at least the results of one year did not differ significantly. It can be concluded, that HTP in the case of BRIX, while CTP in the case of hue was the advantageous processing methodology, resulting in significant differences in both years. No significant differences were found in C* and TPC values in any year.

Using principal component analysis, the subsets of each treatment can be separated with minimal overlapping (Figure 1.). The first three principal components (PCs) describe 83.96% of the total variance (PC1: 44.39%, PC2: 28.00%, PC3: 11.55%). PC1 is responsible for color parameters (C*, L*, a*, b*), PC2 contains water-soluble constituents (BRIX, ascorbic acid, FRAP, TPC), while PC3 is attributed to h^o and lycopene. Both heat treatments have a significant impact on puree color. The subset of HTP samples is relatively closer to FRAP, BRIX and TPC data points, while the CTP group can be characterized by lycopene data. Raw samples are the closest to color parameters, which means, that both hot and cold processing treatments have an impact on color parameters. In the case of RCAT057829, hot and cold treatment-related data points are situated close to each other, suspecting, that this accession is not sensitive to processing temperature. The results of the varieties further support the above findings, and highlights the importance of variety-optimized processing.

Table 3. Summary of treatments with more favorable effect as the function of samples and measured parameters. Treatments in boldface indicate significant differences ($p<0.05$).

Parameter	**'Kecskeméti 549'**		**RCAT030278**		**RCAT030587**		**RCAT057829**		**RCAT079310**	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
C*	CTP	HTP	**HTP**	**HTP**	**HTP**	**CTP**	**HTP**	**CTP**	**CTP**	**CTP**
h^o	**CTP**	CTP	**CTP**	CTP	CTP	CTP	CTP	CTP	CTP	CTP
BRIX	**HTP**	HTP	CTP	**HTP**	**HTP**	**HTP**	CTP	**HTP**	**HTP**	**HTP**
Lycopene	CTP	**HTP**	HTP	**HTP**	CTP	CTP	**CTP**	**CTP**	CTP	**CTP**
Ascorbic a.	HTP	CTP	**HTP**	CTP	HTP	CTP	**CTP**	CTP	**CTP**	CTP
FRAP	**HTP**	**HTP**	HTP	**HTP**	CTP	CTP	**CTP**	**HTP**	**CTP**	**HTP**
TPC	HTP	**HTP**	HTP	HTP	**HTP**	CTP	HTP	CTP	**CTP**	CTP

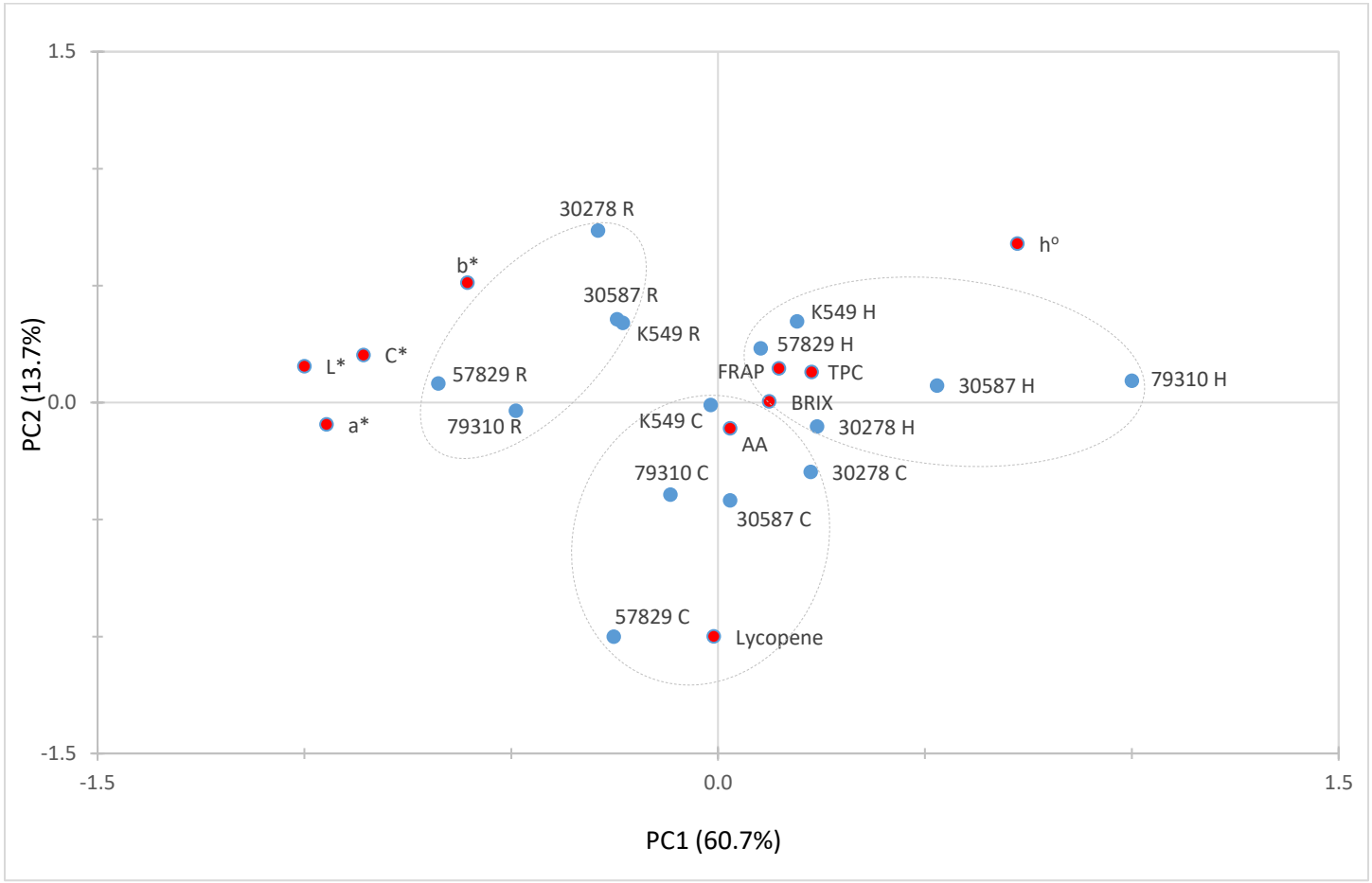

Figure 1. Bi-plot of the investigated tomato variety and accessions based on nutritional values over thermal treatments. The last five digits of the RCAT code and a capital letter indicates the position of the values. R, C, and H refers to raw, cold break, and hot break treatments, respectively. Ellipses indicate the sub-groups of treatments.

4. Conclusions

This study was designed to investigate how the content of several bioactive compounds and characters of tomato puree naturally occurring in tomatoes were affected by industrial processing focusing on tomato accessions.

The hypothesized genetic difference between the variety and the accessions did not result in a drastic phenotypic difference; however, the differences are observable in the RTP data, as well as in the case of changes of nutritional values due to heat treatments.

Therefore, besides economic and technological aspects, it is suggested to take the nutritional background of the raw material into consideration when deciding about processing methodology.

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INVESTIGATION OF VEGETABLE OILS TO OXIDATIVE DEGRADATION OF VARYING DEGREES OF SATURATION WITH TOCOPHEROL

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ABSTRACT
The content of tocopherol isomers and their activity in oils with different depth of refining - by pressing up unrefined and refined deodorized. It is shown that the isomeric composition of tocopherols influence on the rate and kinetics of elongation, and termination of radical chain oxidation. The relationship between the fatty acid composition of the oil, in particular the content of unsaturated fatty acids, the flow rate of peroxidation reactions and content of oxidation inhibitors. It is shown that a critical factor when braking oxidation reactions is the inhibitory effect of tocopherol, namely its greatest when the total content of α-isomer content.

1. Introduction

Radical oxidation reactions occupy an important place in physical chemistry, they underlie a number of technologies. One of the main tasks of physical chemistry is to study the relationship between the reactivity of reagents in such reactions with their strings. Among these reactions, reactions involving peroxide radicals are of considerable importance (Choe and Min, 2009; Fomin V., 2010; Laguerre et al., 2015). They cause oxidative destruction of organic compounds, which can be prevented by natural and synthetic inhibitors (Denisov, 2000; Guseva, 2010).

From the theoretical and practical point of view, the features of the oxidation acylglycerols of fats, as well as the regularities that regulate the rate and direction of this process, are great interest (Lee et al., 2015; Kovtun and Pluzhnikov, 1995). Therefore, the process of oxidation vegetable oils and fats is an urgent task for both the food industry and physical chemistry. It allows in practice to solve the basic task of physical chemistry (to predict the course of the chemical process and the final result) and makes it possible to control this process.

The problem of increasing the resistance of vegetable oils to oxidation is associated with the investigation of the activity and mechanism of action of the antioxidants contained in them. Tocopherols are natural antioxidants for oils and fats.

They increase the induction period and reduce the rate of oxidation (Rubalya et al., 2015; Tyutyunnikov, 2002). The purpose of this paper is to investigate the relationship between the degree of unsaturation of the triglycerol composition of natural oils, as well as their antioxidants, and the rate of oxidation under auto-oxidation conditions.

2. Materials and methods

We chose samples of the most used vegetable oils: sunflower, corn, olive, walnut, palm and palm kernel.

Chromatographic investigation of the composition of methyl esters of fatty acids were carried out on a gas chromatograph Hewlett-Packard HP 6890 with flame ionization detector, the injector S / S with allocation of flows, Sp2380 column length of 100 m, an inner diameter of 0.25 mm, coating thickness 0.2 micron . Tocopherols Detection was performed using a liquid chromatograph Hewlett-Packard HP1100 with fluorescence and diode array detectors, reciprocating-phase column Hypersil MOS 2,1mm in diameter, 200 mm length.

Activity was determined by natural antioxidants with stable chromogen radical DPPH (2,2-diphenyl-1-picrylhydrazyl) in spektrofotometre Helios Omega UV-VIS (Thermo scientific). To prepare working solutions, 96% methyl alcohol was used, the initial concentration of DPPH in the reaction mixture was $7 \cdot 10^{-5}$ mol / l. The degree of decolorization of DPPH solutions after application of oils was determined spectrophotometrically at 515 nm. The reaction was carried out without access to light in quartz cuvettes 10 mm thick.

3. Results and discussions

Under real conditions, the oils contain hydroperoxides, which accumulate in fats during their production (Waraho et al., 2011; Denisov, 1996). Therefore, the main source of radicals in the system is the decomposition of hydroperoxides. The process of oxidation of vegetable oils is a chain free-radical process, which can be described by the following stages (Semenov, 1986; Liebler et al.,1990; Khrapova, 2010):

Initiation:

$$RH + O_2 \rightarrow R\cdot + OH$$

Development:

$$R\cdot + O_2 \rightarrow ROO\cdot$$
$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$

Clipping:

$$R\cdot + R\cdot \rightarrow RR$$
$$R\cdot + ROO\cdot \rightarrow ROOR$$
$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$$

In the first initiating phase, a free radical is formed from the lipid substrate under the action of the initiator. Chain branching occurs as a result of the radical decomposition of hydroperoxides, which are the only primary oxidation products.

If the oil contains an inhibitor, then an additional channel for the consumption of peroxide radicals appears:

$$ROO\cdot + InH \rightarrow ROOH + In\cdot$$
$$ROO\cdot + In\cdot \rightarrow Molecular\ products$$

The isomers of tocopherol are not equivalent in their anti-radical activity.

It is known (Stromberg, 1988; Farhush et al., 2011; Paradiso et al., 2010) that the anti-radical efficacy of tocopherols, which rises from α- to γ-isomer, is not proportional to their vitamin activity, which decreases in this order.

Table 1 - Content of tocopherols in the samples of the tested oils

Sample of vegetable oil	Total content, mg%	Isomers,% of the total			Total amount, mg%
		α	B	γ+δ	
Sunflower unrefined	95	91,5	8,5	-	40,3–102,1
Walnut press	109	46,4	48,0	5,6	56,0–113,0
Olive of the first pressing	10	73,7	26,3	-	13,0–45,3
Sunflower refined-deodorized	61	92,2	-	7,8	44,0-152,0

Corn refined deodorized	655	49,1	42,4	8,5	31,4–347,2
Palm	9	0,4	74,0	52,6	15,0–150,0
Palm-core	56	27,0	73,0	-	30,0–180,0

The inhibitory effect of tocopherol is due to the presence in its structure of an O-H group, which is attached to the aromatic nucleus and shielded by methyl groups. Due to the presence of the π-electron system, the negative charge shifts to oxygen. As a result, it is possible to easily tear off the hydrogen atom in the hydroxyl group -OH (a radical form (T˙) of tocopherol is formed). Tocopherols can give a hydrogen atom from the 6-hydroxy group of the chroman ring to the lipid peroxide radical and neutralize it. Tocopherol (T) with a reduction potential of 500 mV gives hydrogen to the peroxide radical of the lipid ROO˙, which has a reduction potential of 1000 mV. Lipid hydroperoxide (ROOH) and tocopherol hydroxy radical are formed, which is more stable than the lipid radical due to its resonant structure.

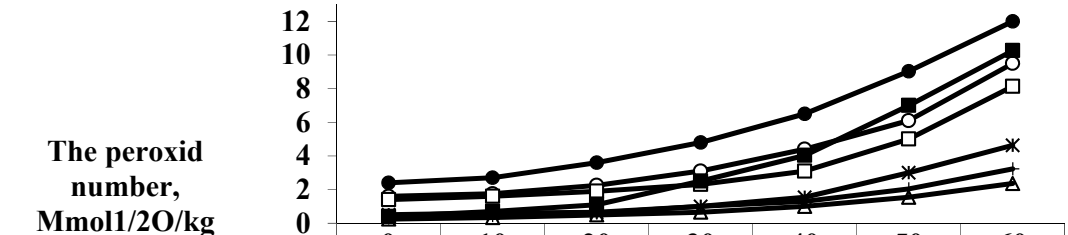
This leads to a slowing down of the oxidation rate at the branching stage of the chain during auto-oxidation (Lutterodt et al., 2011; Piven, 2007). To determine the content of tocopherols, the liquid chromatography method of high-partition capacity was used (Kim, 2015; Goupy et al., 2003) (Table 1).

It was found that the content of tocopherols depends on the type of oil and on the depth of its refining.

Oil refining is a complex of successive stages of oil processing by physical methods (sedimentation, filtration, centrifugation), chemical (neutralization) and physico-chemical (wax freeze, bleaching, hydration, deodorization).

Each of these stages leads to a decrease in the content of tocopherols, which leads to accelerated oxidation of the oil during storage.

The high content of tocopherols among the test samples was found in corn refined oil.



	0	10	20	30	40	50	60
sunflower unrefined	1.6	1.75	2.25	3.1	4.4	6.1	9.5
olive	2.4	2.7	3.6	4.8	6.5	9.03	12
walnut	1.4	1.6	1.9	2.3	3.09	5	8.13
sunflower refined	0.4	0.7	1.1	2.5	4.03	7	10.26
corn	0.5	0.6	0.65	1	1.54	3	4.63
palm	0.24	0.33	0.49	0.65	1	1.54	2.36
palm kernel	0.31	0.45	0.6	1	1.3	2.04	3.23

Auto-oxidation time, days

Figure 1 - Dynamics of auto-oxidation of test oils

The smallest content of tocopherols was recorded in palm oil. In sunflower unrefined oil, the total concentration of tocopherols does not exceed 95 mg%, which is represented by α-tocopherol by 91.5%. γ - and δ-isomers in the samples under study are represented by an insignificant amount from 5.6% in walnut oil to 52.6% in palm oil, this is due to the natural characteristics of the oils.

The fatty acid composition of oils triglycerols was studied by the chromatography of high-specific ability to study the features of the course of oxidative degradation (Table 2).

The composition of tropical oils is dominated by saturated fatty acids, while sunflower oil, walnut oil, and corn are among the group with the largest mass fraction of polyunsaturated linoleic acid C18: 2, 62.59%, 61.36% and 59.99%, respectively. Olive oil contains the most quantity of monounsaturated oleic acid C18: 1, namely 72.09%.

Linoleic and linolenic acids are actively involved in the reactions of isomerization, cyclization and polymerization. Linoleic acid and more highly unsaturated acids begin to oxidize at temperatures below 60 ° C, oleic acid at temperatures above 100 ° C.

Therefore, the oleic fraction of palm oil has been widely used as a highly profitable oil for use at elevated temperatures. It has a high resistance to oxidation in comparison with other types of modified fats (Jeong, 2012).

The kinetics of oxidation of oil samples at each control point was investigated under conditions of free access of light and air (autoxidation) at a temperature of 22 ± 2 ° C by the accumulation of hydroperoxides in them after changing the peroxide number (PN) according to the standard procedure (Kulisic et al., 2004) (Fig.1).

The most stable were oils of tropical origin - palm and palm kernel: the value of the PN at the end of the experiment for them is 2.36 and 3.23 mmol1 / 2O / kg, respectively.

This can be explained both by the fatty acid composition of the oils, and by the low initial value of the IF in the oils, which is within the period of induction of oxidation. Similar results were obtained for corn oil - at a low initial value of the PN, high stability after 60 days of oxidation was revealed.

The course of oxidation of sunflower and walnut oil occurs in a similar way - an induction period of 30 days and rapid accumulation of hydroperoxides to the end of oxidation.

Refining of oils includes a complex of successive stages of oil processing by physical methods (sedimentation, filtration, centrifugation), chemical (neutralization) and physico-chemical (wax freeze, bleaching, hydration, deodorization).

Each of these stages leads in particular to a decrease in the content of tocopherols, which leads to an accelerated oxidation of the oil during storage.

Determination of oxidation indexes of oils made it possible to apply the Tsepalov graphical method for calculating the kinetic parameters of the oxidation process.

According to the theory of chain radical processes involving inhibitors, the equation of oxidation rate has the form (Rubalya, 2015; Scherer and Godoy, 2009):

$$W = \frac{k_{p2} \times [RH]}{k_7 \times f \times [lnH]} \times W_i \qquad (1)$$

where k_7 – is the rate constant of the chain termination reaction (the rate constant of the interaction of peroxide radicals with natural antioxidants);

f - is inhibition coefficient (the capacity of the inhibitor), the number of free radicals "dying" on one molecule of the inhibitor;

$[lnH]$ - is concentration of the antioxidant (inhibitor);

W_i - is initiation rate;

k_{p2}- is constant of the chain extension rate;

$[RH]$ - is the product concentration.

Table 2 - Fatty acid content in the samples of the tested oils

Fatty acid	Content in oils,%					
	Sunflower	Walnut	Olive	Corn	Palm	Palm-core
Saturated fatty acids						
C 6:0	-		-	-	-	0,81
C 8:0	-		-	-	-	2,43
C 10:0 Capric	-		-	-	-	2,67
C 12:0	-		-	-	0,91	49,67
C 14:0	0,08	0,02	-	-	1,95	15,12
C 16:0	6,73	6,06	11,95	6,47	59,60	6,55
C 17:0	-	-	-	-	0,11	-
C 18:0	3,55	2,02	3,15	3,59	5,59	-
C 20:0	0,23	0,08	0,37	0,25	0,43	0,11
C 22:0	0,58	0,02	0,10	0,56	0,07	0,02
C 24:0	0,20	-	-	0,19	0,08	-
Total	11,37	8,2	15,57	11,06	68,74	77,38
Monounsaturated fatty acids						
c9-C16:1	0,10	0,10	0,94	0,10	-	0,11
C 18:1w12t Petroselaidic	-	16,56	-	-	-	-
t9-C 18:1 Elaidic	-	-	-	-	0,16	-
C 18:1w9c Oleic	24,61	-	72,09	27,77	25,31	1,00
C 18:1w7c	1,05	-	-	-	0,70	18,99
C 20:1w9	0,17	0,18	-	-	0,07	-
C 24:1	-	-	0,36	-	-	-
Total	25,93	16,84	73,39	27,87	26,24	19,99
Polyunsaturated fatty acids						
9,12-c, t-C18:2	-	-	-	-	0,12	-
9,12- t, c-C18:2	-	-	-	-	0,12	-
C 18:2w6c Linoleic	62,59	61,36	7,13	59,99	4,78	2,54
C 18:3w3 a-Linolenic	0,10	13,60	0,60	0,12	-	-
C 22:2	-	-	0,10	-	-	-
Total	62,69	74,96	7,83	60,11	5,02	2,54
Amount	100,0	100,0	100,0	100,0	100,0	100,0

The concentration of the inhibitor varies with time:

$$f \cdot [InH]_t = f \cdot [InH]_0 - W_i \cdot t, \quad (2)$$

$[\mathrm{InH}]_t$- is the concentration of the inhibitor at time t;

$[\mathrm{InH}]_0$- is the inhibitor concentration at the beginning of the process.

An important characteristic of the chain process is the induction period τ. The induction period is the greater, the higher the rate constant of the chain termination reaction and the less the chain extension rate.

The value of the induction period τ is the time during which the chain termination passes on the inhibitor molecules:

$$\tau = f \cdot [InH]_0 / W_i, \quad (3)$$

provided that $[InH]_t$=0 and $t = \tau$.

According to the kinetic curves (Fig. 2), the induction period is graphically determined by approximating the tangents in the initial region for each product sample.

For each time point in the induction period, the value ln (1-t / τ) is calculated and the dependence: PN-ln (1-t / τ) is constructed (Fig. 2).

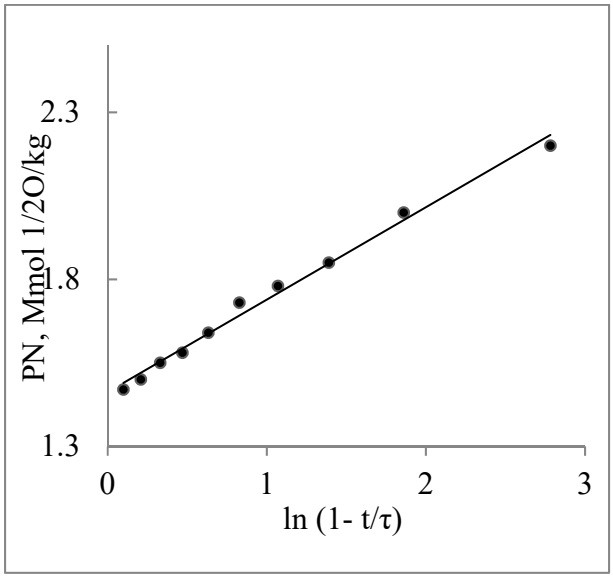


Figure 2 - Oxidation rate in logarithmic coordinates (for example, walnut oil)

The parameter k_{p2}/k_7 , which corresponds to the ratio of the rate constants of the reactions, elongation and termination of the circuits in the induction period is calculated as t $^{g\alpha}$ of the slope angle of the forward PN-ln (1-t / τ). The results are given in Table. 3

Table 3 - Kinetic parameters of oxidation of test oils

Sample of oils	$(k_{p2}/k_7)\cdot 10^2$, (L / mol · s)
Sunflower unrefined	2,82
Walnut press	3,07
Olive of the first pressing	4,55
Sunflower refineddeodorized	2,93
Corn refined deodorized	3,88
Palm	1,84
Palm-core	2,66

The best kinetic parameters are palm and palm kernel oil: the values of the constants k_{p2}/k_7 are 1.84 and 2.66, respectively, which indicates the prevalence of the reaction speed of chain termination on the inhibitor molecules before the extension reactions. The rate of initiation with autoxidized oils is determined by their fatty-acid composition (with other equal conditions), primarily by the content of mono- and polyunsaturated acids (Fomin, 2010; Khrapova, 2010). So, as the content of saturated fatty acids in the triglycerol structure increases, the initiation rate W_i decreases.

Since the chain initiation rate (initiation rate) for linoleic acid is about an order of magnitude higher than for oleic acid (Tyutyunnikov et al., 2002; Denisov, 1996), the rate of initiation for oils with a high content of polyunsaturated fatty acids should be higher, and the induction period, respectively, lower. This is observed in the oxidation of saturated tropical oils.

To determine the activity of natural antioxidants as free radical scavengers, a method with DPPH was used (Jeong et al., 2012; Rubalya and Neelamegam, 2015; Scherer and Godoy, 2009). The standard substance was α-tocopherol. DPPH, soluble in methanol, reacts with the antioxidant sample according to the scheme:

$$DPPH^{\cdot} + AH = DPPH\text{-}H + A^{\cdot}$$

As a result of the reduction of the DPPH with the antioxidant of the sample, the purple-blue color of DPPH decolours, and the reaction is controlled by a change in the optical density. The determination was carried out every 15 minutes for 1 hour.

The antioxidant activity of the oil samples was calculated by the formula:

$$AA = [1 - (A_1 - A_2)/A_3] \times 100\%, \quad (4)$$

where A_1- is the absorbance of the solution of the test sample with the DPPH solution, A_2- is the amount of absorbance of the solution of the test sample with the methanol solution, A_3- is the amount of absorption of the DPPH solution in methanol.

The value of the antioxidant activity of the oil samples was determined from the calibration curve (Fig. 3).

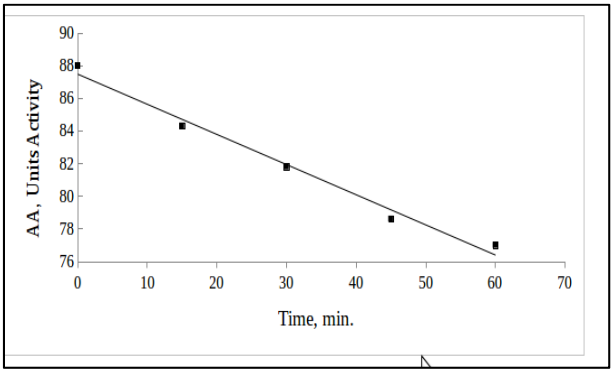


Figure 3. Calibration curve of antioxidant activity (on the example of palm oil).

The significance of the results is expressed through the values of the EC50-concentration of the antioxidant, at which 50% inhibition of the DPPH radical occurs. The results are shown in Table. 4.

Table 4 – Antioxidant activity of test samples

Sample of oils	AA, Units activity
Sunflower unrefined	50,0
Walnut press	23,2
Olive of the first pressing	73,1
Sunflower refined deodorized	58,2
Corn refined deodorized	45,0
Palm	81,8
Palm-core	77,3

According to the reaction with DPPH, corn refined oil is the most stable to oxidation processes, since about half of its vitamin activity is realized due to α-tocopherol, which acts as an inhibitor of oxidation reactions. Tropical oils, which contain a small amount of natural antioxidant, are rapidly oxidized already in the initial stages of the reaction with DPPH. This is clearly seen in the comparison: palm oil with a content of 9 mg% tocopherol is spoiled rather than palm kernel oil with a tocopherol content of 56 mg%. The total content of tocopherol in olive and palm oils is practically the same, but in the isomeric olive oil composition, highly active α-tocopherol predominates. This explains the great antioxidant activity of olive oil at approximately equal oxidation rates of the oils under consideration. Sunflower and nut oils differ little in speed of the initial stages of oxidation. This can be explained both by a similar extraction method (press) and by a similar value of the inhibitor content.

4.Conclusions

The dependence of the value of the induction period of oxidation of vegetable oils with the natural content of inhibitors under autoxidation conditions was studied. It has been shown that the inhibitory effect of tocopherol, namely its total content with the highest content of α-isomer in comparison with the degree of unsaturation of fatty acid composition of oils, is the decisive factor in inhibition of oxidative reactions. The results of the auto-oxidative stability of oils correlate with the parameter of antioxidant activity, which takes into account the mutual influence of the factors of elongation and termination of oxidation chains. The continuation of research on the chemistry of the interaction of peroxide radicals on molecules of fatty acids under the conditions of the inhibited process is a promising direction.

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