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CONTENTS

ORIGINAL PAPERS

CHARACTERIZATION OF RHEOLOGICAL, SENSORIAL AND ANTIOXIDANT PROPERTIES OF BREAD ENRICHED WITH CARROT POWDER

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ABSTRACT

The relationship between health and food has a growing impact on food innovation. The aim of developing food product by adopting nutritional knowledge is to improve the consumer health. Consumers prefer innovative products with convenience and quality. Effects of the addition of carrot powder at various levels of 0, 5, 10, 15 and 20% were examined to get a bread enriched with antioxidants, minerals, having good rheological and sensorial properties. Dynamic rheological measurements were used to assess the bread rheology, total phenolics were estimated by using Folin-Ciocalteau reagent, antioxidant activity was recorded in terms of DPPH (% inhibition), mineral profiling conducted by atomic absorption spectroscopy and sensory properties of the bread sample were evaluated. The textural properties of the supplemented bread were observed in terms of hardness, gumminess and chewiness of bread. The highest values of TPC (57.41 mg GAE/100g) and DPPH (83.14%) were achieved for the samples with higher percentage of carrot powder (20%). But it showed poor results concerning the rheological properties and texture by indicating a tougher structure with darkest color after sensory evaluation. The mineral contents were exceptionally increased with increasing percentage of carrot enrichment in bread. Among the studied samples, a range of 5-10% added carrot powder exhibited good antioxidants and mineral contents, with better rheological characteristic and sensorial acceptability.

KEYWORDS:
Texture, characterization, rheology, sensory, antioxidants, minerals

INTRODUCTION

Carrot (*Daucus carota* L.) is a root crop and one of the significant vegetables grown round the world. It is a good source of phenolics, carotenoids and polyacetylenes [1-3]. The major biological role of carotenoids is as a precursor of vitamin A. Carotenoids are effective antioxidants found in carrots and help the body in neutralizing radicals [4]. Carrot also helps in reducing the high blood pressure risk, stroke and heart disease. Now a days, consumption of carrot-based products has significantly increased due to its anticancer and antioxidant potential [5]. Carrot is an excellent source of vitamins and carotenes. Its utilization includes as cooked vegetable, raw, beverages and juices [6]. Its consumption would also be very beneficial in alleviating the deficiency of vitamin A specifically, among the adults and children under six years.

Functional bakery products such as bread enriched with various vegetables, spices and herbs are very important for human diet. Inclusion of these stuffs find a significant place as non-caloric and cheaper agents for the partial replacement of wheat flour [7, 8]. Additionally, decrease in calorific value, increase in fiber and mineral content are important features which prolong the freshness of baked product due to its capacity to retain moisture. It also primarily reduces economic losses [8, 9].

In current scenario, the consumers prefer healthier foods with antioxidative and medicinal values in order to prevent non-communicable diseases. Maintaining the rheological and sensorial properties of such baked products is also challenge. Hence, researchers and industry are struggling to optimize and develop the bakery products to improve quality, variety and availability [10]. Keeping in mind all these factors, the present study was designed to develop the carrot enriched bread and to evaluate its antioxidant, rheological as well as sensorial characteristics.

MATERIALS AND METHODS

Carrot Powder Preparation. Fresh raw carrot samples were procured from local wholesalers. The samples were washed under tap water, peeled and sliced into a thickness of 56 mm. Blanching of carrot was conducted for 2-3 minutes in hot water. Sodium metabisulphite (0.1%) was added to avoid discoloration and browning. Cooling of sulphited samples by exposing to air was done and dried by using cabinet drier at 60°C for 10 hours. The dried samples were ground (Black & Decker Model, BX600G) and passed through a sieve of 2 mm then 1 mm accordingly, to get a fine powder.

Dough and Bread Preparation. Carrot powder was incorporated in wheat flour dough at the rate of 0, 5, 10, 15 and 20% level. Bread prepared without carrot powder (0%) was used as control. The main ingredients of the dough were kept constant for each bread sample *i.e.* white flour (500 g), water (220 ml), baker's yeast (17.5 g), bread improver (5 g), vegetable oil (15 g) and salt (4 g). Bread prepared from straight dough method with some modifications. Dry ingredients were manually mixed in a bowl. Water and shortening were added to all ingredients and then added to electric mixer (Kenwood KM240 Stand Mixer, UK). The whole ingredients were thoroughly mixed with electric mixer for 15 min. The dough was taken out from mixer and allowed to stay for 15 min. The molded dough was placed in a pan and proofed for 45 min at 35°C and 85% relative humidity. The proofed dough was baked at 220°C for 20 min in a baking oven (Doyon, CA6X). Cooling was done for 1 hour at room temperature, sliced and packed in polyethylene bags for further analysis.

Farinograph Test. Farinograph test was carried out to determine the arrival time (min), water absorption (%), dough stability (min), degree of softening (dough weakening, BU) and dough development time (min) of the dough made from various treatments under evaluation. Test was performed by using farinograph apparatus (Chopin Technologies, France) with 250 gm sample (triplicate) based on 14% moisture content. This method was obtained from farinograph instrument [11].

Mixograph Test. Rheological properties of raw material were determined by Chopin Mixolab, (Villeneuve-la- Garenne, France) using Chopin+ protocol with slight modifications in dough weight from 70g to 80g. The mixolab allows the mixing of dough under the controlled temperature with a temperature sweep until 90±1°C followed by a cooling step. It calculates in a real time the torque (Nm) produced by the passage of dough between two kneading arms, hence allowing the understanding of physicochemical behavior of the dough. In mixograph test, the Mixolab curve showing the different parameters: water absorption (%), mixing time (min), dough development time (min), viscosity (Nm) and retrogradation (Nm).

Total Phenolic Content (TPC). Total phenolic content was evaluated by using Folin-Ciocalteau reagent with analytical grade Gallic acid as standard. One milliliter of standard solution (0-500 mg L^{-1}) was added to 10 ml deionized water and 1.0 ml of Folin-Ciocalteau phenol reagents. After 10 min, 2 ml of 20% sodium carbonate was added to the mixture and put in the dark for 1 hour. The absorbance measured at 750 nm. The concentration of total phenols was expressed as mg g^{-1} of dry extract [11].

DPPH (2,2-diphenyl-1-picrylhydrazyl). Free radical scavenging activity of carrot bread was measured according to the DPPH method [12] with some modification. Three dilutions of carrot bread in the ration of 5/100, 10/100 and 15/100 were prepared in ethanol/water (v/v). An aliquot of 0.5 mL of each sample of carrot bread extract was added to 3 mL of DPPH solution and then added to 2 mL ethanol. The absorbance of DPPH at 517 nm was observed spectrophotometrically (Specord 200 plus).

Texture Analysis. Texture analysis of bread samples were conducted by texture analyzer equipped with 10 mm diameter aluminum cylinder probe and 10 kg load cell. Texture profile analyzer (TA-XT plus, Stable Micro Systems, UK) in a double compression cycle was carried out with 50% penetrate depth and a 20 sec gap between compression on slice 6 mm/sec test speed. The textural parameter considers the hardness (N), gumminess (N) and chewiness (N) of bread. For textural measurements of each sample, 2 slices (triplicate) were cutdown from the central portion of three different bread loaves [14].

Mineral Composition. Samples were mineralized in a hot air oven (Universal UN30, Memmert). Aliquots of 0.5±0.1 g of minced bread powder (triplicate) was taken in Teflon cup. The sample was diluted with 10 mL of 500 mL L^{-1} HNO_3. The solution was placed for 24 hr and kept in microwave oven for 30 min. The mineralized sample was collected after cooling with 4 mL L^{-1} HNO_3 in 150 mL vessel. A blank sample was also prepared. All the minerals were evaluated by using atomic absorption spectrometry [14, 15].

Sensory Evaluation of Bread Loaf. Carrot powder enriched bread was organoleptically assessed for its internal and external characteristics [16, 17]. Sensory analysis of carrot enriched bread was carried out by 10 panelists. The slice of each bread type codded with a number was served to each panelist under normal light. The sensory characteristics i.e. crust and crumb color, texture, aroma, taste

TABLE 1
Mixing properties of dough supplemented with carrot powder

Treatments	Mixing Properties of Dough				
	Water absorption %	Dough development time (min)	Dough stability (min)	Dough Softening (UF)	Mixing tolerance index (BU)
Control	62.37±1.86^{de}	3.57±0.61^{e}	5.33±0.91^{e}	62±2.1^{a}	35.16±2.82^{a}
5%	63.09±1.39^{d}	4.07±0.59^{d}	6.57±0.47^{d}	57±3.7^{b}	29.37±1.26^{b}
10%	66.57±2.19^{bc}	5.1±0.6^{bc}	7.53±0.49^{c}	46±5.2^{c}	22.19±1.08^{c}
15%	67.23±1.55^{b}	5.33±0.86^{b}	8.27±0.91^{b}	37±3.6^{d}	17.21±0.19^{c}
20%	69.03±1.66^{a}	9.27±0.97^{a}	10.21±0.9^{a}	27±2.1^{e}	11.27±0.23^{d}

Data presented by means ± standard deviation of 3 replicates. Means within a column sharing different letters are significant at $p \leq 0.05$

and overall acceptability were evaluated. Nine-point Hedonic scale (1 being dislike extremely to 9 as like extremely) was used to score the sensory characteristics of bread.

Data Analysis. Results were presented with means and standard deviation. Each experiment was conducted with three replicates. Analysis of variance was done to evaluate the significant observations and interactions followed by Least Significant Difference (LSD) test to identify the significant differences among means ($P < 0.05$), using statistical software (SAS V.9.1, SAS Institute, NC, USA).

RESULTS AND DISCUSSION

Rheological and Mixing Properties of Dough. The dough mixing properties and rheological characteristics of dough supplemented with carrot powder are depicted in Table 1. These parameters are useful to predict the potential of carrot powder along with wheat flour for bakery products such as bread. Wheat flour enriched with varying level of carrot powder were the principal components of the dough and their effects are summarized in Table 1. It was observed that water absorption increased (62.37-69.03%) with increasing level of carrot powder (0-20%) as compare to the control sample. These results were in agreement with the findings of Tańska [18] as well as Ashoush and Gadallah [19] when the dried mango peel and carrot powder were mixed with wheat dough. The elucidation behind this phenomenon partly depends on the factor that the fiber structure holds large number of hydroxyl groups, that interact with hydrogen bonds of water [20, 21].

The dough development time was increased from 3.57 to 9.27 with 20% incorporation of carrot powder. During the mixing phase, water molecules hydrate the flour components to develop the dough [9-22]. Similar pattern was noticed by Borchani [23]. Dough stability is associated with protein matrix quality and the incorporation of other ingredients easily damaged this [9-21]. Addition of carrot powder found to enhance the dough stability and a linear increasing trend (5.33-10.21) was observed as depicted in Table 1. These results were alike with those concluded for orange waste products, potato fiber and wheat dough supplemented with rice bran [24, 25]. From the results it was concluded that with increasing concentration of carrot powder in wheat flour, the mixing tolerance index gradually decreased as illustrated in Table 1.

Figs. 1 (a-e) showed the effects of the substitution of wheat flour with carrot powder 0%, 5%, 10%, 15% and 20% on farinograph. The obtained results showed that the arrival time and dough stability were found to be higher in wheat flour (control sample) compared with all the substitutes. The apparent mentioned results were indicated in Table 1. Substitution of carrot powder primarily enhanced the absorption of water by increasing the carrot powder level from 0% to 20%. The highest rise in the water absorption was found with the addition of 10% carrot powder compared to the other fractions as well as the control sample (62.37%). Same effects were also observed by Abou-Zaid [26] on water absorption, when the fiber rich matter was substituted and reported its cause that fiber retain more water. Replacement of wheat flour with chickpea flour enhanced the requirement of water for optimal breadmaking absorption from 58.6% for wheat flour to 62.3% for 30% chickpea flour). Water absorption was amplified with the increase in the amount of added chickpea flour added [22]. Furthermore, the higher hydration capacity of water also enhanced the water absorption of wheat flour [27].

Mixolab Measurements. Mixolab measurements allow the description of physicochemical response of the dough when submitted to dual temperature and mixing constraints. It was made possible to estimate the mechanical changes due to heating and mixing. Figs. 2 (a-e) showed a typical Mixolab curve for control as well as varying level of carrot powder supplemented dough in which various stages can be differentiated pertaining to the dough changes due to both the temperature and mixing force.

Initially during the mixing stage, dispersal of the material, hydration of flour compounds and the distribution of the primary spherical protein particles happens along with the alignment and stretching proteins. It resulted into the development of three-dimensional viscoelastic structure along with gas retaining properties [28]. At this stage, increase in the torque (C1) was detected till highest was reached, after that the dough resisted the deformation for a

while. The phase of persistent torque determines the stability of dough. If there is excessive mixing, the properties of dough go from good (elastic and smooth) to poor (sticky and slack) [29]. The reduction was observed in the torque at second stage (Cs), showing the weakening of protein network. The mutual effect of the temperature as well as mechanical shear stress resulted in further reduction in torque (C2, third stage) that could be associated with the commencement of the protein unfolding and destabilization. The lowest torque was noticed in the range of 42-52°C, showed that more protein changes might be masked by heating due to the modification of the physicochemical properties of starch. During the heating phase, unfolding and destabilization of protein may facilitate sulphidryl–disulphide interchange reactions [30].

The role of protein shifted to a secondary place due to the increase in temperature. The starch gelatinization was responsible at this stage for further differences in torque. As the temperature increased, the proteins go to secondary place, due to the gelatinization of starch which is the major responsible for further variations in torque (C3, fourth stage). At this stage, the starch granules swell by absorbing the water available in the medium. Leaching down of the amylose chains occur in the intergranular aqueous phase promoting the increase in torque due to the increase in viscosity. This enhancement in torque continued till the temperature and mechanical shear stress lead to the physical breakdown of the granules which is related to the reduction of viscosity (C4, fifth stage). In slurries which comprised of wheat flour that breakdown has been associated to the cooking of starch [31]. A reduction of temperature that is resulted in an enhancement of the torque due to increase in the dough resistance (C5, sixth stage).

Mixograph Profiler Index. One can see the ranking of the index in Figs 3(a-e) defined by Mixolab Profiler: Water absorption index (absorption potential), Mixing index or dough behavior at 30°C during mixing, Gluten+ index or behavior of the gluten after heating the dough, maximum viscosity during heating which relies on both starch quality (viscosity index) and amylase activity (amylolysis index), the ability to withstand amylolysis and retrogradation index (starch retrogradation).

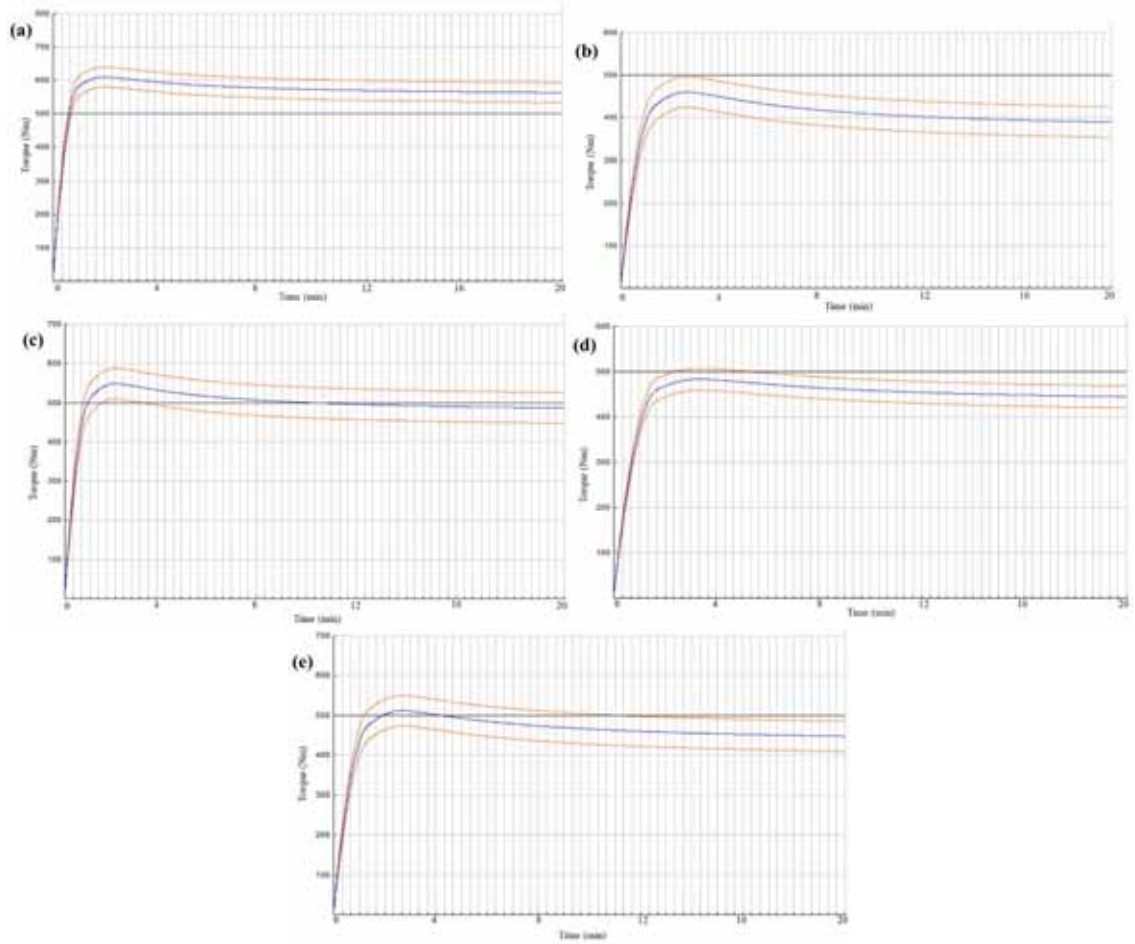
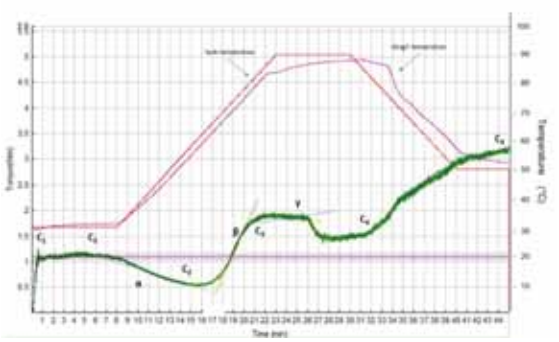
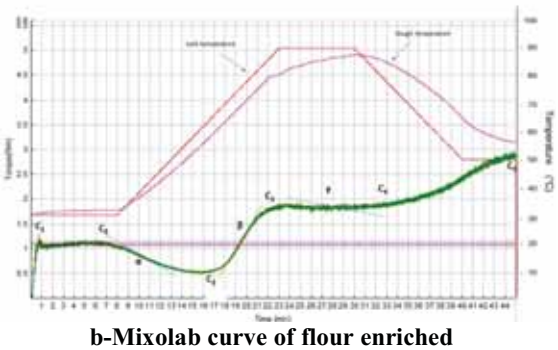


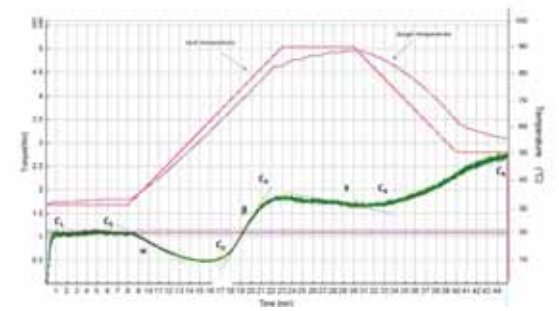
FIGURE 1
Farinograph of wheat flour substituted with carrot powder (a) 0% (b) 5% CPP (c) 10% CPP (d) 15% CF and (e) 20%



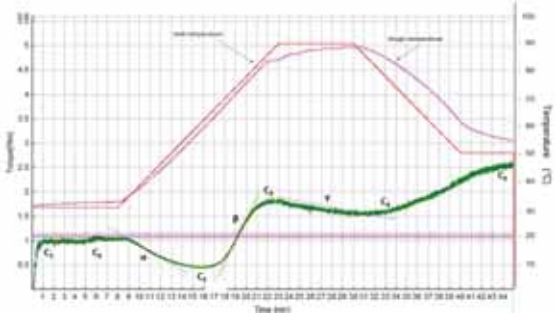
a-Mixolab curve of flour without carrot powder



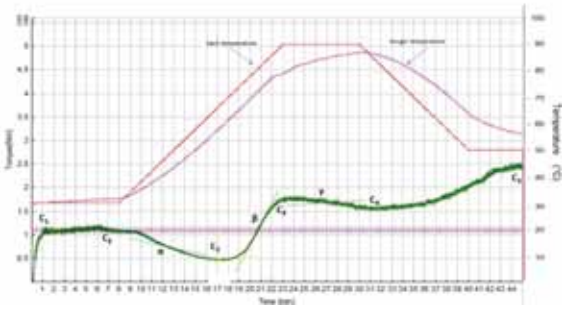
b-Mixolab curve of flour enriched with 5% carrot powder



c-Mixolab curve of flour enriched with 10% carrot powder



d-Mixolab curve of flour enriched with 15% carrot powder



e-Mixolab curve of flour enriched with 20% carrot powder

FIGURE 2
Mixolab curve

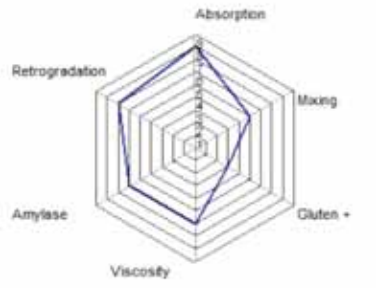
The maximum value of the Gluten+ index and Mixing index corresponds to high gluten resistance to heating with more dough stability in mixing, respectively. The high dough viscosity corresponds to high value of viscosity index during heating. On the other hand, the high values of Retrogradation index and the Amylolysis index corresponds to low amylase activity, ultimately the low shelf life of end product [32]. Comparing with the control sample, it can be observed that mixing index is higher in the case of flour with 10% carrot powder but with low Gluten+ index. Gluten index reduced from 96% to 90% with the rise in wheat bran stream incorporated in wheat flour. This parameter gives information both on quality and quantity of gluten and is generally based on the ratio of low/high molecular weight proteins [33]. High gluten index suggests superior share of high molecular weight proteins exist in gluten [34].

Whereas the higher Viscosity index and Retrogradation index were noticed for control sample with 0% carrot enrichment (Fig. 1) and highest Amylase index was observed (Fig. 2) for 5% carrot powder enriched flour. Optimum viscosity during heating depends on both starch quality and amylase activity (viscosity index), starch ability to withstand starch retrogradation (retrogradation index) and amylolysis/amylolysis index [33]. The highest value of viscosity index is associated with the high viscosity of dough during heating, whereas the high values of the Retrogradation index and Amylolysis index relates to the low shelf life of the product and low amylase activity [32].

a-Mixograph profiler index of flour without carrot powder

b-Mixograph profiler index of flour enriched with 5% carrot powder

c-Mixograph profiler index of flour enriched with 10% carrot powder



d-Mixograph profiler index of flour enriched with 15% carrot powder

e-Mixograph profiler index of flour enriched with 20% carrot powder

FIGURE 3
Mixograph profiler index

TABLE 2
Effect of carrot powder on textural properties of bread

Treatment	Hardness (N)	Gumminess (N)	Chewiness (N)
Control	19.44±2.22^{d}	18.47±1.73^{d}	9.30±1.93^{d}
5%	27.51±3.19^{c}	25.47±3.38^{d}	19.74±8.54^{c}
10%	45.93±6.14^{b}	43.29±6.34^{c}	35.35±6.11^{b}
15%	52.20±3.66^{b}	50.66±2.87^{b}	40.57±3.42^{ab}
20%	60.01±4.31^{a}	58.32±4.14^{a}	48.78±2.90^{a}

Data presented by means ± standard deviation of 3 replicates. Means within a column sharing different letters are significant at $p \leq 0.05$

Texture Analysis. The results of texture properties of bread enriched with carrot powder showed an evident effect. The hardness of bread crumb was increased significantly with increasing percentage of added carrot powder. Maximum hardness (60.01N) was observed in bread enriched with 20% carrot powder compared with the control sample as indicated in Table 2. Similar pattern of bread hardness was reported by Kumar and Kumar [35] from 41.07 N to 116.10 N in 0% and 9% carrot powder enriched bread. High fiber composition of bread was supposed as the major reason of increased hardness [36]. Increased in the gumminess and chewiness characteristics of bread was observed with increasing level of carrot percentage from (18.47N, 9.30N) for control samples to (58.32N, 48.78N) for the bread enriched with 20% carrot powder. The highest gumminess was also reported by Kumar [37] for the cutlets enriched with bread crumb and carrot powder. Similarly, the chewiness of bread was increased after the supplementation of extruded and unextruded millet flour [38]. Chewiness of bread was directly linked with the fiber content and dilution of gluten by adding of other components.

Sensory Analysis. Information on the sensory characteristics of the bread is useful to predict the possible supplementation of carrot powder in wheat flour and also it reflects the end product. The effect of carrot enrichment on the sensorial properties of bread are summarized in Table 3. It was noticeable that standard brown color of bread crust was obtained in control sample, however it gradually increased with increasing level of carrot powder. The control sample showed a crust color with a score of 8.52 and it increase from 7.3 for 5% carrot powder to 3.5 for 20%. Similarly, the texture of the bread become more and more softer with increasing percentage of carrot powder compared with the control sample. The more likely crumb color was obtained

in bread samples enriched with 0-5% carrot powder and it gradually increased with varying the level of carrot powder. Non-significant results were recorded for the aroma of all bread samples as depicted in Table 3. Taste and overall acceptability of bread samples revealed that control sample (0%), 5% and 10% carrot powder enriched bread has satisfactory results as compared to other treatments.

Additional values of carrot powder resulted in considerable darker crust color of wheat rolls. Similar results were also reported by [18-35] for the dried carrot powder incorporated in cookies and bread. Results of the sensory evaluation also suggested that addition of the carrot powder in wheat flour would not interfere with the bread acceptability in fact the samples with lower concentration reflected the overall acceptability.

This result was compliance with the results of Bolarinwa [39] whereby fortification of Moringa seed powder in bread was ranged from 5 to 20%. The results of Moringa seed powder fortified bread showed that score was significantly decreased by the addition of Moringa seed powder in all sensorial attributes. Results of sensory analysis showed that 0% and 5% Moringa seed powder bread was graded same nearly in all quality parameter judged by panelists. Similar results showed in the research of [40]. In this study, black carrot flour was added in the sponge cake by the percentage from 0 to 9% black carrot cake. The results showed that 6% black carrot cake acceptable judged by panelists, while other black carrot cakes were unsatisfactory.

Total Phenolic Content. Total phenolic content of control sample and carrot enriched powder bread are presented in Table 4. The results depicted that phenolic content were considerably increased with the addition of carrot powder in bread samples. The values showed phenolic content of 26.06 mg GAE $100g^{-1}$ DW for control sample following the 32.41, 39.06, 44.12 and 57.41 mg GAE $100g^{-1}$ DW for 5%, 10%, 15% and 20% respectively. Similar trend was proposed by Pekmez and Yılmaz [41] for the flat bread enriched with black carrot fiber content at various levels. The given results are also in agreement with the findings of Konrade and Klava [42] concluded an increasing trend of phenolic content in crispbread by adding the pumpkin, carrot and apple dry extracts. Total phenolic content in fresh carrot are always higher than dried form, either by microwave or sundried. The reduction could be due to thermal treatments [43, 44].

Antioxidant Activity (% Inhibition). The antioxidant activity of bread enriched with carrot powder has been explained in Table 4. The results indicated that as the carrot supplementation increased in all bread samples, the antioxidant activity towards the DPPH activity also increased gradually. Estimation of antioxidant activity of bread samples revealed that addition of carrot powder had a significant effect on all treatments compared with the control sample. Highest antioxidant activity in terms of DPPH activity (83.14%) was observed in 20% carrot enriched bread sample following the (79.16%) for 15% sample, (68.23%) for 10% sample, (54.21%) for 5% and 36.19% for the control sample (no added carrot), respectively.

In preceding studies, results showed that antioxidant activity was increased by the addition of by-products [42]. Similar trend for antioxidant activity was reported by other researchers for sponge cake enriched with black carrot flour [40], flat bread enriched with Jamun [45] and black carrot flour incorporated cookies [46]. Few researchers showed a decreasing trend during heat treatment whereas, some scientists reported an increase or no change for both

TABLE 3
Sensory characteristics of carrot powder enriched bread

Treatment	Crust color	Texture	Crumb color	Aroma	Taste	Overall acceptability
Control	8.52 ± 1.00^{a}	6.38 ± 1.00^{a}	8.67 ± 1.15^{a}	8.21 ± 1.53^{a}	8.19 ± 0.58^{a}	8.42 ± 0.58^{a}
5%	7.31 ± 1.53^{a}	7.67 ± 1.53^{ab}	8.01 ± 1.00^{ab}	8.63 ± 0.58^{b}	7.26 ± 1.53^{a}	7.56 ± 1.53^{a}
10%	6.21 ± 1.53^{ab}	8.21 ± 1.00^{bc}	7.52 ± 1.15^{bc}	8.72 ± 0.58^{bc}	6.52 ± 1.00^{b}	5.52 ± 0.58^{b}
15%	5.82 ± 1.00^{bc}	8.73 ± 0.58^{c}	6.19 ± 1.00^{cd}	7.29 ± 1.00^{bc}	5.76 ± 0.58^{b}	4.17 ± 1.00^{c}
20%	3.57 ± 0.58^{c}	9.00 ± 1.00^{c}	5.21 ± 1.53^{d}	7.67 ± 1.15^{c}	3.32 ± 1.15^{c}	3.71 ± 0.58^{c}

Data presented by means ± standard deviation of 3 replicates. Means within a column sharing different letters are significant at $p \leq 0.05$

TABLE 4
Effect of carrot powder on TPC and scavenging activity of bread

Treatment	TPC (mg GAE/100g DW)	DPPH (%)
Control	26.06 ± 4.57^{c}	36.19 ± 2.03^{d}
5%	32.41 ± 2.87^{bc}	54.21 ± 5.68^{c}
10%	39.06 ± 4.70^{b}	68.23 ± 3.14^{b}
15%	44.12 ± 2.83^{ab}	79.16 ± 4.36^{ab}
20%	57.41 ± 6.91^{a}	83.14 ± 3.24^{a}

Data presented by means ± standard deviation of 3 replicates. Means within a column sharing different letters are significant at $p \leq 0.05$

TABLE 5
Mineral contents of bread enriched with carrot powder

Treatment	Na	Ca	K	Fe	Zn	Cu
Control	413.21±3.68^{e}	101.31±12.76^{d}	221.51±3.08^{e}	3.67±0.43^{d}	1.74±0.27^{d}	1.21±0.19^{d}
5%	429.42±5.78^{bc}	121.43±11.25^{cd}	342.62±6.89^{d}	5.31±0.49^{c}	3.28±0.59^{c}	1.62±0.19^{c}
10%	437.87±4.92^{ac}	143.10±14.84^{bc}	463.79±7.19^{c}	8.41±0.73^{b}	4.15±0.34^{bc}	2.07±0.28^{bc}
15%	451.15±7.37^{ab}	162.33±13.18^{ab}	581.31±2.74^{b}	9.08±0.69^{b}	4.81±0.53^{ab}	2.41±0.37^{b}
20%	483.18±3.13^{a}	169.27±16.90^{a}	701.06±6.81^{a}	12.57±0.83^{a}	5.41±0.83^{a}	3.07±0.23^{a}

mg/kg; Data presented by means ± standard deviation of 3 replicates. Means within a column sharing different letters are significant at $p \leq 0.05$

antioxidant activity and phenols [47, 48]. Changes in antioxidants and phenolic contents may attributed to the composition of phenolic substances (carotenoids) as well as the nature of raw material being used. In current study, the results indicated that addition of various level of carrot powder could be a great source of antioxidants.

Mineral Analysis. Significant results were noticed for the mineral analysis of carrot enriched bread as depicted in Table 5. The highest concentration of Na (413.21 mgkg^{-1}) was observed in control sample followed by Ca (101.31 mgkg^{-1}), K (223.41 mgkg^{-1}, Fe (3.27 mgkg^{-1}), Zn (1.74 mgkg^{-1}) and Cu (1.17 mgkg^{-1}), respectively. It was observed that mineral contents exceptionally increased with increasing percentage of carrot enrichment in bread. For each treated sample, the highest mineral contents were found in 20% carrot enriched bread with Na (483.18 mgkg^{-1}), Ca (169.27 mgkg^{-1}), K (701.06 mgkg^{-1}), Fe (12.57 mgkg^{-1}), Zn (5.41 mgkg^{-1}) and Cu (3.07 mgkg^{-1}), respectively. Carrot being a root crop have high mineral contents. Higher mineral contents of K (224 mgkg^{-1}), Ca (334 mgkg^{-1}) and Fe (13.9-26.4 mgkg^{-1}) were also reported by Bolarinwa [39], by adding the moringa seed powder in bread. Enrichment of various varieties of mushroom powder in wheat bread resulted in optimum level of Na (33.50-60.81 mg/g) as reported [49]. Supplementation of pumpkin powder in wheat bread stimulated the Na content (460.9 mgkg^{-1}) compared with the bread without pumpkin powder (426.1 mgkg^{-1}) [50]. Similar trend was noticed for Zn (0.5-1.1 mgkg^{-1}) and Cu (0.1-0.2 mgkg^{-1}) in bread fortified with oat, rye and buckwheat [14].

CONCLUSIONS

Supplementation of carrot powder in wheat bread appeared useful and efficient strategy to get an enhanced quality of bread. The purpose of using various fractions of carrot powder was clarified in this study with a conclusion that bread enriched with 5-10% carrot was found to be effective in terms of texture, sensory properties, mineral profile as well as antioxidant properties. Although higher proportions of carrot resulted in more antioxidant and mineral contents but their textural and rheological characteristics were decreased. This study was also helpful in exploring the nutritional quality of baked goods with efficient use of vegetable waste to be reuse for value addition of various food stuff.

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REFERENCES

[1] Babic, I., Amiot, M.J., Nguyen-The, C. and Aubert, S. (1993) Changes in phenolic content in fresh ready-to-use shredded carrots during storage. J. Food Sci. 58, 351-356.

[2] Block, G. (1994) Nutrient source of pro-vitamin a carotenoid in American diet. Am. J. Epidemiol. 139, 290–293.

[3] Hansen, S.L., Purup, S. and Christensen, L.P. (2003) Bioactivity of falcarinol and the influence of processing and storage on its content in carrots (Daucus carota L). J. Sci. Food Agric. 83(10), 1010-1017.

[4] Dias, J.S. (2012) Nutritional quality and health benefits of vegetables: A review. Food Nutr. Sci. 3(10), 1354.

[5] Lee, S.K., Mbwambo, Z.H., Chung, H., Luyengi, L., Gamez, E.L., Mehta, R.G., Kinghorn, A.D. and Pezzuto, J.M. (1998) Evaluation of the antioxidant potential of natural products. Comb. Chem. High T Scr. 1(1), 35-46.

[6] Baljeet, S.Y., Ritika, B.Y. and Reena, K. (2014) Effect of incorporation of carrot pomace powder and germinated chickpea flour on the quality characteristics of biscuits. Int. Food Res. J. 21(1), 217.

[7] Almana, H.A. and Mahmoud, R.M. (1994) Palm date seeds as an alternative source of dietary fiber in Saudi bread. Ecol. Food and Nutr. 32(3-4), 261-270.

[8] Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. and Attia, H. (2011) Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. Food Chem. 124(2), 411-421.

[9] Kohajdová, Z., Karovičová, J. and Jurasová, M. (2012) Influence of carrot pomace powder on the rheological characteristics of wheat flour dough and on wheat rolls quality. Acta Sci. Pol. Technol. Aliment. 11(4), 381-387.
[10] Hathorn, C.S., Biswas, M.A., Gichuhi, P.N. and Bovell-Benjamin, A.C. (2008) Comparison of chemical, physical, micro-structural, and microbial properties of breads supplemented with sweetpotato flour and high-gluten dough enhancers. LWT-Food Sci. Tech. 41(5), 803-815.
[11] Balestra, F., Cocci, E., Pinnavaia, G., Romani, S. (2011) Evaluation of antioxidant, rheological and sensorial properties of wheat flour dough and bread containing ginger powder. LWT-Food Sci. Tech. 44(3), 700-705.
[12] Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) Antioxidative activity of phenolic composition of commercial extracts of sage and rosemary. LWT. 28, 25-30.
[13] Kelebek, H., Selli, S., Canbas, A. and Cabaroglu, T. (2009) HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchem. J. 91(2), 187-192.
[14] Angioloni, A. and Collar, C. (2011) Nutritional and functional added value of oat, Kamut®, spelt, rye and buckwheat versus common wheat in breadmaking. J. Sci. Food Agric. 91(7), 1283-1292.
[15] Isserliyska, D., Karadjov, G. and Angelov, A. (2001) Mineral composition of Bulgarian wheat bread. Eur. Food Res. Technol. 213, 244-245
[16] Lawless, H. and Heymann, H. (1999) Sensory syrup in baking and confectionery. Evaluation of Food: Principles and Practices. Pak. J. Nutr. 11(1), 688-695.
[17] Almasodi, A.G.S. (2018) Production and Evaluation of Some Bakery Products Containing Ginger Powder. J. Food Nutr. Res. 6(1), 205-215.
[18] Tańska, M., Zadernowski, R. and Konopka, I. (2007) The quality of wheat bread supplemented with dried carrot pomace. Pol. J. Nat. Sci. 22(1), 126-136.
[19] Ashoush, I.S. and Gadallah, M. (2011) Utilization of Mango Peels and Seed Kernels Powders as Sources of Phytochemicals in Biscuit. World Journal of Dairy and Food Sciences. 6(1), 35-42.
[20] Bouaziz, M.A., Amara, W.B., Attia, H., Blecker, C. and Besbes, S. (2010) Effect of the addition of defatted date seeds on wheat dough performance and bread quality. J. Texture Stud. 41(4), 511-531.
[21] Gómez, M., Jiménez, S., Ruiz, E., Oliete, B. (2011) Effect of extruded wheat bran on dough rheology and bread quality. LWT-Food Sci. Tech. 44(10), 2231-2237.
[22] Mohammed, I., Ahmed, A.R. and Senge, B. (2012) Dough rheology and bread quality of wheat–chickpea flour blends. Ind Crops Prod. 36(1), 196-202.
[23] Borchani, C., Besbes, S., Masmoudi, M., Blecker, C., Paquot, M., Attia, H. (2011) Effect of drying methods on physico-chemical and antioxidant properties of date fibre concentrates. Food Chem. 125(4), 1194-1201.
[24] Nassar, A.G., AbdEl-Hamied, A.A. and El-Naggar, E.A. (2008) Effect of citrus by-products flour incorporation on chemical, rheological and organolepic characteristics of biscuits. World J. Agric. Sci. 4(5), 612-616.
[25] Anil, M. (2012) Effects of wheat bran, corn bran, rice bran and oat bran supplementation on the properties of pide. J. Food Process. Pres. 36(3), 276-283.
[26] Abou-Zaid, A.A.M., El-Bandy, M.A.S., Ismaeil, H. (2012) Rheological properties and quality evaluation of pan bread and biscuits supplemented with mushroom micelles flours. Aust. J. Basic App. Sci. 6(6), 237-245.
[27] Abd-El-Moniem, G.M. and Yaseen, A.A.E. (1993) High dietary fibre cookies from several sources of bran or husk. Egypt. J. Food Sci. (Egypt). 21, 157-170.
[28] Aussenac, T., Carceller, J.L. and Kleiber, D. (2001) Changes in SDS solubility of glutenin polymers during dough mixing and resting. Cereal Chem. 78(1), 39-45.
[29] Sliwinski, E.L., Kolster, P., Van Vliet, T. (2004) On the relationship between large-deformation properties of wheat flour dough and baking quality. J. Cereal Sci. 39(2), 231-245.
[30] Li, M. and Lee, T.C. (1998) Effect of cysteine on the molecular weight distribution and the disulfide cross-link of wheat flour proteins in extrudates. J. Agric. Food Chem. 46(3), 846-853.
[31] Rojas, J.A., Rosell, C.M. and De Barber, C.B. (1999) Pasting properties of different wheat flour-hydrocolloid systems. Food Hydrocoll. 13(1), 27-33.
[32] Chopin Applications Laboratory (2009) Mixolab applications handbook. Rheological and enzymatic analysis. Villeneuve la Garenne, France.
[33] Banu, I., Stoenescu, G. and Aprodu, I. (2012) Effect of the addition of wheat bran stream on dough rheology and bread quality. Food Tech. 36(1), 39–52.
[34] Collar, C., Bollain, C. and Rosell, C.M. (2007) Rheological behaviour of formulated bread doughs during mixing and heating. Food Sci. Tech. Int. 13(2), 99-107.
[35] Kumar, N. and Kumar, K. (2011) Development of carrot pomace and wheat flour based cookies. J. Pure Appl. Sci. Tech. 1(1), 5-11.

[36] Ktenioudaki, A. and Gallagher, E. (2012) Recent advances in the development of high-fibre baked products. Trends. Food Sci. Tech. 28(1), 4-14.
[37] Kumar, S., Kumar, P. and Kerkhi, S.A. (2017) Genetic analysis for various yield components and gluten content in bread wheat (*Triticum aestivum* L.). J. Appl. Nat Sci. 9(2), 879-882.
[38] Patil, S.S., Rudra, S.G., Varghese, E. and Kaur, C. (2016) Effect of extruded finger millet (*Eleusine coracan* L.) on textural properties and sensory acceptability of composite bread. Food Biosci. 14, 62-69.
[39] Bolarinwa, I.F., Aruna, T.E. and Raji, A.O. (2019) Nutritive value and acceptability of bread fortified with moringa seed powder. J. Saudi Soc. Agric. Sci. 18, 195–200.
[40] Song, K.Y., Hyeonbin, O., Zhang, Y. and Kim, Y.S. (2016) Quality characteristics and antioxidant properties of sponge cakes containing black carrot (Daucus carota ssp. sativus var. atrorubens Alef) flour. Prog. Nutr. 18(2), 176-183.
[41] Pekmez, H. and Yılmaz, B.B. (2018) Quality and Antioxidant Properties of Black Carrot (Daucus carota ssp. sativus var. atrorubens Alef.) Fiber Fortified Flat Bread (Gaziantep Pita). J. of Agricultural Sci. and Techn. B. 8, 522-529.
[42] Konrade, D. and Klava, D. (2017) Total Content of Phenolics and Antioxidant Activity in Crispbreads with Plant By-product addition. Rural Sustain. Res. 38(333), 24-31.
[43] Rickman, J.C., Barrett, D.M. and Bruhn, C.M. (2007) Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. J. Sci. Food and Agric. 87(6), 930-944.
[44] Ruiz-Rodriguez, A., Marín, F.R., Ocaña, A. and Soler-Rivas, C. (2008) Effect of domestic processing on bioactive compounds. Phytochem. Rev. 7(2), 345-384.
[45] Kapoor, S., Ranote, P.S. and Sharma, S. (2015) Antioxidant potentials and quality aspects of Jamun (*Syzygium cumini* L.) supplemented unleavened flat bread (Indian chapatti). J. Appl. Nat Sci. 7(1), 309-315.
[46] Turksoy, S., Keskin, S., Ozkaya, B. and Ozkaya, H. (2011) Effect of black carrot (Daucus carota L. ssp. sativus var. atrorubens Alef.) fiber addition on the composition and quality characteristics of cookies. J. Food Agric. Environ. 9(3-4), 57-60.
[47] Sojak, M.J., Jaros, M. and Głowacki, S. (2014) Analysis of giant pumpkin (Cucurbita maxima) quality parameters in various technologies of convective drying after long-term storage. Drying Tech. 32(1), 106-116.
[48] Zargar, F.A., Kumar, S., Bhat, Z.F. and Kumar, P. (2014) Effect of pumpkin on the quality characteristics and storage quality of aerobically packaged chicken sausages. SpringerPlus. 3(1), 39.
[49] Oyetayo, V.O. and Oyedeji, R.R. (2017) Proximate and Mineral Composition of Bread Fortified with Mushroom (*Plerotus ostreatus* and *Calocybe indica*). Microbiology Research Journal International. 19(4), 1- 9.
[50] Winiarska-Mieczan, A. and Kwiecień, M. (2011) Evaluation of the mineral composition of breadstuff and frequency its consumption. Acta. Sci. Pol. Technol. Aliment. 10(4), 487-495.

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USE OF STABILIZED RICE BRAN FOR PREPARING FISH FINGERS AS A FUNCTIONAL FOOD

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ABSTRACT

Fish fingers were produced using minced Atlantic mackerel fish (*S. scombrus*) meat as a functional healthy food. Rice bran that subjected to different stabilization techniques (Microwave oven, toasting by dry heat at 130 °c for 10 and 20 min. and autoclave steaming for 10 and 20 min.) was used as an additive material for preparing fish fingers. Microwave treatment was found to be the best technique among stabilization techniques. So, microwave stabilized rice bran was chosen to be used as an additive material for preparing fish fingers. Moderate amounts of γ-oryzanol were found in microwave stabilized rice bran which in turn will help to produce fish finger stable against lipid oxidation, since γ-oryzanol is considered a good antioxidant agent. The produced fish fingers showed a good chemical and physical properties, meantime they were acceptable by the panelists. So, it can be recommended to produce fish fingers as a healthy and functional food.

KEYWORDS:
Fish fingers, stabilization techniques, rice bran, γ-oryzanol

INTRODUCTION

High protein contents, essential amino acid profile and less stroma make fish meat easily digestible. so, it can be considered an important raw material to make different healthy food products [1]. In recent years, the increase of civilization and numbers of working women led to direct consumers preference to ready to eat foods such as fish fingers [2]. Fish fingers are made of different ingredients, one of them is rice bran that considered a by-product of rice milling was considered unfit for prolonged storage and consumption. Due to new stabilizing technology to inactivate the enzyme lipase, rice bran is no longer used as waste material. The stabilized rice bran (SRB) is an allergen-free functional ingredient which can be used as a traditional binders in fish products. Moreover, rice bran is one of the valuable by products of rice milling process, particularly rich in dietary fibers and contains significant quantities of starch and protein, where its oil is rich in gamma-oryzanol [3], γ-oryzanol has been suggested to have properties beneficial to health including reducing total plasma cholesterol. Moreover, it was reported that γ-oryzanol exhibited antioxidant properties [4]. However, there are many natural antioxidants which can be added to the fish products to improve their health benefits, one of these is rice bran [1]. This work was undertaken to study the possibility of using rice bran as a food additive for preparing fish fingers as a healthy functional food.

MATERIALS AND METHODS

Materials. Rice bran. Rice bran samples were obtained as a by-product of the milling of a mixture of three varieties of Egyptian rice (*O. sativa* L.) namely, Sakha 101, 105 and 106 which are popular short-grain japonica cultivars for consumed in Egypt. Rice bran samples were obtained during the growing season of 2016 from the Rice Research and Training Center (RRTC) in Sakha, Kafrelsheikh Governorate, Egypt.

Fish meat and other ingredients. Atlantic mackerel fish (*S. scombrus*), spices (Salt, sugar, carbonate, red pepper, black pepper, curry, cumin, thyme, garlic powder, onion powder, breadstick crumbs and sunflower oil that used as ingredients for preparing fish fingers)., were bought from the local market of Kafrelsheikh City, Egypt.

Chemicals. The chemicals used in the study were of analytical grade, and purchased from El-Gomhouria for Trading Chemicals and Medical Appliances, Tanta City, Egypt.

Methods. Processing of rice bran. The full fat raw bran was sieved to remove the husk, and the obtained samples were free from impurities.

Stabilization of rice bran. Rice bran samples were subjected to different stabilization method as follows:

The rice bran was first divided into six portions. The first portion was unstabilized rice bran (Un-RB). The second portion was stabilized using a microwave oven (MW LG – 900W) for 2 min. and manually mixed every minute according to [5]. The third and fourth portions were separately roasted by a hot-air oven at 130 ºC for l0 min (HAO1) and 20 min (HAO2), respectively [6]. The fifth and sixth ones were steamed by an autoclave under atmospheric pressure for 10 min. (Autoclave 1) and 20 min. (Autoclave 2), respectively [7]. Finally, the bran samples were stored in the dark at -10°C in water-tight containers until further analyses.

Preparation of fish fingers. The meat of mackerel fish (*S. scombrus*) was grinded with thorns in the grinder. Patented products were used as pasting and coating materials, respectively. The fish fingers produced contained 20% of additive substances and 80% of minced fish meat. Additive substances consisted of breadstick crumbs, sugar, sunflower oil (16%) and other spices (4%) were added as outlined by [2]. The mixed formula was enriched with5%, 10%,15% and 20% levels of microwave stabilized rice bran, where 0% rice bran was used as a control then subjected to further processing.

Rice bran analysis. Determination of gross chemical composition. Moisture, crude protein (N × 5.95), ether extract, and ash contents were determined according to [8]. Total carbohydrates were calculated by the difference.

Extraction of rice bran oils. Rice bran oil was extracted according to the method described by [9].

Determination of physical and chemical properties of extracted oils. The refractive index, specific gravity, acid value, peroxide value, iodine value, saponification value and unsaponifiable matter of rice bran oils were performed according the methods of [8]. γ- oryzanol in microwave stabilized rice bran oil extract was carried out by a spectrophotometric method [10]. 0.01Ml of sample was dissolved in 10Ml of hexane, where the absorption was taken at 314 nm in a 1-cm cell (SPECTROUV-VISAUTO, UV-2602). The oryzanol content was calculated by using the formula:

(A/W) × (100/358.9).

Where A is the absorbance of the sample, W is the weight of the sample in gram/100 ml, 358.9 is specific extinction coefficient for γ-oryzanol.

Determination of the fatty acid composition of rice bran oil samples. The methyl esters were prepared using benzene: methanol: concentrated sulfuric acid (10:86:4) according to [11]. Identification of the fatty acid methyl esters was performed by a gas liquid chromatography (GLC) (model 4550) equipped with a flame ionization detector and coiled glass Column (1.6m × 4mm) packed with 10% polyethylene glycol adipate (PEGA) supported on chromocarb W-AW 100-200 mesh. Samples of 1-1.5 ML were injected into the column using a Hamilton micro syringe. The gas chromatographic conditions used for isothermal analysis were as follows: column temperature 190 ºC, flow rates: hydrogen 33ml/min., nitrogen 30 ML/min., and air 330 ML/min. The peak areas were measured using petrophysics integrator as described in [8].

Chemical Analysis of prepared fish fingers. Moisture, ash, crude protein, ether extract were estimated using the methods of [8]. where carbohydrates were calculated by difference.

Physical Properties of fish fingers. Physical properties of fish fingers were calculated using the equations given in [12].

Cooking properties of fish fingers. Cooking properties of fish fingers were determined following the procedures of [13].

Organoleptic properties of fish fingers. Organoleptic evaluation of prepared fish fingers was performed as described by [13].

Statistical analysis. The data were statistically analyzed using analysis of variance (ANOVA), and the means were further compared using a least significant difference test as described by [14].

RESULTS AND DISCUSSION

Proximate chemical composition of rice bran samples subjected to different stabilization procedures. Many factors such as the variety of rice, variation in organic compounds in the soil, the fertilizers applied, climatic and environmental factors, the degree of milling and the treatments used affect the chemical composition of rice bran [15]. Therefore, chemical compositions were determined to study the previously mentioned treatments and their effects on the quality of the different samples.

The chemical composition of Un-RB and rice bran subjected to the different stabilization procedures MW, HAO 1, HAO 2 and Autoclave 1 and 2 is presented in Table (1) The moisture content values of rice bran samples were significantly different at ($P \leq 0.05$). The moisture content of HAO2 rice bran was lower than that of Un-RB and rice bran subjected to the other stabilization procedures, this procedure may be effective for samples with a longer shelf life, less microbial contamination and some nutrient preservation. The obtained results are in accordance with those of [16] who reported that, the moisture content of stabilized oils depended on

TABLE 1
Gross chemical composition of rice bran as affected by different stabilization procedures. (Organic matters were calculated on a dry weight basis)

Treatment	Moisture	Crude Protein	Ether Extract	Ash	Carbohydrates*
Un-stabilized	8.56a+	15.34d	18.52d	8.7b	57.44a
Microwave	6.65c+	16.04c	20.32a	9.31a	54.33b
Hot Air Oven1	6.60c+	16.16c	19.42c	9.20a	55.22b
Hot Air Oven2	6.10d+	16.65b	19.82b	9.40a	54.13b
Autoclave 1	7.30b+	16.94a	19.32c	9.27a	54.47b
Autoclave 2	7.50b+	17.04a	20.02a	9.40a	53.54d

+ Means with the same small superscript letters in a column are not significantly different at p≤ 0.05.
* Total carbohydrates were calculated by the difference.
(1) Un-stabilized (Un-RB). (2) Microwave (MW) 900 W for 2min. (3) Hot Air Oven1 (HAO1) 130°C for 10 min. (4) Hot Air Oven2 (HAO2) 130°C for 20 min. (5) Autoclave 1: Steaming for 10 min. (6) Autoclave 2: Steaming for 20 min.

TABLE 2
Some physical and chemical properties as well as γ-oryzanol of rice bran oil as affected by different stabilization procedures.

Parameters	Stabilization Treatments					
	Control	MW	HAO1	HAO2	Autoclave 1	Autoclave 2
Refractive index (25ºC)	1.4603a+	1.4671a	1.4603a	1.4602a	1.4602a	1.4703a
Specific gravity (25ºC)	0.9152b+	0.9281a	0.9144b	0.9162b	0.9252a	0.9201ab
Acid value (%)	1.34a+	1.11d	1.29b	1.21c	1.15d	1.13d
Peroxide value (meq/kg oil)	0.901a+	0.655e	0.838b	0.805c	0.751d	0.731d
Iodine value (gI/100g oil)	107.20a+	100.61f	106.10b	105.22c	102.10d	101.41e
Saponification value (mg KOH/g oil)	196.12a+	195.41b	195.55b	194.77c	194.10d	194.30d
Unsaponifiable matter (%)	4.52a+	4.37b	4.31b	4.19c	4.16c	4.11c

+ Means with the same small superscript letters in rows are not significantly different at p≤ 0.05.
(1) Un-stabilized (Un-RB). (2) Microwave (MW) 900 W for 2 min. (3) Hot Air Oven1 (HAO1) 130°C for 10 min. (4) Hot Air Oven2 (HAO2) 130°C for 20 min. (5) Autoclave 1: Steaming for 10 min. (6) Autoclave 2: Steaming for 20 min.

the processing temperature and duration of heating. Furthermore, moisture content plays a key role during storage [17]. Also shown in Table (1) the autoclaved rice bran samples (Autoclaved 1 and 2) contained the highest content of crude protein contents 16.94 and 17.04 %, respectively, followed by HAO2 (16.65%) while the lowest crude protein content (15.34%) was found in the control (Un-RB). These results are in line with those of [6].

Based on ether extract, Un-RB and stabilized rice bran contained 18.52 to 20.32% crude fat (Table 1). Furthermore, stabilization by either dry or moist heat helped to increase the ether extract levels in rice bran. The augmentation of oil extractability could be related to the ability of heat to cause the fat in the cells to coalesce into oil droplets and break down cell structure, thereby improving the speed and extent of oil extraction, or causing degradation of lipoproteins. These results are in agreement with those reported by [18]. As previously reported by other authors, different stabilization techniques play an active role in reducing enzymatic activity to a greater or lesser degree, hence, increasing or decreasing oil extraction [19].

As shown in Table (1) the ash content of Un-RB and stabilized rice bran ranged from 8.70 to 9.0%, and the total carbohydrate content ranged from 54.13to57.44 %. The present findings are similar to those reported by [6, 16].

Some physical and chemical properties of oils extracted from rice bran as affected by subjecting to different stabilization procedures. Physical properties. Some physical properties of rice bran oil were determined and the obtained results are presented in Table (2). The refractive index is one of the most important physical parameters used in the identification of fat and oils; it can be used to estimate of the degree of saturation of oils. No remarkable changes were recorded in the refractive index of Un-RB (1.4603) or stabilized rice bran oils (1.4671 - 1.5703) at ($p \leq 0.05$) (Table, 2). These results are in accordance with those obtained by [20]. The specific gravity of oils extracted from untreated rice bran at 25ºC was 0.9152. These results are in line with those obtained by [21]. The heat treatments, especially the MW treatment led to an increase in the specific gravity of oil extracted from rice bran to 0.9281. In addition, the heat treatments had a slight effect on specific gravity. However, there is a non-linear relationship between temperature and the specific gravity of oils as found by [22]. The obtained results are in agreement with those of [23].

Chemical properties and γ-oryzanol content. Some chemical properties of rice bran oil were determined and the results are given in Table (2). The acid and peroxide values of oil extracted from unstabilized (Un-RB) rice bran were (1.34% and 0.901 meq O_2/kg oil), respectively. These results are com-

parable to those reported by [24]. The heat treatments especially MW led to a decline in both acid and peroxide values. Such results may be due to the inhibition of lipase activity in rice bran by heat. Acid and peroxide values reflect oil quality degradation as a function of triacylglycerol hydrolysis and the further breakdown of hydroperoxides [25]. Furthermore, Autoclave 2 had a stronger effect on the acid and peroxide values than Autoclave 1. In addition, Autoclaving was more effective than the use of a hot-air oven. [26]. stated that steam treating of some grains for 5 min. led to a reduction in the acid value of their oils and that steaming rice bran for one minute reduced the peroxidase activity to 40-60% of that in the control, moreover, the reduction reached 95% of the original activity after steaming for 3 min. Additionally, [27]. stated that, heat treatments especially steaming, led to the decline of both acid and peroxide values, in addition heat treatments for 20 min. were more effective than those for 10 min.

The iodine value also indicates the stability of oil against oxidation, since it represents the degree of unsaturation of oils and measures their vulnerability to oxidation [25]. The iodine values of the rice bran samples varied from 107.20 to 100.61 g /100g (Table, 2). These results are comparable to those reported by [21] and [26]. Furthermore, heat treatment had an obvious effect on the iodine value of rice bran oil, and played an active role in decreasing the iodine value. This result could be related to the destruction of double bonds in the unsaturated fatty acids of oils as a function of heating as found by [25]. The results in Table (2) showed that the saponification values of rice bran oils ranged from 194.10 to 196.12 mg KOH/g. Crude rice bran oil contains about 96% saponifiable matter and approximately 4% un-saponifiable matter. These results are in agreement with those of [15]. The stabilization process especially in MW had a slight effect on the saponification value. These results are comparable to those found by [27]. who reported that, heat treatment had a small effect on saponifiable matter. In addition, the reduction of saponification value of oils as a result of heating could be attributed to the chemical reactions that led to the degeneration of products other than free fatty acids [25]. In addition, unsaponifiable matter including hydrocarbons, sterols, vitamins and pigments, usually play an important role in oil stability. Table (2) shows that, the unsaponifible matter of rice bran oil extracted from the Un-RB and stabilized rice bran oil samples ranged from 4.11 to 4.52%. These results confirmed that, rice bran oil has a large amount of unsaponifible matter. The results of unsaponifiable matter content were in accordance with those reported by [9] and [27]. Based on the a forementioned results, it was founded that the best physical and chemical properties were found in microwave stabilized rice bran oil so, this sample was used for γ-oryzanol determination since microwave stabilized rice bran was chosen among all the stabilized rice bran samples for preparing fish fingers, hence, extra crude fiber and starch will be added to fish fingers which in turn will improve the quality of fish fingers. In addition, the presence of γ-oryzanol in the added microwave stabilized rice bran help to improve the stability of fish fingers lipids against oxidation since γ-oryzanol is considered a good antioxidant. This will help finally to produce healthy and functional food in the form of fish fingers. From the date of Table (3) it was found that the amount of γ-oryzanol in variety Sakha 105 is (11.25%) was lower than that of Sakha 106 which was (14.44%).and both are contained a moderate amounts of antioxidant that will help to improve the quality and nutritional properties of the final product (fish fingers).

Fatty acids composition. The results presented in Table (4) show the fatty acid composition of rice bran oil extracted from Un-RB and stabilized (MW, HAO 1, HAO 2, Autoclaved 1 and 2) rice bran. Oleic acid (C18:1), linoleic acid (C18:2), and palmitic acid (C 16:0) are the dominant fatty acids in Un-RB and stabilized rice bran oil were in the ranges of 41.06- 42.40; 34.55- 36.40 and 17.40- 19.96%, respectively. The concentrations of the major fatty acids, namely C18:2, C18:1and C16:0 of the investigated rice bran oils generally agreed with those of [9, 28], who studied the fatty acid composition of 204 rice varieties and found that the main fatty acids in rice bran oil were palmitic, oleic, and linoleic acids, which were in the ranges of 13.9–22.1, 35.9–49.2, and 27.3– 41.0%, respectively. The level of these fatty acids depends on the variety and cultivation location of the rice [29]. The reduction in oleic and linoleic acids represents a decrease in the yielded free fatty acids that might be expected during storage. Conditions especially conditions that promoting oxidation reactions. The results of the present study agree with those obtained by [30]. Concerning to thermal processing, the results in Table (3) revealed that thermal processing of rice bran caused a decrease in unsaturated fatty acids especially after (HAO 2), while saturated fatty acids increased. In contrast, MW processing caused a slight decrease in unsaturated fatty acids. The results of the present study were in agreement with those obtained by [16, 27].

TABLE 3
γ-Oryzanol content of microwave stabilized rice bran oil

Sample:	Sakha 105 Microwave stabilized rice bran oil	Sakha 106 Microwave stabilized rice bran oil
γ-Oryzanol:	11.25%	14.44%

TABLE 4
Fatty acids profile of o rice bran oil as affected by different stabilization procedures.

Fatty acid	Control*	MW oil	HAO1 oil	HAO2 oil	Auto1 oil	Auto2 oil
Myristic C14:0	0.55	0.57	0.75	0.77	0.69	0.72
Palmitic C16:0	17.40	17.80	19.65	19.96	19.0	19.30
Palmitoleic C16:1	0.13	0.14	0.23	0.23	0.16	0.20
Stearic C18:0	1.72	1.76	2.3	2.34	1.79	2.09
Oleic C18:1	42.40	42.10	41.17	41.06	41.72	41.42
Linoleic C18:2	36.40	36.30	34.75	34.55	35.30	35.0
Linolenic C18:3	0.80	0.76	0.67	0.63	0.76	0.73
Eicosenoic C20:1	0.60	0.57	0.48	0.46	0.58	0.54
TSFA** (%)	19.67	20.13	22.70	23.07	21.48	22.11
TUSFA** (%)	80.33	79.87	77.30	76.93	78.52	77.89

*- Control = oil extracted from Un-RB.
**TSFA = Total saturated fatty acids, **TUSFA = Total unsaturated fatty acids

TABLE 5
Chemical composition of fish fingers supplemented with different levels of microwave stabilized rice bran.

Chemical properties	moisture	Ash	Crude Protein	Crude Fat	Carbohydrates*
control	77.40**a	1.7d	49.77e	8.4e	40.13a
5% rice bran	75.30b	1.9d	51.35d	8.9d	37.85b
10% rice bran	74.60b	2.3b	53.11c	9.4c	35.19c
15% rice bran	73.50c	2.5b	54.46b	10.2b	32.84d
20% rice bran	72.20d	2.8a	56.24a	10.6a	30.36e

*- carbohydrates were calculated by difference.
**Value in column with the same letters are not significantly different at $p \leq 0.5$.

TABLE 6
Some physical and cooking properties of fish fingers supplemented with different levels of microwave stabilized rice bran.

Physical properties	Control	5% rice bran	10% rice bran	15% rice bran	20% rice bran
Water holding capacity (**WHC**)	22.6d	24.7c	25.4c	26.5b	27.8a
Protein water coefficient (**PWC**)	0.64a	0.68a	0.71a	0.74a	0.77a
Protein water -fat coefficient (**PWFC**)	0.58a	0.61a	0.63a	0.65a	0.68a
Feder value	0.84a	0.83a	0.82a	0.81a	0.80a
Cooking properties	Control	5% rice bran	10% rice bran	15% rice bran	20% rice bran
Cooking Yield	101.72 d+	101.63 d	102.38 c	127.77 b	133.96 a
Cooking Loss%	28.89 a	27.89 b	26.89 c	25.87 d	25.38 d
Shrinkage %	25.15 a	21.86 b	18.89 c	15.89 d	12.69 e

+- Values in rows with the same letter are not significantly different at $p \leq 0.5$.

Chemical composition of fish fingers. The data given in Table (5) showed that moisture content of fish fingers supplemented with different levels of microwave stabilized rice bran decreasing with increasing the level of rice bran added to prepare fish fingers. This could be attributed to the level of moisture content of rice bran. Similar results were found elsewhere [31]. Similar trend was recorded in case of the level of carbohydrates content of fish fingers. As for ash, crude protein and crude fat contents of fish fingers, it was found from the same Table (5) that, these values were increased with increasing the level added of microwave stabilized rice bran. This leads to improve the nutritional value of the final product, since minerals, proteins, and fat are very important from the nutritional point of view. The results of [1] support our findings.

Some physical and cooking properties of fish fingers. The results of some physical and cooking properties of fish fingers are given in Table (6). The obtained results showed that water holding capacity (WHC) increased with increasing the rate of microwave stabilized rice bran added to prepare fish fingers. This could be related to the increment in protein content of fish fingers with increasing the level of rice bran added. The results of [32] support our findings since there are a positive relationship between water holding capacity and the amount of protein as found by [32]. Same trend was recorded in case of

TABLE 7
Organoleptic properties of fish fingers supplemented with different levels of microwave stabilized rice bran.

Treatments	Control	5% Rice bran	10% Rice bran	15% Rice bran	20% Rice bran
Properties					
Color	8.2 a*	8.0 b	10.3 ab	8.42 c	8.1 cd
Taste	8.0 a	8.2 a	9.8 a	8.3 ab	7.5 c
Oder	8.0 a	8.0 a	10.2 a	8.1 ab	8.2 c
Texture	7.6 a	7.8 ab	9.4 ab	8.0 a	8.0 c
Tenderness	7.7 a	7.8 ab	9.6 ab	8.0 ab	8.0 c
Overall-Acceptability	7.9 a	8.0 a	9.9 a	8.2 ab	7.9 c

*- Values in rows with the same letter are not significantly different at $p \leq 0.5$.

protein water coefficient (PWC), protein water fat coefficient (PWFC) and Feder value of fish fingers supplemented with different levels of microwave stabilized rice bran. These results may be due to the increment of ash, protein and lipid contents of fish fingers with increasing the levels of rice bran added. Similar results were found elsewhere [33]. Apparent also from the same Table (6) that cooking yield of fish fingers increased with increasing the levels of microwave stabilized rice bran added, where the loss percent of fish fingers as a function of cooking was decreased. Similar trend of shrinkage percent was recorded. These results were in agreement with those of [34].

Organoleptic properties of fish fingers. Date given in Table (7) represent the organoleptic properties scores of fish fingers supplemented with different levels of microwave stabilized rice bran. From the given data it should be noted that significant changes were found upon increasing the level of microwave stabilized rice bran added except at the level of 10% where the scores of all the organoleptic properties were the highest among all the other levels of rice bran added At $P \leq 0.05$. Similar results were found by [1] and [31]. So, it can be strongly recommended to use such level (10%) of microwave stabilized rice bran for preparing fish fingers as a functional healthy food and acceptable for human consumption from the organoleptic properties point of view.

CONCLUSIONS

Atlantic mackerel *(S. scombrus*) minced meat fish was used for preparing fish fingers. adding of microwave stabilized rice bran that rich in crude fiber, starch and γ-oryzanol helped to improve the final product from the nutritional point of view. Using microwave stabilized rice bran as a food additive to prepare fish finger especially at the level of 10% gave fish fingers as a functional food acceptable for human consumption.

REFERENCES

[1] Izci, L. (2010) Utilization and Quality of Fish Fingers from Prussian Carp (*Carassius gibelio* Bloch, 1782). Pak. Vet. J. 30(4), 207-210.

[2] Cakli, S., Taskaya, L., Kisla, D., Celik, U., Ataman, C.A., Cadun, A., Kilinc, B. and Maleki, R.H. (2005) Production and quality of fish fingers from different fish species. Eur. Food Res. Technol. 220, 526-530.

[3] Saenjum, C., Chaiyasut, C., Chansakaow, S., Suttajit, M. and Sirithunyalug, B. (2012) Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. Journal Medical Plants. 6(6), 1070-1077.

[4] Vorarat, S., Managit, C., Lamthanakul, L., Soparat, W. and Kamkaen, N. (2010) Examination of Antioxidant Activity and Development of Rice Bran Oil and Gamma-Oryzanol Microemulsion J. Health Res. 24(2), 67- 72.

[5] Faria, S.A.S.C., Bassinello, P.Z. and Penteado, M.V.C. (2012) Nutritional composition of rice bran submitted to different stabilization procedures. Brazilian Journal of Pharmaceutical Sciences. 48(2), 650–657.

[6] Bagchi, T.B., Adak, T. and Chattopadhyay, K. (2014) Process standardization for rice bran stabilization and its' nutritive value. Journal of Crop and Weed. 10(2), 303-307.

[7] Rosniyana, A., Hashifah, M.A. and Shariffah Norin, S.A. (2009) Nutritional content and storage stability of stabilised rice bran – MR 220. Journal of Tropical Agriculture and Food Science. 37(2), 163–170.

[8] A.O.A.C. (Association of Official Analytical Chemists) (2005) Official Methods of Analysis of the Association of Official Analytical Chemists. 18th Ed. Washington, DC, USA.

[9] Kahlon, T., Chow, F., Sayre, R. and Betschart, A. (1992) Cholesterol-lowering in hamsters fed rice bran at various levels, defatted rice bran and rice bran oil. Journal of Nutrition. 122, 513-519.

[10] Gopal, K.A.G., Khatoon, S. and Babylatha, R. (2005) Frying performance of processed rice bran oils. J. Food Lipids. 12, 1-11.

[11] Stahl, E. (1967) Thin Layer Chromatography. A Laboratory Handbook. Springer Verlag, Berlin. Heidelberg, New York, USA. 359p.
[12] Hussein, S.H. (2015) Utilization of Black Rice and Red Sorghum Hull as Natural Antioxidants on the Quality Attributes of Fish Fingers. Middle East Journal of Agriculture Research. 4(3), 509-518.
[13] Izci, L., Bilgin, E. and Gunlu, A. (2011) Production of fish finger from sand smelt (*Atherina boyeri*, RISSO 1810) and determination of quality changes. African Journal of Biotechnology. 10(21), 4464-4469.
[14] Steel, R.G. and Torrie, J.H. (1980) Principles and procedures of statistics. 2nd Ed. McGraw Hill, New York, USA. 120p.
[15] Orthoefer, F.T. (2005) Rice bran oil. In: Shahidi, F. (ed.) Bailey's Industrial Oil and Fat Products. John Wiley and Son, Inc., USA: Hoboken, NY. 465–489.
[16] Abd El-Hady, S.R. (2013) Effect of some thermal processing on stability of rice bran during storage at room temperature. J. Agric. Res. Kafr El-Sheikh Univ. 39(1), 92-106.
[17] Amorim, J.A., Eliziário, S.A., Gouveia, D.S., Simões, A.S.M. (2004) Thermal analysis of the rice and by-products. Journal of Thermal Analysis and Calorimetry. 75, 393-399.
[18] Vissier, M.N., Zock, P.L., Meijer, G.W., Katan, M.B. (2002) Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans. The American Journal of Clinical Nutrition. 72, 1510-1515.
[19] Lakkakula, N.R., Lima, M. and Walker, T. (2004) Rice bran stabilization and rice bran oil extraction using ohmic heating. Bioresour. Tech. 92, 157–161.
[20] Kawase, R., Azami, S. and Maed, A.Y. (2010) Rice bran-like composition and food. PCT international Patent Application Publication. 2010, 5:1-9, US20100196583A1
[21] Iskander, M.H., Awad, A.H. and Hamam, A. M. (1990) Effect of storg condition on the stability of fatty acid composition of corn. 1st Alex. Conf. Food Sci. Tech. 21-36.
[22] Rotimi, D. (2016) Effect of the Temperature on Dynamic Viscosity, Density and Flow Rate of Some Vegetable Oils. J. Scient. Res. Eng. Technol. 1(1), 14-24.
[23] Rizk, L.F., Doas, H.A. and El-Sakr, A.S. (1994) Chemical composition and mineral content of rice bran of two Egyptian rice varieties heated by microwave. Nahrung. 38(3), 273-277.
[24] Sanghi, D.K. and Tiwle, R. (2015) A review of comparative Study of Rice Bran Oil and Rice Bran Wax. International Journal of Pharmacy Review and Research. 5(4), 403-410.
[25] Nagassapa, F.N., Nyandoro, S.S. and Maeaisaka, T.R. (2012) Effects of temperature on the physicochemical properties of traditionally processed vegetable oils and their blends. Tanz. J. Sci. 38(3), 166-176.
[26] Abdel-Aal, E.S.M. and Sosulski, F.W. (1993) Rapid tests for residual lipase and peroxidase activities in a steam of heated cereals. Alex. Agric. Res. 2, 161-179.
[27] El-Sayed, M.E.A. (2015) Effect of some heat treatments on canola seed and rice bran characteristics. Journal Agricultural Research Kafrelsheikh University. 41(1), 246-265
[28] Amarasinghe, B.M.W.P.K. and Gangodavilage, N.C. (2004) Rice bran oil extraction in Sri Lanka: Data for process equipment design. Food Bioprod. Process. 82(C1), 54-59.
[29] Gupta, H. (1989) Rice bran offers India an oil source. Journal of the American Oil Chemists' Society. 66(5), 620-623.
[30] Krishna, A.G.G. (2002) Nutritional components of rice bran oil in relation to processing. Lipid Technology. 14, 80–84.
[31] Praneetha, S.S., Dhanapal, K., Reddy, G.V.S. and Balasubramanian, A. (2015) Development of fish fingers from Rohu (Labeo rohita) and its quality evaluation during refrigerated storage condition. International Journal of Science Environment and Technology. 4(6), 1457-1468.
[32] Bowker, B. and Zhuang, H. (2015) Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poultry Science. 94, 1657–1664
[33] Praneetha, S.S., Prakask, C.B., Dhanapal, K. and Reddy, G.V.S. (2016) Quality evaluation in fish fingers from *Labeo Rohita* during refrigerated storage. J. Exp. Zool. India. 19(1), 225-229.
[34] Bechtel, P.J., Bland, J.M., Woods, K., Lee, J.M., Brashear, S.S., Boue, S.M., Daigle, K.W. and Bett-Garber, K.L. (2018) Effect of pan Frying on composition and texture of breaded and battered catfish. Foods. 7(4), 46.

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DETERMINATION OF MICROBIOLOGICAL QUALITY AND VOLATILE COMPOUNDS OF DIFFERENT BRANDS ROSE WATERS IN TURKEY

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ABSTRACT

Rose water is the colorless liquid the is obtained as a byproduct while obtaining rose oil by water distillation. There are also non-natural products in the market with synthetic rose scent. These synthetic products are scented by adding rose oil into tap water and offered to the market. In this study, rose water samples from 15 brands sold in the market in the province of Afyonkarahisar in Turkey were collected and examined in terms of the volatile compounds and microbiological quality characteristics of the rose waters. In the volatile compound analyses of the rose water samples, 48 different compounds were determined, while phenethyl alcohol, geraniol, and citronellol were found as the main compounds in most samples. While no phenethyl alcohol was found in 7 of the rose water samples, the highest value by 32.212% was found in one sample. In the microbiological analyses, total aerobic mesophilic bacterium numbers were determined in 6 samples. Coliform numbers were determined in 3 numbers, while *E. coli* numbers were determined in 2 samples. Enterococci, Pseudomonas and coagulase (+) staphylococci could not be determined in any sample. The findings of the study showed that the synthetic and natural rose waters that are sold contain chemical substances and microorganisms that they need to not contain. These findings also revealed the necessity to periodically and regularly conduct screening studies towards determination of the status of rose waters that are sold in Turkey and to certificate and inspect production.

KEYWORDS:
Rose water, microbiological analysis, volatile compounds, GC/MS

INTRODUCTION

The most significant distillation and extraction products obtained from rose flowers are rose oil, rose water, rose concrete and rose absolute [1]. Rose oil and rose water are obtained by distillation, while concrete and absolute are obtained by extraction [2]. In addition to its usage as a scent source and fixator in cosmetics and perfumery, rose oil is also used in the food industry (liqueur, confectionery, chewing gums and puddings (maximum usage rate 0.002%)), as an additive in scent providing fruit oils, to provide tobacco with scent and flavor, in the soap and detergent industries and pharmacy (in toothpaste) and in production of pomades due to its antiseptic effects. Rose water is used to produce rose ointments and shaving lotions in the cosmetics industry, in desserts, candies, and syrups in the food industry and in religious ceremonies as it has a pleasant smell and cooling effect. Among the public, due to its antiseptic effect, it is used for toothaches, eye inflammations, eczema treatment and as a laxative [2-4]. Different methods and solvents are used to obtain rose water. In different studies that have been conducted in this context, the chemical composition of rose water has been investigated by using different solvents and extraction methods. Studies which used ethanol as a solvent found the main compounds of rose water as phenyl-ethyl alcohol (69.7-81.6%), citronellol (1.8-7.2%) and geraniol (0.9-7%) [5]. Eikani et al., used the method of simultaneous extraction-distillation and found the ratios of the main compounds of rose water as 81.27% for phenyl ethyl alcohol, 5.72% for citronellol and 4.43% for geraniol [6].

This study conducted a volatile compound analysis on 15 different brands of rose water samples obtained from the province of Afyonkarahisar in Turkey. Additionally, the study investigated the microbiological characteristics of these products which are usually obtained by the method of vapor distillation and expected to be sterile.

MATERIALS AND METHODS

15 different brands of rose water samples were purchased in their original packaging and brought to the laboratory in the cold chain.

TABLE 1
Microbiological Analysis Methods

Parameter	Broth Medium	Incubation Conditions	Method
Total Mesophilic Bacterium Count	PCA (Plate Count Agar, Oxoid CM0325)	At 32ºC for 48 h	TS 3834 ISO 2293 [7]
Total Coliform Bacteria	Violet Red Bile Agar (VRBA)	At 37ºC for 24 h	TS EN ISO 9308-1 [8]
Escherichia coli	Tryptone Bile X-glucuronide Agar (TBX Agar)	At 42ºC for 24 h	ISO 16649-2 [9]
Enterococci	Salanetz and Bartley Agar	At 37ºC for 24 h	TS EN ISO 7899-2 [10]
Pseudomonas spp	Pseudomonas Selective Agar	At 37ºC for 24 h	TS EN ISO 16266 [11]
Coagulase Positive Staphylococci	Baird Parker Agar	At 37ºC for 24 h	TS 6582-2 EN ISO 6888-2 [12]

TABLE 2
Microbiological Analysis Results in Rose Water Samples (cfu/100 ml)

Sample No	Total Aerobic Mesophilic Bacteria	Coliform	*E. coli*	Enterococci	Pseudomonas	Coagulase (+) Staphylococci
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	**42**	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	**80**	**42**	**18**	-	-	-
8	**14**	-	-	-	-	-
9	-	-	-	-	-	-
10	**162**	**115**	**12**	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	**36**	**21**	-	-	-	-
14	**62**	-	-	-	-	-
15	-	-	-	-	-	-

Microbiological analyses. The membrane filtration method was used to analyze the rose waters. For each bacterium count, 100 ml of the rose water sample was added onto the funnel of the membrane filtration setup under sterile conditions. In the membrane filtration process, each rose water sample was filtered through sterile membrane filters with pore diameters of 0.45 µm. The filters were then put on the relevant broth media and left for incubation for the necessary time at the necessary temperature (Table 1).

Volatile Compound Analysis. For determination of volatile compounds by GC/MS, extraction was carried out with the method reported by Moein et al. [13], and after extraction, measurements were made in an AGILENT 5975 C AGILENT 7890A gas chromatography device with the method reported by Baydar et al. [14]. CP-Wax 52 CB (50 m x 0.32 mm; 0.25 µm) was used as the column. The oven temperature was increased from 60°C to 220°C at a rate of 2°C per minute and kept at 220°C for 20 minutes. Injection port temperature was set at 240°C, detector temperature was set at 250°C, and the detector energy flow was set at 70 eV. Helium gas (20 mL/min) was used as the mobile phase.

RESULTS AND DISCUSSION

Table 2 shows the findings of the microbiological analyses of the rose water samples obtained from the province of Afyonkarahisar in their original packages on the counts of total aerobic mesophilic bacteria, coliform bacteria, *Escherichia coli*, Enterococci, *Pseudomonas* spp. and coagulase (+) staphylococci.

The total aerobic mesophilic bacterium numbers were determined in the samples numbered 3, 7, 8, 10, 13 and 14 respectively as 42, 80, 14, 162, 36 and 62 cfu/100 ml. The numbers of coliform bacteria were determined in the samples numbered 7, 10 and 13 respectively as 42, 115 and 21 cfu/100 ml. *E. coli* was determined in only 2 samples as 18 and 12 cfu/100 ml. Enterococci, Pseudomonas and coagulase (+) staphylococci were not determined in any rose water sample (0 cfu/100 ml). Additionally, in the rose water samples other than those reported above, the total aerobic mesophilic bacterium, coliform, and *Escherichia coli* numbers were determined as 0 cfu/100 ml.

In their study in Eskişehir in Turkey, Kırımer et al. [15], found the total mesophilic bacterium numbers in 4 different brands of rose water as >100, >100, 0 and 7 cfu/100 ml, they found the coliform and fecal coliform bacterium numbers in two samples as >100 cfu/100 ml, and in other two samples as zero. While they found the number of

Enterococci in one sample as >100, they found it as 1 cfu/100 ml in another sample. They determined the number of Pseudomonas as >100 cfu/100 ml in one sample, while they reported that they did not find any *E. coli* and pathogenic staphylococci in any of the samples. In their study in Iran in 2012-2013, Arani et al. [16] determined the mean number of total aerobic mesophilic bacteria in 191 rose water samples as 1.6×10^3 CFU/ml, while they determined *Enterococcus* spp. in 1 sample. They also reported that they did not determine total coliform, *Enterococcus*, *Pseudomonas* and sulfite reducing *Clostridium* in any samples.

In their study in Morocco on 12 traditionally and 8 industrially produced rose water samples, Zebarjad et al. [17], reported the presence of coliform in 3 traditional products, that all samples were contaminated with yeast-mold, one sample contained *Enterococcus* spp., and they did not determine *P. aeruginosa* in the samples. A comparison of the findings of this study to those of others shows that rose water samples may contain unwanted microorganisms based on production technology and conditions, and higher levels of indicator microorganisms may be found depending on the raw material and especially water that is used in production. Total aerobic mesophilic bacterium number, coliform, *E. coli*, Enterococci, etc. are known as indicator bacteria, and they show the hygienic quality of products. Indicator microorganisms are an indicator of especially fecal contamination [18]. These bacteria that are determined also indicate inadequate hygiene conditions and the presence of contamination during or after production. These bacteria that risk public health may be caused by inadequacy or lack of heat treatment during production or contaminate glass or plastic bottles.

Table 3 shows the main compounds (≥5%) that were determined based on the GC and GC/MS analyses on 15 rose waters belonging to different brands sold in Afyonkarahisar. Baydar and Göktürk-Baydar [19], investigated volatile oil compounds of main samples such as rose oil and rose water and pulp water samples obtained from the distillation of fresh oil roses by using GC/MS analyses. As a result of the analyses, the main aromatic compounds of rose oil were found as monoterpenoid alcohols as linalool (1.15%), citronellol (35.27+), nerol (8.69%) and geraniol (21.55%), long-chain hydrocarbons as nonadecane (12.77%), 9-nonadecane (3.38%), eicosane (1.58%) and heneicosane (6.96%), oxides and ethers as methyl eugenol (2.43%), esters and aldehydes as geranyl acetate (1.89%) and phenols as eugenol (0.61%). Moreover, in rose water, phenyl ethyl alcohol was determined as 60.71%, and this was the main volatile compound. Phenylethyl alcohol, which is the most noticeable aromatic compound of fresh rose flowers, could be determined by only 0.69% in rose oil, which is a distillation product. Determination of phenyl ethyl alcohol much more in rose water than in rose oil is attributed to the transition of a large part of phenyl ethyl alcohol into the rose water during distillation. As a large part of phenyl ethyl alcohol, which is an aromatic alcohol that is highly soluble in water, passes to the pulp water during distillation and to the rose water during condensation, the volatile oils of rose water and pulp water have a very high phenylethyl alcohol content [2, 5, 20, 21].

There are studies that have examined the chemical composition of rose water by using different solvents and extraction methods. In their study where they examined the main compounds of rose water in ethanol as the solvent, Agarwal et al., found the levels of phenyl ethyl alcohol as 69.7-81.6%, linalool as 1.5-3.3%, citronellol as 1.8-7.2%, nerol as 0.2-4.2% and geraniol as 0.9-7.0% [5]. Eikani et al. [6], reported the main compounds of rose water as phenyl ethyl alcohol (81.27%), citronellol (5.72%) and geraniol (4.43%).

Kırımer et al. [15], carried out liquid-liquid extraction of 4 different brands of rose water with n-hexane, and according to their GC/MS results, phenyl ethyl alcohol, geraniol, and citronellol were the main compounds in rose water. Ulusoy et al. [22], found in their GC/MS analysis that the main compounds of rose water were 30.7% geraniol, 29.4% citronellol, 16.2% nerol, and 23.7% phenyl ethyl alcohol.

In their study in Iran, Moein et al. [13], emphasized that, as a result of increased demand for rose water in the market, producers resorted to producing rose water containing synthetic plant compounds or other plants' volatile oils, or rose water diluted with water. They reported that rose waters produced this way are distinguished from original rose waters as they are weaker in terms of color and scent. Boskabady et al. [23], stated that, in Iran, sometimes synthetic compounds or the volatile oils of other aromatic plants are added to rose water to reduce the production cost of this product, and this practice leads to reduction of the amount of phenyl ethyl alcohol, which is one of the important compounds of rose water, in the product. Moein et al. [13], reported that the main compounds in ten different brands of rose water in Iran were phenyl ethyl alcohol, geraniol, and b-citronellol, and they determined a total of 22 compounds. Additionally, they attributed the higher geraniol, b-citronellol and linalool content in some samples to the addition of geranium extract of clove extract into rose water [13].

In our study, in the GC/MS findings on the rose waters, 48 different compounds were determined, while 15 were the main compounds (≥5%) (Table 3). It is seen that the main compounds of especially rose waters produced in laboratory conditions were phenyl ethyl alcohol (69.7-81.6%),

linalool (1.5-3.3%), citronellol (1.8-7.2%), nerol (0.2-4.2%) and geraniol (0.9-7.0%). These findings showed that products that may be considered as a mere scent that is synthetic are being sold, consumers purchase these as if these are natural rose waters, but most of these rose waters are not natural. Additionally, it was reported that 80% of the rose waters in Turkey are synthetic [24]. It was determined in our study that the main compounds of the rose water samples varied a lot. While phenyl ethyl alcohol was not encountered at all in 7 of the 15 samples, the highest value of 32.212% was found in one sample, and the lowest value was determined as 11.556%. In another sample, 60.115% 1,2-propanediol, 38.02% ethanol and trace amounts of citronellol, nerol and geraniol were found.

CONCLUSION

Considering all this literature and our findings, it is thought that the content standards of rose waters are very different, there may be risky contents, and this situation will directly affect both the quality of rose water and habits of safe production and consumption. It was revealed that label information is not adequately explanatory, there is no sufficient information especially on the type of distillation, and there is no certain standard in terms of analysis results on the chemical compositions of the products that are sold. In the microbiological analyses, it was determined that there were higher than standard bacterium numbers in some of the products that were expected to be sterile as products of distillation. This shows that bottle cleanliness and hygiene rules during bottling were not complied with. In rose water production, it is important to carry out production by providing good hygiene practices (GHP) and good manufacturing practices (GMP), use pasteurization in production, prevent contaminations during and especially after production, pay attention to the selection of raw materials, and additionally, raise awareness among producers and consumers. The finding that the collected samples complied with standards in terms of some parameters and did not comply with hygiene and production standards in terms of others showed that it is needed to discuss the sustainability of standard and inspection efforts towards products on the shelves and production facilities.

TABLE 3
Levels of Volatile Compound (≥5%) in Rose Water Samples (%)

Component (Rt)	1	2	3	4	5	6	7	8
Dihydromyrcenol	-	10.78	-	-	-	-	-	-
Linalool	-	5.946	-	-	-	-	-	-
1,2-propendiol	-	-	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-	-	-
Fenchol	-	18.961	-	-	-	-	-	-
oCimene	-	-	-	-	-	-	-	-
Citronellol	25.023		18.455	38.978	48.986	61.407	62.3	60.221
Nerol	9.765	5.942	14.068	13.075	32.898	-	8.513	-
Geraniol	9.923	5.497	11.574	22.471	10.474	13.728	12.083	13.778
Phenyl ethyl alcohol	32.212	-	30.084	15.439	-	13.204	-	14.316
Beta Ionone	-	8.528	-	-	-	-	-	-
Eugenol	4.297	-	6.538	1.047	0.85	1.719	1.414	1.712
Methyl Eugenol	2.039	-	1.345	0.986	2.152	0.455	1.962	0.335
Benzene pentanol, gamma methyl acetate	-	5.146	-	-	-	-	-	-

Component (Rt)	9	10	11	12	13	14	15
Dihydromyrcenol	-	-	-	-	-	8.547	-
Linalool	-	6.83	-	74.323	-	8.501	-
1,2-propendiol	60.115						-
Ethanol	38.02	-	-	-	-	-	-
Fenchol	-	-	-	-	-	17.462	-
oCimene	-	-	-	-	-	12.765	-
Citronellol	-	52.495	59.915	8.27	57.52		57.312
Nerol	-	-	5.11	-	-	9.335	5.453
Geraniol	-	16.168	19.095	11.179	13.88	7.709	11.428
Phenyl ethyl alcohol	-	16.274	-	-	17.225	-	11.556
Beta Ionone	-	-	-	-	-	8.111	-
Eugenol	-	0.098	1.421	0.02	1.502	-	2.147
Methyl Eugenol	-	0.155	1.923	0.015	0.015	-	2.26
Benzene pentanol, gamma methyl acetate	-	-	-	-	-	6.22	-

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REFERENCES

[1] Akdaş, M. and Baydar, H. (2018) Productivity and Scent Analysis of Concrete and Absolute Obtained by Different Solvent Extractions of Oil-Bearing Rose (Rosa damascena Mill.) Flowers. Süleyman Demirel University Journal of Natural and Applied Sciences. 22, 149-155.

[2] Göktürk-Baydar, N. and Baydar, H. (2005) Essential oil compositions of Turkish oil rose (Rosa damascene Mill.) products. 36th International Symposium on Essential Oils. 5-7 September 2005. Budapest-Hungary.

[3] Kineci, S. (2005) Investigation of new yield increasing techniques in rose oil production. Doctoral Thesis. Süleyman Demirel University, Graduate School of Natural and Applied Sciences.

[4] Baydar, H. (2017) Agriculture and Industry of Rose Oil. http://ziraat.sdu.edu.tr/assets/uploads /sites/27/files/yag_gulu-20012015.pdf. Acces Date: 01.05.2017. (In Turkish).

[5] Agarwal, S.G., Gupta, A., Kapahi, B.K., Baleshwar, T.R.K., Suri, O.P. (2005) Chemical composition of rose water volatiles. J. Essent. Oil Res. 17, 265-267.

[6] Eikani, M.H., Golmohammad, F., Rowshanzamir, S., Mirza, M. (2005) Recovery of water-soluble constituents of rose oil using simultaneous distillation–extraction. Flavour and Fragrance Journal. 20(6), 555-558.

[7] TS 3834 ISO 2293 (1996) Enumeration of Microorganism Colony Count Technique At 30°C (Reference Method).

[8] TS EN ISO 9308-1 (2014) Water quality - Enumeration of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method.

[9] ISO 16649-2 (2001) International Standart Organisation (ISO 16649-2), Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of ß-glucuronidase- positive Escherichia coli. Part 2: Colony-count technique a 44°C using 5-bromo-4-chloro-3-indoyl-beta-D-glucuronide 07/2001.

[10] TS EN ISO 7899-2 (2002) Water quality - Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method.

[11] TS EN ISO 16266 (2009) Water quality - Detection and enumeration of Pseudomonas aeruginosa - Method by membrane filtration.

[12] TS 6582-2 EN ISO 6888-2 (2001) Horizontal method for the enumeration of coagulase - Positive staphylococci.

[13] Moein, M., Zarshenas, M.M. and Delnavaz, S. (2014) Chemical composition analysis of rose water samples from Iran. Pharmaceutical Biology. 52(10), 1358-1361.

[14] Baydar, H., Kuleasan, H., Kara, N., Secilmis-Canbay, H. and Kineci, S. (2013) The effects of pasteurization, ultraviole tradiation and chemiclal preservatives on microbial spoilage and scent composition of rose water. Journal of Essential Oil Bearing Plants. 16(2), 151-160.

[15] Kirimer, N., Köseoğlu, Ç., İşcan, G., Kürkçüoğlu, M., Can-Başer, K.H. (2012) Microbiological safety controls of selected hydrosols and their volatile compounds. Marmara Pharmaceutical Journal. 16, 23-28.

[16] Arani, N.M., Chaleshtori, R.S. and Rafieian-Kopaei, M. (2014) Microbialquality of some medicinal her bal products in Kashan, Iran. J HerbMed Pharmacol. 3(2), 113-117.

[17] Zebarjad, Z., Nazemosadat Arsanjani, Z. and Moein, M.R. (2017) Microbial quality of commercial rose water products in Fars, Iran. First Iranian Pharmacognosy Congress.

[18] Jayakody, P., Parajuli, P.B. and Brooks J.P. (2014) Evaluating spatial and temporal variability of fecal coliform bacterial oads at thepelahatchie watershed in Mississippi. Hum Ecol Risk Assess. 20, 1023-1041.

[19] Baydar, H. and Göktürk-Baydar, N. (2017) Essential Oils and Phenolic Compounds, Antiradical and Antioxidant Activities of Distillation Products in Oil-bearing Rose (Rosa damascena Mill.). Journal of Agricultural Sciences. 23(1), 1-9.

[20] Baydar, H. and Kazaz, S. (2013) Oil Rose and Isparta Rose. Gülbirlik Publications. No: 1, Isparta (in Turkish).

[21] Baydar, H. and Erbaş, S. (2016) Comparison of Volatile Compounds using Headspace Solid-phase Microextraction (HSSPME) and Conventional Hydro-distillation Method in Oil-bearing Rose (Rosa damascena Mill.). Süleyman Demirel University Journal of Natural and Applied Sciences. 20(1), 27-36.

[22] Ulusoy, S., Tinaz, G. and Seçilmis-Canbay, H. (2009) Tocopherol, carotene, phenolic contentsand antibacterial properties of rose essential oil, hydrosoland absolute. Current Microbiology. 59, 554-558.

[23] Boskabady, M.H., Shafei, M.N., Saberi, Z. and Amini, S. (2011) Pharmacological effects of Rosa Damascena. Iran J Basic Med. Sci. 14, 295–307.

[24] Anonymous (2018) https://www.haberler.com/piyasadaki-gul-suyunun-yuzde-80-i-sentetik-6256013-haberi/. Acces Date: 12.06.2018.

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