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ORIGINAL PAPERS

FORMULATION AND PHYSICOCHEMICAL AND SENSORY EVALUATION OF HERBAL TEA MADE FROM HIBISCUS SABDARIFFA, CAMELLIA SINENSIS AND ZIZIPHUS JUJUBE

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ABSTRACT

The objectives of the study were to conduct physicochemical and sensory evaluation on three herbs *Hibiscus Sabdariffa*, *Camellia sinensis* and *Ziziphus jujube* in order to assess their potential for making herbal tea and to evaluate different drying method of jujube fruit. For this purpose, jujube fruits were dried using oven, microwave and spray drying methods and then mixed with *Hibiscus Sabdariffa* and *Camellia sinensis* to make infusions. Some physiochemical and sensory attributed were studied. The results revelled that drying methods had significant effect on pH, acidity, total sugar, moisture, water activity, and sensorial properties as well as colour of infusions. Application of black tea increased pH in oven dried or microwave dried pure jujube infusion, while sour tea significantly decreased infusion pH. The highest overall acceptability was related to pure jujube infusion, when jujube fruits were oven dried.

KEYWORDS:
Sour tea, Black tea, Jujube, Physicochemical and Sensory properties, spray drying.

INTRODUCTION

Jujube fruit (*Ziziphus jujube* Mill.), which has been described as the ‘‘fruit of life” [1] is a species of *Ziziphus* in the buckthorn family (Rhamnaceae), used primarily as a shade tree that also bears fruit. It is native to southern Asia, China and India. The plant has distributed widely in China, South Korea, India, and Iran and has been cultivated for over 3000 years and used as food and food additives [2].

Jujube is a key constituent in the Chinese herb that is known for its hepatoprotective effects. It contains flavonoids, saponins, tannins, vitamins, polysaccharide, trace metals, and multiple amino acids [1]. Jujube fruits are rich in various essential nutrients with carbohydrates contributing in largest portion (55–85%), followed by moisture (25–30%), crude fiber (2.4–8.4%), crude protein (2.9–6.6%), crude fat (0.4–1.0%), and several other essential vitamins and minerals [3].

In recent years, natural polysaccharides, which were found largely in fruits and vegetables, have been confirmed to play an important part as free radical scavengers in the prevention of oxidative damage in living organism and can be exploited as novel potential antioxidants, and the effects have something to do with their chemical properties and architectural characteristics [2]. Other studies have revealed that jujube contains abundant phenolic compounds with high antioxidant activity. Recently, jujube juice milk has been highlighted because it contains different food components that may provide health benefits beyond those arising from individual food components. The popularity of this beverage in the Chinese market indicates that consumers have incorporated it in their diet [4]. Its fruits are not only good-tasting food but also are traditional Chinese medicine. The Chinese jujube is a high-quality honey source and Chinese jujube leaves have been exploited as tea [5]. The fruiting body of Chinese jujube is a kind of favourable and profitable fruit, which has been commonly used as a crude drug in traditional Chinese medicine. Jujube is has been used as analeptic, palliative, antibechic, food, food additives and flavours for thousands of years [2]. This plant possesses multiple medicinal properties such as anti-fertility, antimicrobial and antioxidant. [6]. On the other hand, jujube is used widely for the treatment of various diseases such as chronic fatigue; loss of appetite, anemia, and irritability demonstrated that jujube attenuated carbon tetrachloride–induced hepatic injury in mice by modulating oxidative stress.

Aqueous extracts of jujube inhibits lipid peroxidation by mice liver homogenates, and decreases radical induced hyaluronic acid depolymerization [7]. In addition, jujube reduces the lipid peroxides that are associated with diabetes and display anticancer activity in neoplastic human liver cells [3]. In China, many people drink jujube tea and believe in the synergic effects of jujube and

tea for better health. Jujube tea is prepared from fresh high quality jujube fruits. The fresh jujubes are collected and boiled in water for several hours. Finally, the extract obtained after these long hours of boiling is used to prepare the tea. The tea has an original, sweet flavour of jujube fruits and is enriched with loads of vitamins and nutrients. Jujube tea contains sugar, vitamin A, B_1, B_2 and C and is commonly used to treat various health ailments, ranging from sore throat to anemia. Hence, for its numerous health benefits, this herbal tea is one of the most priced tea worldwide.

Roselle (*Hibiscus sabdariffa* L.) is an erect annual herb belonging to the family Malvaceae. It originated from Malaysia and is cultivated mainly in tropical and subtropical regions of the world [8]. The calyces of roselle are used in tropical Africa, West Indies, the Philippines and Indonesia to make refreshing drinks, tea, syrups, puddings, sauces, condiments and perfume [9]. Roselle extracts are used as raw material of soft drink and medicinal herb preparations [10]. Roselle contains a wide range of vitamins and minerals including Vitamin C, calcium, niacin, riboflavin and flavonoids. Roselle calyces contain brilliantly red, water-soluble, flavonoid pigments known as anthocyanins [11]. Wang et al. [1] suggested that daily consumption of Hibiscus anthocyanins might be effective in lowering oxidative damage in living systems. Aqueous extracts of roselle calyces have been demonstrated to have strong antioxidant effects [12, 13].

Black tea is consumed throughout the world for its unique taste, briskness and flavour. Tea is the most widely consumed and cheapest non-alcoholic drink like next to water. Black tea is manufactured from the tender leaves of *Camellia sinensis* L. [14]. Catechins are the major biochemical constituents (amounting to ca. 20% on dry weight basis) present in tea leaves and they get oxidized to form theaflavins and thearubigins during fermentation [14, 15, 16]. Catechins and their oxidation products are mainly responsible for the taste and astringent character of black tea [14, 17]. Apart from quality characters, catechins are also found to possess properties of benefit to human health [14].

Drying is considered as a critical factor for the postharvest management and the merchantability of herbs [18]. The drying of herbs inhibits microbial growth and forestalls biochemical changes but, at the same time, it can give rise to other changes that affect the herb quality. The changes in appearance and aroma are caused by losses in volatiles or the formation of new volatiles as a result of oxidation or esterification reactions. In addition, the drying of herbs is often accompanied with the loss of bioactive compounds, which may possess antioxidant activity and other health-promoting properties [19]. In tea manufacturing, drying is a necessary process to remove moisture, stop fermentation, reduce volume and increase shelf life. Air-drying is a traditional, low cost technique that is used to lower the water content of herbs at low temperatures. The drying at low temperatures protects against the degradation of the active constituents, but it is slow and metabolic processes may continue longer, which may lead to quality loss of the aromatic plants and subsequently of the produced added value products [20]. Many drying methods such as convection oven drying, freeze-drying, microwave drying etc. are also used to preserve medicinal herbs [21]. However, there is still a lack of study on the drying methods of herbal tea. Therefore. The main objective of the study was to explore alternative uses for *Hibiscus Sabdariffa*, *Camellia sinensis* and *Ziziphus jujube* by blending the three herbs to produce an herbal tea with acceptable sensory properties. Also, different methods of jujube drying were investigated.

MATERIALS AND METHODS

In order to investigate physicochemical and sensory aspects of herbal tea made from *Hibiscus Sabdariffa*, *Camellia sinensis* and *Ziziphus jujube* and different jujube drying methods an experiment was conducted based on completely randomized design with three replications. Freshly ripened jujube fruits were purchased from local market, sorted and washed with tap water several times. The fruits were preheated with steam for 5 min. The seeds were separated from the fruits and finally pulp materials were subjected to different drying methods.

Oven drying was performed using a laboratory oven (Jeto Tech, OF-O2G, South Korea) at 70° C for 3 days. Granulated jujube was mixed with certain amount of silicon dioxide as anti-cake agent, to prevent the formation of lumps.

Similar procedure was done for microwave drying. The fruits were dried in a 300 W microwave for 7 min, until the moisture content reduced to 6% and then powdered using dry grinder and passed through sieve.

Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. For this purpose, the jujube pulps were extracted using thermal process method and then jujube extracts (BX=22) were sent to Golshad Food Industries Company, Mashhad Iran, to be spray dried with maltodextrin.

Finally the products were sent to Zarrin Shahsavand Company (PVT. LTD.), Mashhad Iran, to be mixed with black and sour tea fannings (1:6 w/w jujube and sour tea an 1:4 w/w jujube and black tea) and packed as tea bags by tea bag production machine (Miflex, Poland). Spanish

cellulose filter paper, Turkish label and Chinese string were used for manufacturing the tea bags.

As mentioned above, the three dried and milled herbs were mixed in varying proportions to obtain nine different formulations. All bagged samples were stored in glass jars at between 28° C and 34° C away from sunlight.

Infusions were prepared from all bagged samples. The tea bags were placed in a glass jar and boiling water was poured into the jar. The formulations were allowed to infuse for 5 min. The bags were then removed from the infusions.

The pH was measured using pH meter (Metrohm 827) after cooling to room temperature. Titratable acidity was determined according to National Standard, of Iran (Number 373). Briefly, 5 ml of sample was mixed with 0.1 N NaOH in a flask and then titrated with phenolphthalein until the solution colour changes to stable orange. The results were expressed based on citric acid using folloing formula:

$$A = \frac{V \times 0.0064 \times 100}{m}$$

Where A: total acidity based on citric acid (g $100g^{-1}$) V: consumed NaOH (ml), m: sample weight (g)

Total sugar was assayed using Lane and Eynon method. In order to determine total ash, samples were kept in an oven at 525°C until they became total ash, then moisturized with distilled and dried again in the oven at 525°C for 1 h, repeating until reaching the point where the difference between two successive estimates was less than 0.001. Total ash was calculated according to following formula:

$$M = m_1 \times \frac{100}{m_0} . \frac{100}{RS}$$

Where M: Total ash (mass percent), m_0: sample weight (g), m_1: ash weight (g), RS: mass percent of dried material in softened sample according to standard number 3272.

Moisture of samples was determined by drying samples at 103°C until reaching the point where the difference between two successive estimates was less than 0.005. Weight loss was calculated using following formula:

$$M = (m_0 - m_1) \times \frac{100}{m_0}$$

Where M: weight loss, m_0: primary weight, m_1: second weight

The water activity was measured using a water activity meter (Novasina ms1-aw Axair Ltd, Switzerland) at room temperature.

The colour of samples was measured using a scanner (HP Scanjet G3010) and Image J software. For this approach, 10 ml of sample was poured onto a plate and scanned at 300 pixels. The pictures were introduced to the software and *L**, *a** and *b** values were calculated.

The sensory evaluation was carried out according to A.O.A.C. methods [22]. An internal panel of ten expert members of Technical and Engineering Researches Section, Agricultural and Natural Resources Research Center, Mashhad, Iran was recruited to complete the questionnaire. Sensory attributes of herbal teas, included, aroma, taste, colour and overall acceptability were measured using a five-point hedonic scale where 5 = excellent, 4 = very good, 3 = good, 2 = fair, and 1 = poor.

The experimental data were subjected to an analysis of variance for a completely randomized design using SAS software. Duncan's multiple range tests were used to determine the difference amongst means at the level of 0.05.

RESULTS AND DISCUSSION

Analysis of variance indicated that the treatments differ significantly, however ash content was not significantly affected by the treatments (Table 1), this means that within the means there were no significant differences in response. Oven or spray drying methods significantly reduced moisture content compared with microwave drying method (Table 2). The highest moisture content was observed when microwave dried jujube was stewed with black tea (Table 2). Spray drying method caused the lowest moisture content in all mixtures (Table 2). There was no significant difference

TABLE 1
Analysis of variance on some physicochemical and sensory characteristics of herbal tea

Source of variations	d.f	pH	Acidity	Ash	Total sugar	Moisture	Water activity	Aroma	taste	colour	Overall acceptability	L*	a*	b*
Treatment	8	0.92**	0.02**	0.26ns	1363.62**	48.48**	0.01**	1.00**	1.14**	0.89**	7.08**	765.65**	125.76**	370.33**
Error	18	0.0006	0.0002	0.15	0.12	0.55	0.0001	0.25	0.18	0.11	0.29	0.89	0.47	0.26
C.V (%)		0.58	3.29	16.27	0.94	12.57	5.18	14.47	12.62	8.91	5.10	2.93	13.86	2.00

*, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively.

TABLE 2
Comparison of means of different herbal teas on some physicochemical characteristics

Treatments (infusions)		Moisture	Water activity	pH	Acidity	Ash	Total sugar
Oven dried	Pure Jujube	7.66^{c}±0.58	0.27^{c}±0.02	4.63^{d}±0.01	0.52^{bc}±0.02	2.32^{a}±0.30	49.00^{d}±0.44
	Jujube + Black tea	2.66^{d}±0.58	0.18^{e}±0.01	4.75^{b}±0.02	0.52^{bc}±0.02	2.90^{a}±0.10	58.90^{a}±0.26
	Jujube + Sour tea	2.33^{d}±0.58	0.11^{g}±0.00	3.80^{f}±0.02	0.30^{e}±0.02	2.20^{a}±0.20	10.73^{g}±0.24
Microwave dried	Pure Jujube	10.33^{b}±0.58	0.30^{b}±0.02	4.31^{e}±0.01	0.53^{b}±0.01	2.83^{a}±1.01	50.51^{c}±0.49
	Jujube + Black tea	12.00^{a}±1.00	0.32^{a}±0.00	4.71^{bc}±0.02	0.59^{a}±0.01	2.13^{b}±0.12	56.99^{b}±0.39
	Jujube + Sour tea	10.00^{b}±1.00	0.27^{c}±0.01	3.83^{f}±0.06	0.52^{bc}±0.02	2.16^{a}±0.15	11.10^{g}±0.26
Spray dried	Pure Jujube	3.00^{d}±1.00	0.20^{d}±0.01	5.12^{a}±0.01	0.53^{b}±0.02	2.50^{a}±0.30	43.85^{f}±0.33
	Jujube + Black tea	2.66^{d}±0.58	0.150^{f}0.00	4.67^{cd}±0.02	0.38^{d}±0.02	2.16^{a}±0.15	47.93^{e}±0.40
	Jujube + Sour tea	2.66^{d}±0.58	0.17^{e}±0.01	3.44^{g}±0.01	0.49^{c}±0.01	2.20^{a}±0.20	6.93^{h}±0.25

Values within each column and followed by the same letter are not different at P < 0.05 by an ANOVA protected Duncan's Multiple Range Test.

between oven dried jujube, stewed with black tea or sour tea, and all spray dried treatments (Table 2). The differences in moisture content of the samples may be attributable to differences in structure of the samples. For instance, roselle is fleshy and cup-shaped in nature [23] implying reduced surface area. It may therefore have allowed the least penetration of heat during drying hence the relatively high moisture content after drying.

Application of black tea or sour tea in oven dried and spray dried jujube decreased water activity (Table 2). By contrast, black tea increased water activity when it was added in to microwave dried jujube (Table 2). In other words, the highest water activity was related to microwave dried jujube stewed with black tea.

According to the comparison of means for pH value, the highest pH was observed when spray dried pure jujube was stewed (Table 2). On the other hand, the lowest pH value was related to spray dried jujube stewed with sour tea (Table 2). The results revealed that black tea increases jujube tea pH, while sour tea decreases this value (Table 2). However, when spray dried jujube was applied; both black and sour tea decreased tea pH so that the effect of sour tea was more pronounced (Table 2). This finding confirms report that roselle is rich in organic acids [4]. The pH of a sample affects the sensory character of the sample. Low pH results in sour and astringent products.

In case of acidity, the highest and lowest values were observed when microwave dried jujube and oven dried jujube were stewed with black tea and sour tea, respectively (Table 2). Irrespective of drying methods, sour tea application decreased acidity. Although there were no significant differences between herbal tea formulations in terms of ash content, the highest and the lowest numerical values were recorded from oven dried and microwave dried jujube, respectively, both stewed with black tea (Table 2). Ash content refers to the total mineral composition of a sample. The differences in ash value may be attributable to differences in the mineral composition of the soils within which they were cultivated. For example the ash value of roselle was recorded approximately 6.8% by Babalola [24].

Total sugar content increased on account of black tea application when compared to pure jujube tea or jujube and sour tea treatments in all drying methods (Table 2). Oven dried jujube stewed with black tea showed the highest total sugar content whereas spray dried jujube stewed with sour tea resulted to the lowest sugar content (Table 2). Tea biomolecules mainly consists of non-protein amino acid theanine and free sugars [25]. It has been reported that sugar contents and monosaccharide compositions of tea leaves polysaccharides and tea flower polysaccharides were all significantly affected by extraction methods [26].

Panelists showed the highest preference for the aroma of oven dried pure jujube and microwave dried pure jujube followed by oven dried jujube stewed with black tea, oven dried jujube stewed with sour tea and spray dried pure jujube (Table 3). Spray dried jujube stewed with sour tea was also the least preferred in aroma (Table 3). Because of high concentration of aromatic oils in black and sour tea [27], it was expected that samples with black or sour tea would record higher mean scores for aroma. From the results, oven drying method was the most effective method in increasing aroma.

Irrespective of drying method, application of black tea of sour tea decreased taste scores; however differences in taste scores were insignificant in some cases (Table 3). This may be as a result of the absence of any significant differences in the taste characteristics of the herbal teas, or panelists' inability to clearly distinguish between the taste characteristics of the infusions.

Consumer appetite for food is stimulated or dampened by its colour. This is because the colour of food indicates the flavour of food [28]. Oven dried pure jujube was the most preferred colour, followed by oven dried jujube stewed with black tea, oven dried jujube stewed with sour tea,

TABLE 3
Comparison of means of different herbal teas on some sensory characteristics

Treatments (infusions)		Aroma	Taste	Colour	Overall acceptability
Oven dried	Pure Jujube	$4.33^{a}\pm0.57$	$4.66^{a}\pm0.57$	$4.66^{a}\pm0.57$	$13.66^{a}\pm0.57$
	Jujube + Black tea	$3.66^{ab}\pm0.57$	$3.66^{bc}\pm0.57$	$4.00^{b}\pm0$	$11.33^{bc}\pm0.57$
	Jujube + Sour tea	$3.66^{ab}\pm0.57$	$3.00^{cd}\pm0$	$4.00^{b}\pm0$	$10.66^{bc}\pm0.57$
Microwave dried	Pure Jujube	$4.33^{a}\pm0.57$	$4.00^{ab}\pm0$	$3.33^{cd}\pm0.57$	$11.66^{b}\pm0.57$
	Jujube + Black tea	$3.00^{bc}\pm0$	$3.33^{bcd}\pm0.57$	$4.00^{b}\pm0$	$10.33^{cd}\pm0.57$
	Jujube + Sour tea	$3.00^{bc}\pm0$	$3.00^{cd}\pm0$	$3.66^{bc}\pm0.57$	$9.66^{de}\pm0.57$
Spray dried	Pure Jujube	$3.66^{ab}\pm0.57$	$3.33^{bcd}\pm0.57$	$4.00^{b}\pm0$	$11.00^{bc}\pm0$
	Jujube + Black tea	$3.33^{bc}0.57$	$3.00^{cd}\pm0$	$3.00^{d}\pm0$	$9.33^{e}\pm0.57$
	Jujube + Sour tea	$2.66^{c}\pm0.57$	$2.66^{d}\pm0.57$	$3.00^{d}\pm0$	$8.33^{f}\pm0.57$

Values within each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test

TABLE 4
Comparison of means of different herbal teas on colour parameters

Treatments (infusions)		L*	a*	b*
Oven dried	Pure Jujube	$19.42^{g}\pm0.67$	$-0.13^{e}\pm0.03$	$12.76^{g}\pm0.67$
	Jujube + Black tea	$29.55^{d}\pm1.16$	$2.68^{d}\pm0.47$	$31.38^{c}\pm0.70$
	Jujube + Sour tea	$22.24^{f}\pm0.69$	$12.03^{c}\pm0.93$	$16.85^{f}\pm0.47$
Microwave dried	Pure Jujube	$13.67^{h}\pm1.26$	$0.38^{e}\pm0.23$	$9.02^{h}\pm0.67$
	Jujube + Black tea	$25.30^{e}\pm1.34$	$2.37^{d}\pm0.25$	$28.86^{d}\pm0.35$
	Jujube + Sour tea	$22.60^{f}\pm1.05$	$13.31^{b}\pm1.00$	$21.34^{e}\pm0.51$
Spray dried	Pure Jujube	$58.03^{a}\pm088$	$-0.64^{e}\pm0.03$	$39.49^{a}\pm0.33$
	Jujube + Black tea	$52.95^{b}\pm0.34$	$-0.20^{e}\pm0.04$	$36.34^{b}\pm0.08$
	Jujube + Sour tea	$46.04^{c}\pm0.55$	$14.89^{a}\pm1.43$	$35.66^{b}\pm0.51$

Values within each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test

microwave dried jujube stewed with black tea and spray dried pure jujube in that order (Table 3). Conversely, the two least preferred herbal teas were included spray dried jujube contained black tea or sour tea. This indicates that oven drying method is the best method to gain preferred colour. Roselle infusion has been described as a red, transparent, liquid [29] which many people find attractive [30]. Roselle is also known as Red Sorrel due to the unique red colour of its calyx [31]. Researchers [11, 32] have attributed the reddish colour of Roselle calyx to the presence of anthocyanins – highly water-soluble, brilliantly red pigments.

Oven dried pure jujube had the highest mean score in overall acceptability (Table 3). This was expected as it was the most preferred infusion in aroma, taste and colour. There was no signification difference between black tea and sour tea application when jujube was oven dried or microwave dried, whereas black tea application increased overall acceptability when jujube was spray dried (Table 3). Spray dried jujube stewed with sour tea scored the lowest preference for overall acceptability (table 3). Difference in L^* value among all the infusions was significant; however there was no significant difference between oven dried jujube and microwave dried jujube, both stewed with sour tea (Table 4). The highest L^* value was related to spray dried pure jujube, while the lowest value was observed in microwave dried pure jujube infusion (Table 4). Regarding a^* value, spray dried jujube stewed with sour tea and spray dried pure jujube showed the highest and the lowest a^* value, respectively (Table 4). In addition, b^* value significantly decreased, when jujube was dried using microwave and stewed purely. On the contrary, spray drying method increased b^* value in pure jujube infusion (Table 4). Colour of medicinal and aromatic plants is considered as a primary quality criterion to the consumers, who prefer leaves with a natural appearance. In this study, discolouration may be caused by degradation of chlorophylls to pheophytins. Rocha et al. [33] examined the effect of drying temperature on the preservation of chlorophyll pigments. It was found that the conversion rate of chlorophylls to pheophytins was decreased by drying at low temperatures.

CONCLUSION

In conclusion, using microwave drying method and application of black tea increased moisture and water activity. In addition, pH, b^* and L^* values increased when jujube fruits were spray

dried. The highest *a** value was found when spray dried jujube was mixed with sour tea. Sensorial evaluation indicated that the highest overall acceptability is related to pure jujube infusion, dried by oven, microwave and spray drying method, respectively. In sum, the best infusion was pure jujube dried using oven or microwave.

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EFFECT OF COATED BRAN ON THE PHYSICAL PROPERTIES OF BARBARI BREAD

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ABSTRACT

The aim of this work was to assess the influence of coated wheat bran addition on the physical properties and sensory evaluation. Two types of coating materials were used; edible coatings based on lipid stearic acid and beeswax. In this study, wheat bran at the level 10% w/w flour basis was added to Barbari bread. Bread containing wheat bran designated as control and four kinds of coated bran with stearic acid and beeswax at levels of 0.67 % and 1.34 % were compared with the control. The results of this study showed that coated wheat brans had a positive effect on physical properties of bread. In addition, coated bran with beeswax had better properties.

KEYWORDS:
Barbari bread, coated bread, beeswax, stearic acid.

INTRODUCTION

Wheat bran is a low cost and rich resource of dietary fiber, minerals (Ca, Fe, Zn, e.g), antioxidants, flavonoids, vitamins and other bioactive compounds which is lost as a by-product in milling industries. So, wheat bran can be used as a natural and cheap source for magnification of certain products [1-4]. Amongst different foods, bread is a suitable option to carry fiber in human diets. If bread properly formulated, it can increase daily fiber intake. However, addition of bran to bread formulation results in significantly hard samples with lower volumes as compared to the control [1, 5]. These effects are strongly related to negative effect of bran on gluten network formation. Fiber-gluten interactions have the negative effects on the formation of the gluten network via dilution of gluten, penetrating of gas cells or particles disturbing the gluten network [6]. It has been indicated that the bran particles damage to the foam structure of the dough and cause a reduction in bread volume. One way to reduce the negative effect of bran is modifying the property of wheat bran by its coating [1]. The aim of this research is coating of wheat bran by stearic acid and beeswax that have hydrophobic properties. Those cause the reduction of water-binding capacity and allow more incorporating wheat bran into foods, without significant adverse textural effects. Sroan and Macritchie showed that addition of free fatty acids at level of 150% to 200% native lipids at flour defatted, will not increase volume of bread [7]. Thus in this study, to prevention of the effects of free fatty acids on bread volume, 0.67% and 1.34% used for bran coating and the physical and textural properties of Barbari bread prepared using the coated bran were investigated.

MATERIALS AND METHODS

Materials. Hard red wheat bran with an average particle size of 280 μm, and wheat flour with an extraction rate of 87% were supplied from bakery. Active dried bakery yeast (Razavi Yeast Co) and iodine free table salt were purchased from local market. Stearic acid and beeswax were obtained from Scharalu (Spain) and Samchun (South Korea), respectively.

Methods. Preparation Of Coated Wheat Bran. At first 0.75 gr and 1.50 gr stearic acid and beeswax separately were cast in containers and melted in the oven at 80℃, then 11.1 g wheat bran was added to the melted material and mixed.

Preparation of dough. 100 g of flour with 2 g of yeast and coated bran and uncoated for each sample were mixed. 2 g of salt dissolved in 75 ml of water 30 ± 2 °C and were added to mix flour, yeast, coated bran and uncoated bran and stirred.

Bread making. Barbari bread was prepared according to Maleki and Milani [9], with slight modifications. After dough fermentation for 1.5 hours at 38℃ and 80-90% RH, the dough was punched and rested for 10 min. Then it was sheeted to form an oval at approximately 20cm×10cm, with a thickness of approximately 1.5cm. One teaspoon of Roomal (a boiling mixture of 5 gr of flour and 100 ml of water) was spread across the bread, and three grooves were made along its length, mainly for joining crust to crumb and for the sake of a better appearance. The dough was proofed for 5

min (last fermentation) and baked for 10 min at 260℃.

Bread characterization. For all experiments, bread samples were placed at the room temperature (25℃) for 1 hour after baking. Each bread sample was evaluated based on the following characteristics:

Specific volume, crumb to crust ratio, oven spring and moisture content crumb were determined according to the method used by Maleki and Milany [9]. Specific volume was determined using a rapeseed displacement. For evaluate the crumb to crust ratio (expressed as w/w ratio), the specific amount of bread was noted and the crust was separated from the crumb using a razor blade. Oven spring was determined by recording the height of the fermented dough and height of the baked bread samples for moisture content of the crumb, 1 gr of crumb was placed in to a petri dish, which had previously been weighed. The petri dish and sample were transferred in to the oven set at 105 C to dry to a constant weight for 2 h. At the end of the 2 hours, the petri dish and sample were removed from the oven and transferred to desiccators, where they were cooled, the samples were weighed. The amount of moisture content is determined by the difference between the weight of the petri dish containing the sample before and after being placed in the oven.

Crumb texture analysis was determined according to the method used by Gomes et al [10] by a TA-CT3 (Brookfield-CT3). A 25 mm diameter cylindrical probe was used in a texture profile analysis (TPA) with double compression test to penetrate to depth of 50% with speed of 1 mm/s and with a 30s delay between first and second compressions. Hardness (gr), cohesiveness, chewiness (m J), springiness (mm) and resilience were calculated from the TPA graph. Texture measurements were performed on 20 mm thick slices of Barbari bread.

Crumb microstructure by using scanning electron microscope EM3200 (KYKY, Korea) was analyzed. Samples of bread were dried by freeze drier. Dried samples were mounted on aluminum stubs and coated with gold. Then specimen were observed with scanning electron microscope.

Sensory evaluation according to the method used by maleki and milani [9] is done. Sensory analysis was carried out by five trained panelist using semi-structure scales scoring 1 as a lowest to 5 as a highest. Several attributes of the bread such as shape appearance, underside surface, porosity, chewing ability, hardness and flavor were evaluated. Overall acceptability was calculated by weighted arithmetic mean, with the following weights given to each attribute: shape appearance 10%, underside surface 5%, upper surface 10%, porosity 15%, chewing ability 15%, hardness 20%, and flavor 25%, based on the influence of each attribute on acceptance of the product by consumers.

Data analysis all the results reported are an average of three replicates. Experimental data were analyzed by analysis of variance (ANOVA) with significance defined at $p<0.05$. Significant differences among mean values were determined by Duncan' s test. Analysis of variance and the duncan's multiple range was performed using SAS version 9.2 software.

RESULTS AND DISCUSSION

Table 1 shows the results of some physical properties of the bread loaves. There was no significant difference between the moisture content of samples crumb. The specific volume and oven spring were significantly improved with the presence of samples of coated bran.

The loaf-volume depressing effect of fibrous materials is the result of reduced gas retention rather than reduced gas formation [11]. Wheat bran particles can interfere with the gluten network and damage the structure of gas cells and, thus, gas retention [12]. Shittu et al [13] reported that excessive stress on the gas cell, lead to tensile failure and opening up of the cell wall thereby leading to gas cell merger. Due to no significance

TABLE 1
Physical properties of the breads containing coated and uncoated bran.

	Control	Coated bran with beeswax		Coated bran with stearic acid	
Coating (%)		0.67	1.34	0.67	1.34
Moisture Content (%)	44.81±0.64^{a}	44.78±0.07^{a}	44.78±0.3^{a}	44.55±0.62^{a}	44.50±0.32^{a}
Specific volume(cm^3/g)	2.443±0.41^{b}	2.77±0.11^{a}	2.69±0.3^{a}	2.74±0.5^{a}	2.72±0.3^{a}
Oven spring (mm)	7.73±0.3^{b}	9.63±0.31^{a}	9.01±0.08^{a}	9.08±0.57^{a}	8.89±0.41^{a}
Crust/crumb (g/g)	0.113±0.02^{a}	0.114±0.01^{a}	0.128±0. 2^{b}	0.126±0.1^{ab}	0.139±0. 2^{b}
TPA parameter					
Hardness(g)	1121±0.04^{a}	986±0.06^{a}	982±0.02^{a}	1085±0.06^{a}	1174±0.1^{a}
Cohesiveness	0.69±0.01^{a}	0.69±0.05^{a}	0.66±0.01^{b}	0.60±0.02^{c}	0.51±0.01^{d}
Springiness(mm)	9.28±0.001^{a}	9.21±0.03^{a}	9.02±0.06^{a}	8.40±0.03^{b}	7.04±0.3^{c}
Chewiness	70.63±0.03^{a}	61.7±0.06^{ab}	58.1±0.03^{ab}	54.13±0.01^{bc}	41.16±0. 1^{c}

difference of moisture, there is a possibility that the reason of high specific volume and increased oven spring compared with control, is coating bran prevent from injury bran particles to the gluten network and helps keep more gas. This was previously established by Onwulata [8] which observed with coating of bran, the volume was increased. Addition of fiber materials to white flour avoided from the optimal gluten network formation and it causes the loss of more gas during the fermentation and baking of bread [14].

Crust-to-crumb ratio was analyzed as an important quality factor in baking technology. A lower ratio shows thinner crust and higher quality. Thicker crust led to the migration of moisture from crumb to crust [9]. Lower amount of moisture limits the starch gelatinization and form more a porous network comprised of protein, unscathed granules and partially gelatinized starch granules [15]. The more water in the crust makes thicker crust [16]. The lowest ratio was observed in the control and coated bran with beeswax at the level 0.67%. May be the cause is timely absorption of water diffused from gluten after melting of coating by the bran. Previous studies by Zhang and Moore [17] showed that with the addition of bran to bread results thinner crust. It seems that water absorption by bran and shortage of water in the crust during baking is one of the reasons for the formation of thinner crust, that shows coated bran with beeswax at the level 0.67% has better performance and after melting in the heat of the oven, the bran is better able to absorb water evaporates.

The results of the texture analysis showed that in terms of the hardness there is no significance difference between samples coated with control. Cohesiveness is generally a positive character in all types of baked products [18]. A determination of the bread cohesiveness showed that most cohesiveness was observed in the bread control and bread coated bran with beeswax at level 0.67 %. Zhang et al [14] reported similar effects of bran on crumb cohesiveness at 10% addition level. High cohesiveness was observed in the samples of coated bran with beeswax, that represents the better effectiveness of these covers compared with other coated brans. Due to the compression force springiness of Fresh bread crumb partly reduced. A lower springiness was observed in the samples of coated bran with stearic acid. Angioloni and Collar [19] reported that during dough mixing lipids connected to proteins and bound to starch during baking. In the presence of the higher fiber content seems to irritate a reduction of the lipid – protein and lipid starch linkages due to interaction between fibers and endogenous biopolymers. It is one of the reasons for the decrease springiness, despite the presence of fatty acid in the samples. Singh et al [20] reported that addition of stearic acids inhibited the swelling of the starch granules. It seems another reason for the decline in springiness in these samples is reduce swelling granules. Decrease granule swelling, resulted in an initial increase in firmness, which changed little, during storage [21]. Probably this initial firmness makes a lower the springiness, because of crumb springiness is related to the strength of crumb cell wall network [19]. Highest amount of chewiness was observed in the control sample. Chewiness is a negative factor in the bread that should be avoided [18]. Its reduction in all of the samples coated bran confirms the beneficial effects coating on the bran.

The results of the Scanning electron Microscope are shows (Fig. 1) that the control samples have a Compact structure. In the coated brans with beeswax at the level 0.67% and stearic acid at the level 0.67% the structure better formed but in the samples of coated with stearic acid, there are less gelatinized granules. In beeswax sample at the level 1.34 % Compact structure was observed but not as control compaction. Since beeswax has emulsifier properties [22] and emulsifier at levels higher than 1.0% does not allowing gluten formation [29], can be said that a bit compact in this sample is probably related to beeswax emulsifier properties.

Table 2 shows the results of sensory evaluation of the bread loaves. Bread containing coated bran with beeswax at the level 0.67% obtained better scores in all the characteristics than the control and other coatings. But, some cases

TABLE 2
Sensory evaluation of the breads containing coated and uncoated bran.

	Control	Coated bran with beeswax		Coated bran with stearic acid	
Coating (%)		0.67	1.34	0.67	1.34
form	8.62±0.2^{a}	9±0.7^{a}	8.39±0.19^{a}	8.63±0. 2^{a}	8.46±0.1^{a}
Underside surface	3.72±0.1^{a}	3.76±0. 2^{a}	3.76±0.05^{a}	3.73±0.1^{a}	3.66±0.1^{a}
Upper surface	8.4±0.2^{a}	8.65±0.3^{a}	8.65±0.1^{a}	8.57±0.3^{a}	8.05±0.8^{a}
Porosity	11.56±0.29^{bc}	13.2±0.4^{a}	12.6±0. 3^{ab}	12.43±0.5^{ab}	11.1±1. 3^{c}
Chewing ability	11.5±0.1^{b}	12.83±0.2^{a}	12.5±0.3^{ab}	12.3±0.8^{ab}	12.27±0.3^{ab}
Hardness	15.93±0.1^{b}	18.28±0.6^{a}	17.26±0.6^{ab}	15.9±0.5^{b}	15.88±1.09^{b}
Flavor	17.83±1. 04^{c}	23.1±1. 4^{a}	21.66±0. 75^{ab}	20.1±1.1^{bc}	19.16±1.6^{bc}
Overall score	3.69±0. 3^{c}	4.44±0. 16^{a}	4.24±0. 05^{ab}	4.08±0. 1^{bc}	3.93±0. 2^{c}

were not significantly (P<0.05) different such as in appearance, upper and under surface.

Characteristics of standard Barbari bread were explained to the panelists that the texture should be so soft and could easily be torn by hand. When chewing, the bread should not be either sticky or grainy in the mouth. The bread loaves should be palatable without any off-flavor [1]. In this study, fewer points were given to samples that were resistant to chewing. The control achieved lowest score. Bread loaves containing coated bran with beeswax obtained better scores according to the texture softness. Addition of coated bran with beeswax at the level 0.67%, gained higher scores regarding to porosity and softness comparing with the control and other coatings. Bread containing coated bran with stearic acid at the level 1.34% obtained lowest scores in terms of porosity.

Using coated bran results in better flavor. The best flavor was attributed to coated brans with beeswax and the lowest score was obtained by control. Panelists recognized a bitter and unfavorable taste in the sample of control. This was previously observe by other authors [23, 28]. Galliard and Gallagher [24] reported it is likely that lipid oxidation by lipase are responsible for the perceived off- flavor in bread containing bran. It seems coating of bran prevents from off-flavors created by lipase.

Figure 1 shows the microstructure of bread loaves containing coated and uncoated bran. SEM images show the formation of the gluten network is better than this sample.

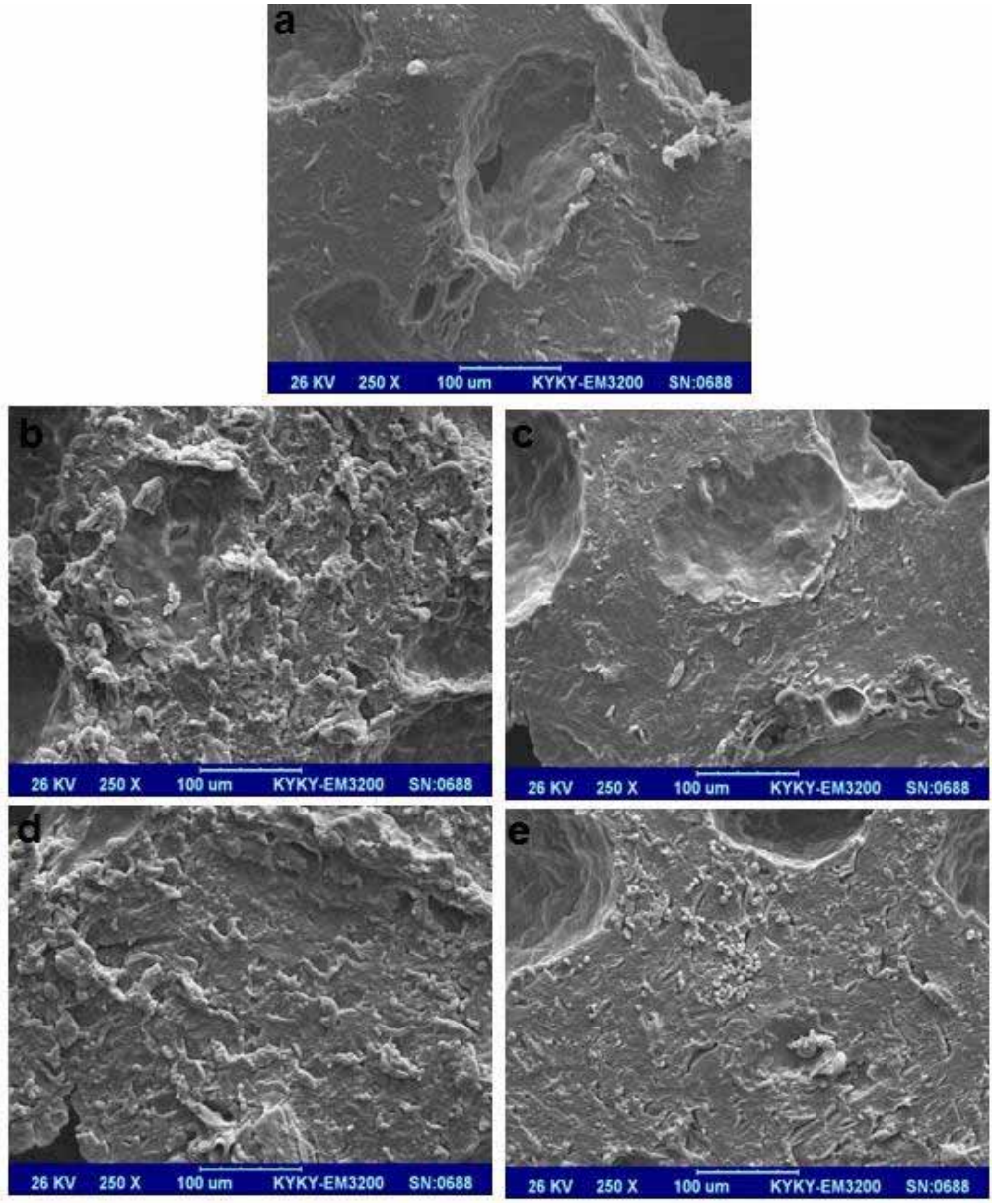

FIGURE 1

Microstructure of bread loaves containing coated and uncoated bran. a: control, b: coated bran with beeswax at level 0.67%, c: coated bran with beeswax at level 1.34%, d: coated bran with stearic acid at level 0.67%, e: coated bran with stearic acid at level 1.34%.

CONCLUSION

The physical properties of bread were improved by using coated bran. All coatings increased specific volume and oven spring. In this study it seems that preventing injury bran particles to the gas cells and helping of keeping more gas caused to increased specific volume and oven spring. The results of crust-to-crumb ratio shows that coated brans with stearic acid caused increased of ratio. Raphaelides and Georgiadis [25] reported that the presence of stearic acid increases the gelatinization temperature. Probably, due to an increase gelatinization temperature, in the crust area that is last stage of baking [26], gelatinization of starch is improved in this area and formed a thicker crust. A thick crust shows low quality. Also a lower cohesiveness in these samples is one of the undesirable cases, because Cai et al [27] reported that low cohesiveness increases bread staling during storage. The best effect on quality and sensory properties were observed with coated bran with beeswax at level 0.67 percent. This coating both increased crumb volume and reduced ratio crust to crumb. Panelists, also have more point than this sample.

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THE EFFECTS OF RED PEPPER SEED FLOUR ON THE SOME QUALITY CHARACTERISTICS OF DEEP FAT FRIED CHICKEN MEATBALLS

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ABSTRACT

The effects of pepper seed flour on the some properties of chicken meatballs were investigated. Meatballs batter was prepared with chicken meat, wheat and red pepper seed flour mixtures and other ingredients. Flour mixtures prepared as 100% wheat flour (W), 100 % pepper seed flour (S), 1:2 W:S, 1:1 W:S and 2:1 W:S. Meatballs were fried at 180 °C for 3, 5 and 7 min and analysed for some physical, chemical and sensory properties. The results of analysis indicated that the effects of S on the frying yield values of meatballs were not significant (p>0.05). The samples that contain 100% W, 2:1 W:S, 1:1 W:S and 1:2 W:S had more high moisture content than 100% S. However, the samples that contain 100% W and 1:2 W:S had lower fat contents and the samples that contain 2:1 W:S, 1:1 W:S and 100% S had higher penetrometer values. The result of instrumental colour analysis indicated that 100% W and 1:2 W:S had higher a values. Increasing frying time decreased L and b values and increased a values. Moreover, increasing frying time decreased moisture content and frying yield. Appearance, colour, odour and texture values of fried samples were not affected from W:S mixes. However, flavour values of the samples that contain S were higher than control samples. According to the results of this study concluded that the red pepper seed flour with wheat flour and 3 or 5 min of frying time can be successfully used to produce chicken meatballs.

KEYWORDS:
red pepper seed, chicken meatball, deep frying, quality characteristics

INTRODUCTION

The food industry is in the effort of evaluation of agricultural waste products. For this purpose, studies on the by-products such as seeds, peels and leaves have performed. These products are used as additives in foods because they are a potential source of bioactive compounds [1]. In general, plant products are added to foods after grinding.

Various additives have been used to improve properties of meat products such as texture, water holding capacity, shape and storage stability. For these purposes, studies on the use of natural additives have increased considerably. Natural plant products in formulations of meat products are the most important factors for technological properties and health. They have been used in the meat products as edible coating materials or batter ingredients [2-4].

Pepper (*Capsicum annuum*) is widely used in food industry. However, the use of pepper seeds is not common enough. Whereas they are rich in protein and diet fibre. Firatligil-Durmus and Evranuz [5] reported that protein content of defatted red pepper seeds is 26.02g/100g dry solids. Technological properties of foods are related to the amount and properties of proteins which can affect water holding and fat retention capacity of food products. However, cooking yield and texture are related to water and fat content of meat products [6]. Thus, the pepper seed flour can be an alternative to food additives for manufacturers.

Different cooking methods are used for the meat products. One of them is deep frying process which is widely used for meatballs cooking. It is a dry cooking and high heat involved method because it does not contain water. So that it is a quick cooking method. One of the considerable features of the deep-frying is oil migration to fried products. However, this process is caused a crust formation on the meatball surface prevented moisture loss and oil migration [7, 8]. Moreover, it can be affected colour values of meat products. It is known as the colour of foods is critical for customers. Some cereal products such as wheat and soy flours were used in the meat products to improve moisture retention and prevent oil migration during deep frying. In addition, different studies recommends the investigation of other materials from vegetable origin [8-10].

The knowledge of pepper seed products usage potential in the meat products are limited. The aim of this research was determined the effects of red pepper seed flour on the chicken meatballs.

MATERIALS AND METHODS

Materials. Wheat flour (W), fresh chicken meat and sunflower oil were obtained from a local market. After the red pepper seeds were obtained from a paprika manufacturer (Adıyaman, Turkey), they were milled into flour.

Methods. Sample preparation. Different flour mixtures were prepared with wheat flour (W) and pepper seed flour (S). W and S were mixed as 100% W (control), 2:1 W:S, 1:1 W:S, 1:2 W:S, 100% S. After the chicken breast meat was minced in a grinder (Tefal, Le Hachoir 1500, France), meat balls were prepared with 84.2% chicken meat, 4.65% flour mixture, 6.98% rusk flour, 1.38% salt, 0.93% onion powder, 0.93% garlic powder and 0.93% baking powder. One kg of each formulation was kneaded by hand to obtain uniform meatball batter. Then, the batter was shaped into 3.2 cm diameter meatballs. The samples were then fried using 1 L of sunflower oil at 180°C for 3, 5, 7 min in a deep fryer (Tefal, FF1024, China).

Determination of moisture content, fat content, and water holding capacity. Moisture contents were determined by oven air method at 105±2 °C, whereas fat contents were determined by using soxhlet extraction method with n-hexane [11]. Protein content was measured by using Kjeldahl analysis [12]. Water holding capacity was determined according to Dogan and Unal [13].

Determination of pentrometer values. Standard penetrometer (Yuksel Kaya Machine, Istanbul, Turkey) equipped with a total 52.6 g lead weight was used to evaluate hardness in fried meatballs. Needle head was placed 3 mm above the surface of the meatballs and released. Penetration depth was read after 5 s of penetration. Four meatballs were used for each replication. Three measurements were taken for each sample. The depth was determined to 1/10 mm for each meatball sample.

Determination of the cooking yield. Cooking yield was determined as follows:

$$Cooking\ Yield\ (\%) = \frac{w_1 \times 100}{w_0}$$

Where, w_0 is the weight of patties before cooking and w_1 is the weight after cooking.

Instrumental colour analysis. The colour values of meatballs were measured by using a portable colorimeter (Minolta CR-400, Osaka, Japan). The instrument was standardised against a white standardisation plate before each measurement. The colour was measured according to CIELAB systems as L (lightness), a (redness) and b (yellowness) values, as described by Dogan [14]. Four meatballs were used for the analysis of each treatment. Three measurements were taken for each sample.

Sensory analysis. The cooked meatballs were coded after 5 min and served in a random order. Ten semi-trained judges assessed the sensory properties using a hedonic scale for the appearance, colour, odour, flavour, and texture for acceptability. Different values in the scale indicated the following reactions: 1: extreme dislike, 2: very much dislike, 3: moderate dislike, 4: slight dislike, 5: neutral, 6: like slightly, 7: like moderately, 8: like very much, 9: like extremely.

Statistical analysis. The data were subjected to analysis of variance (ANOVA), and the results were indicated as mean ± standard deviation (SD). Duncan's multiple-range test was applied on the data to determine differences using Statistical Analysis System Program (SPSS, CHICAGO, IL, USA).

TABLE 1
Water holding capacity, moisture and protein contents of wheat and pepper seed flours.

Flour	Moisture (%)	Protein (%)	Water holding capacity (%)
Wheat flour	13.80	10.24	57
Pepper seed flour	4.30	14.74	101

RESULTS AND DISCUSSIONS

Some properties of wheat and pepper seed flour. As shown in Table 1, water holding capacity and protein content were found to be higher in pepper seed flour than wheat flour. However, the moisture content was found to be higher in wheat flour than pepper seed flour. Higher water holding capacity of pepper seed flour might be due to the amount and type of proteins. Because food proteins played an important role on the water holding capacity. Also, the low level of moisture in seed flour can be increase it [9].

The effects of pepper seed flour on the some properties of fried meatballs. The results of analysis of variance indicated that the effects of pepper seed flour on the moisture values were found to be significant (p<0.05). The addition of pepper seed flour without wheat flour decreased moisture values of meatballs (Table 2). This difference in the moisture values might be due to a lower moisture content of pepper seed flour than wheat flour. However, the mixtures of wheat and pepper seed flour did not cause statistically significant differences when compared to control (100% wheat.

TABLE 2
The effects of pepper seed flour on the some properties of fried chicken meatballs.

Flour mixture	Moisture (%)	Fat (%)	Penetrometer (mm)	Frying yield (%)
100% W	56.96±1.36[a]	3.90±0.32[c]	17.00±3.93[c]	91.45±2.76
2:1 W: S	55.90±2.84[a]	4.96±0.40[b]	22.02±1.74[ab]	88.33±4.01
1:1 W:S	56.39±1.79[a]	4.98±0.49[b]	21.95±2.29[ab]	90.42±3.75
1:2 W:S	56.51±1.87[a]	4.29±0.30[c]	20.87±3.57[b]	90.54±3.30
100% S	53.51±2.36[b]	7.80±0.71[a]	24.93±2.20[a]	87.32±4.65

W: Wheat flour, S:Pepper seed flour. Mean values within a column followed by different letters are significantly ($p<0.05$) different.

TABLE 3
The effects of pepper seed flour on the colour values of fried chicken meatballs.

Flour mixture	*L*	*a*	*b*
100% W	40.44±3.25	15.86±0.32[ab]	18.62±1.79
2:1 W: S	40.29±3.53	15.21±1.10[b]	18.03±2.02
1:1 W:S	42.68±3.52	14.83±1.25[b]	19.51±2.33
1:2 W:S	38.78±2.32	16.32±0.49[a]	17.22±1.63
100% S	39.65±3.27	14.86±0.32[b]	17.77±2.11

W: Wheat flour, S:Pepper seed flour. Mean values within a column followed by different letters are significantly ($p<0.05$) different.

flour). The interactions between pepper seed proteins and wheat proteins and starch might be prevent moisture lost during frying process The effects of pepper seed flours on the fat values were found to be significant ($p<0.01$). As shown in Table 2, the addition of pepper seed flour without 1:2 W:S mixture increased fat values of meatballs (Table 2). However, the effect of 1:2 W:S mixture on fat values was not significantly different from the control (100% wheat flour).

As a result of analysis of variance, the effects of pepper seed flour on the penetrometer values were found to be significant ($p<0.01$). The addition of pepper seed flour increased penetrometer values of meatballs. This effect in the penetrometer values might be due to the increasing fat content of meatballs. As shown in Table 2, the lowest moisture and the highest fat and penetrometer values of meatballs were found with 100% S. Increasing moisture and fat contents can leads to softer structure of food products. Moisture and oil transfer between meatballs and frying oil affects the quality characteristics of fried meat products [15, 16]. Although the addition of pepper seed flour affected moisture and fat content of meatballs, the differences in the frying yield values of meatballs was not found to be significant ($p>0.05$).

The effects of pepper seed flour on the instrumental colour values of fried meatballs. While the effects of pepper seed flour on the *a* values were found to be significant ($p<0.01$), its effects on the *L* and *b* values did not found to be significant ($p>0.05$). The addition of W:S mixtures without the 1:2 W:S decreased *a* values of meatballs (Table 3). However, 1:2 W:S caused the highest *a* value of meatballs. This effect might be due to the effects of interaction wheat and pepper seed components. Moreover, colour pigments of pepper seed might be affected colour values of meatballs. Embaby and Mokhtar [17] reported that pepper seed oil (*Capsicum annuum* L.) had the natural orange red in colour. Kilincceker [18] also obtained similar results on surface of fried fish meatballs.

The effects of frying time on some properties of the fried meatballs. Increasing frying time decreased the moisture content and the frying yield values of meatballs. The results of analysis of variance indicated that the effect of 7 min frying time on the moisture values was found to be significant ($p<0.01$). However, the effects of 3 and 5 minutes on the moisture values were not caused statistically significant ($p>0.05$) differences (Table 4). The decreasing of moisture values might be due to the increasing denaturation of proteins during frying time. Moisture

TABLE 4
The effects of frying time on the some properties of fried chicken meatballs.

Frying time (min.)	Moisture (%)	Fat (%)	Penetrometer (mm)	Frying yield (%)
3	57.55±1.30[a]	5.04±1.22	22.67±2.46	93.14±1.53[a]
5	56.38±0.96[a]	5.20±1.63	20.34±3.86	90.47±1.30[b]
7	53.63±1.90[b]	5.31±1.63	21.05±4.55	85.24±2.7[c]

Mean values within a column followed by different letters are significantly ($p<0.05$) different.

TABLE 5
The effects of frying time on the colour values of fried chicken meatballs.

Frying time (min.)	*L*	*a*	*b*
3	43.76±1.92[a]	14.83±1.30[b]	20.25±1.01[a]
5	40.32±1.84[b]	15.73±061[a]	18.40±1.16[b]
7	37.03±1.41[c]	15.70±0.51[a]	16.05±0.94[c]

Mean values within a column followed by different letters are significantly ($p<0.05$) different.

TABLE 6
The effects of pepper seed flour on the sensory properties of fried chicken meatballs.

Flour mixture	Appearance	Colour	Odour	Flavour	Texture
100% W	5.89±1.45	5.94±2.36	5.83±1.19	5.94±1.08[b]	5.83±1.76
2:1 W: S	6.22±1.70	6.44±1.27	6.21±1.00	7.16±0.81[a]	6.82±1.20
1:1 W:S	6.00±1.71	6.00±1.72	6.11±0.98	6.05±0.85[ab]	6.66±1.07
1:2 W:S	6.66±1.08	6.88±1.19	6.39±0.71	7.16±1.00[a]	7.00±1.35
100% S	6.55±1.07	6.66±0.79	6.77±0.89	7.11±0.83[a]	7.00±1.05

W: Wheat flour, S:Pepper seed flour. Mean values within a column followed by different letters are significantly ($p<0.05$) different.

loss might be decreased frying yield of meatballs (Table 4). Some researchers [3, 19] reported that increasing frying time decreased moisture content and frying yield values of meat balls. However, the effects of frying time on the fat content and penetrometer values of meatballs were not found to be significant ($p>0.05$).

The effects of frying time on the instrumental colour values of fried meatballs. As a result of analysis of variance, the effects of frying time on the *L* and *b* values of meatballs were found to be significant ($p<0.01$). Moreover, the effects of frying time on the *a* values were found to be significant ($p<0.05$). As shown in Table 5, increasing frying time decreased *L* and *b* values and increased *a* values of meatballs. Meatballs became darker red. These colour changes might be due to the moisture loss and structural change of meatball ingredients during increasing heat effect. Structural changes can affect colour values of the meat products during increasing heat process [20, 21].

The effects of pepper seed flour on the sensory properties of fried meatballs. The addition of pepper seed flour did not cause statistically significant differences between the sensory scores, with the exception of flavour scores (Table 6). Flavour scores of the meatballs with pepper seed flour were found to be higher than control (100% wheat flour). Panellists emphasized that pepper seed flour give a pleasant taste. Although the addition of pepper seed flour affected instrumental colour (*a*) values (Table 3) of meatballs, this affect was not found to be significant by the panellists (Table 6). As shown in table 6, the sensory scores of meatballs with pepper seed flour were higher than control. The sensory values of chicken meatballs with pepper seed flour were 6 or higher. In terms of sensory properties of meatballs, pepper seed flour addition to meatballs could be possible. Similar findings were founded by Kilincceker [18].

The effects of frying time on the sensory properties of fried meatballs. As shown in table 7, the effects of frying time on the sensory scores were found to be significant ($p<0.01$), with the exception of odour scores. Frying time up to 5 min did not cause statistically significant ($p>0.05$) differences between sensory scores. However, 7 min of frying time decreased appearance, colour, flavour and texture scores (Table 7). These decreasing scores might be due to the moisture loss and protein denaturation of meatballs during frying time. Similarly, Kilincceker [3] determined that prolonged frying process decreased the some sensory properties of chicken nuggets.

TABLE 7
The effects of frying time on the sensory properties of fried chicken meatballs.

Frying time (min.)	Appearance	Colour	Odour	Flavour	Texture
3	7.06±0.50[a]	7.13±0.57[a]	6.46±0.70	7.16±0.72[a]	7.49±0.63[a]
5	6.80±0.70[a]	7.16±0.55[a]	6.63±0.90	7.16±0.81[a]	7.26±0.60[a]
7	4.93±1.52[b]	4.86±1.65[b]	5.70±1.07	5.73±0.86[b]	5.23±1.09[b]

Mean values within a column followed by different letters are significantly ($p<0.05$) different.

CONCLUSION

The pepper seed flour had the considerable protein content and water holding capacity. However, the results indicated that the effects of pepper seed flour with wheat flour on the chicken meatballs were better than 100% pepper seed flour. The mixtures of wheat and pepper seed flours can be used in chicken meatball production. The long frying time up to 7 minutes caused quality loss of meatballs. The frying time up to 5 minutes can be recommended.

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INFLUENCE OF COLD STORAGE ON ANTIMICROBIAL, ANTIOXIDANT AND PROTEOLYTIC ACTIVITIES OF THREE DIFFERENT PROBIOTIC FERMENTED MILKS

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ABSTRACT

Crude extracts of acidophilus, bifidus and casei fermented milks were screened for their antimicrobial, antioxidant and proteolytic activities during ten day of cold storage (5±1°C). Results showed that the antimicrobial, antioxidant and proteolytic activities of all treatments were increased after ten days of cold storage. Crude extract of casei fermented milk had higher antimicrobial, antioxidant and proteolytic activities than either bifidus or acidophilus during cold storage. Results of organic acid profiles showed that Lactic acid is the major metabolites produced in acidophilus and casei fermented milks. While lactic and acetic acids were the major two metabolites produced in bifidus fermented milk. Also, levels of lactic and acetic acids were increased in comparison with non-fermented milk during cold storage period. At the end of cold storage, levels of amino acids (phenylalanine, histidine, tyrosine, aspartic acid and glutamic acid) were increased in crude extract of casei fermented milks compared two other extracts which fit well with proteolytic activity. Results of this study indicated that with increase the cold storage period, levels of amino acids and organic acids were increased which reflect to increase the antimicrobial, antioxidant and proteolytic activities.

KEYWORDS:
Functional dairy foods, fermented milks, therapeutic effects, metabolites, *Lactobacillus casei*

INTRODUCTION

Different chronic diseases like cancer, coronary heart disease, obesity and diabetes are mostly associated with oxidative damage, life style and diet [1]. Therefore, changes in lifestyle and type of diet consumed by different consumers can lower the risk of different chronic diseases. A functional food is a food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, i.e. they contain bioactive compounds [2]. Fermented milks containing live microbial strains called probiotics have been considered as first functional food. Some species of the genera *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics in fermented and non-fermented dairy products [3].

Consumption of probiotic fermented milks has expanded rapidly because they fulfil many nutritional requirements with therapeutic effects e.g. lowering plasma cholesterol [4,5], anti-obesity [6,7], antioxidant activity [8], reduction the risk of intestinal inflammation [9], decrease risk of foodborne illness and modulating the intestinal microbiota through antibacterial activities [10,11]. However, impact of cold storage on antimicrobial, antioxidant and proteolytic activities of fermented milks has not been assessed. Therefore, the aim of our investigation is to evaluate the antimicrobial, antioxidants and proteolytic activities of three different probiotic fermented milks during cold storage.

MATERIALS AND METHODS

Materials. Bacterial strains. *Bifidobacterium* (*B.*) *longum* NRRL-B-41409, *Lactobacillus* (*L.*) *acidophilus* NRRL-B- 4495, *L. casei* subsp *casei* NRRL-B- 1922 and *Kluyvermyces lactis* NRRL-Y-8279 were obtained from Northern Regional Research Laboratory (NRRL), Peoria, USA. *Bacillus* (*Ba.*) *stearothermophilus*, *Eschericha* (*E.*) *coli* and *Pseudomonus* (*Ps.*) *aerogenosa* were purchased from were obtained from Egyptian Microbial Culture Collection (EMCC), Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Chemicals. Ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co., USA. All other chemicals were of analytical reagent grade.

Methods. Preparation of fermented milks. Three different fermented milks were prepared from cow milk using the procedure reported by Donkor *et al*. [12]. Standardized cow milk (3% fat, 14% total solids) was heated at 85°C for 30 min

followed by cooling to 40°C and inoculated with 1% (v/v) cultures of *B. longum*, *L. acidophilus* or *L. casei* separately. The different mixes were dispended into 200 mL polystyrene cups and incubated at 42°C until pH reaches to 4.62±0.05 whereas counts of *B. longum*, *L. acidophilus* and *L. casei* using MRS agar (Merck Co., Darmstadt, Germany) supplemented with 0.05% L-cystein-HCl were 7.65±0.45, 8.30±0.60 and 8.50±0.30, respectively. Samples of fermented milk were stored at 5±1°C for 10 days.

Preparation of whey fraction. The whey fraction of fermented milks was prepared as described by Virtanen *et al*. [8]. Aliquots were collected from the fermented milks and the pH was adjusted to 4.6 with 1 *M* HCl then centrifuged at 10,000 g for 20 min. The supernatant was filtered using a 0.45 µm filter (Millipore Corp, Billerica, MA, USA). Fresh cow milk (Farm of Faculty of Agriculture, Cairo University, Giza, Egypt) was used as control.

Determination of antimicrobial activity. The antimicrobial activity of three fermented milks was assessed by using agar well diffusion method as described by Varadaraj *et al*. [13]. Briefly, 0.1 mL of sterile whey fraction of each product was added to each well at 0, 5, and 10 days. The Petri plates were incubated at 37°C for 18 h. The diameters of inhibition zones (mm) were measured and the whole diameter was deducted.

Determination of antioxidant activities. Determination of total antioxidant capacity. Total antioxidant capacity of three fermented milks was assayed by the phosphor-molybdenum method as described by Kumaran and Karunakaran [14].

Determination of reducing power. The reducing power of three fermented milks was determined by the method of Mathew and Abraham [15].

Assay of DPPH radical scavenging activity. The antioxidant activity of three fermented milks based on the scavenging activity of the stable DPPH free radical was determined by the method described by Lee *et al*. [16].

Determination of proteolytic activity. The degree of protein hydrolysis (DH) of fermented milks was measured using the OPA method as described by Donkor *et al*. [12].

Determination of organic acid profiles. Lactic, acetic, formic, orotic and propionic acids were determined in fermented milks after 10 days of cold storage 5±1°C using HPLC. The samples were injected after dilution with sulphuric acid 0.0042 *M* (1:25) on a Metacarb 87H column 7.5×300 mm. The mobile phase was sulphuric acid 0.0042 *M* with a flow rate of 0.3 ml/min. The Metacarb HPLC column was coupled with reflective index (RI) detector was heated at temperature 65°C.

Determination of amino acid profiles. Amino acids composition of three fermented milks was analyzed after 10 days of cold storage by automatic amino acid analyzer (AAA 400 INGOS Ltd., Czech Republic).

Statistical analysis. Preparation of fermented milks trials were carried out in duplicate, where each trial for each sample was done in triplicate. The mean was then calculated from these triplicate analyses. Data are presented as the overall mean for the two trials. Statistical analysis for obtained data was carried out using analysis of variance (ANOVA) and Duncan tests with Statistical Analysis System (SAS, 1994). A probability of $P < 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Antimicrobial activity. The potential control of food and intestinal pathogens by several lactobacilli and bifidobacteria is due to different antimicrobial substance that produced by these bacteria. Results of antimicrobial activities (Table 1) showed that all extracts of fermented milks had antimicrobial activities. Also, the antimicrobials activities of all extracts were significantly increased after 10 day of cold storage (5±1°C). These results correlate well with concentrations of different organic acids in crude extracts as seen in Table (6). The antibacterial activities have been studied; Misra and Kulla [17] reported that *Bifidobacterium* spp. exhibited antibacterial activities against pathogenic bacteria. Abd El- Salam *et al*. [18] showed the ability of cell free filtrate of nine strains of lactobacilli to suppress the growth of some harmful bacteria (*E. coli*, *Staph. aureus*, *Ps. aeurogenosa* and *B. cereus*), they indicated that *L. johnsonii* and *L. acidophilus* TISTR 450 were able to suppress all the above mentioned indicator bacteria.

El-Dieb *et al.* [19] found that *L. acidophilus* La-5, *L. casei* -01 and *Bifdobacterium* Bb-12 had antibacterial activities against Gram positive *Staph. aureus* and *B. subtilis* and Gram negative *Enterococcus aerogenes* and *Ps. fluorscence*. Recently, Abd El-Gawad *et al*. [10] found that the *S. aureus* was not detected in the probiotic yoghurt containing *Bifdobacterium* Bb- 1 2 and Bb-46 after ten days of cold storage. However, counts of *E. coli* were disappeared after two days of cold storage of yoghurt containing bifidobacterial stains. They

TABLE 1
Antimicrobial activity of three different fermented milks during cold storage periods

Fermented milks	Storage period, days	Indicator microorganisms Diameter of inhibition zone (mm)*				
		Ba. stearothermophilus	*E. coli*	*Ps. aerogenosa*	*Staph. aureus*	*K. lactis*
Acidophilus	0	10.82±0.52	7.60±0.35	9.35±0.27	10.20±0.32	6.20±0.12
	5	11.20±0.42	8.25±0.61	9.70±0.42	10.52±0.18	6.85±0.40
	10	12.50±0.55	8.75±0.53	10.30±0.35	10.85±0.26	7.25±0.24
Bifidus	0	13.20±0.45	9.20±0.31	9.50±0.25	11.50±0.30	7.30±0.20
	5	13.80±0.32	9.80±0.26	10.30±0.40	12.30±0.42	7.60±0.15
	10	14.25±0.25	10.20±0.35	10.80±0.60	13.10±0.31	7.80±0.14
Casei	0	11.40±0.30	8.30±0.31	11.20±0.40	12.10±0.24	7.50±0.17
	5	12.20±0.24	8.80±0.40	11.50±0.15	13.20±0.28	7.80±0.31
	10	13.10±0.40	9.40±0.30	11.70±0.32	13.50±0.36	8.00±0.27

*: Initial zone diameter was 5mm; ±: Standard deviation

attributed that bactericidal effect of probiotic yoghurt to increase concentration of organic acids more than plain yoghurt during cold storage period.

Total antioxidant activity. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. In the present data (Table 2), total antioxidant capacity of three fermented milks with various probiotic strains and ascorbic acid was demonstrated. The obtained results revealed that the antioxidant activity of the fermented milk is in the increasing trend with the increasing of storage period in comparison with unfermented milk. On the 5th day of refrigerated storage, the highest antioxidant activity was recorded with milk fermented with *B. longum* (0.638±0.007) while the lowest antioxidant activity was recorded with *L. casei* (0.538±0.002). On the 10th day of refrigerated storage, the highest antioxidant activity was recorded with milk fermented with *L. casei* (0.662±0.012), followed by *B. longum* (0.645±0.007) and *L. acidophilus* (0.645±0.022). The activity was compared to ascorbic acid which is employed as the standard (0.886±0.031) and unfermented milk as control (0.460±0.010).

Reducing power. Data in Table (3) show the reducing power of three different fermented milks compared to ascorbic acid. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, a higher absorbance at 700 nm indicates a higher reducing power. The obtained results revealed that the reducing power of each fermented milk varied

TABLE 2
Total antioxidant capacity of three different fermented milks during refrigerated storage periods

Probiotic strain (fermented milk)	Storage period (days)	Total antioxidant capacity ($O.D_{695\ nm}$)
L. acidophilus	0	0.559^{d}±0.015
	5	0.602^{c}±0.010
	10	0.645^{b}±0.022
B. longum	0	0.597^{c}±0.005
	5	0.638^{b}±0.007
	10	0.645^{b}±0.007
L. casei	0	0.498^{e}±0.005
	5	0.538^{d}±0.002
	10	0.662^{b}±0.012
Unfermented milk (control)		0.460^{f}±0.010
Ascorbic acid (standard)		0.886^{a}±0.031

-Values are means of three replicates ± SD. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

TABLE 3
Total reduction capability of three different fermented milks during refrigerated storage periods.

Probiotic strain (fermented milk)	Storage period (days)	Total reduction capability ($O.D_{700\ nm}$)
L. acidophilus	0	0.191^{de}±0.026
	5	0.185^{e}±0.005
	10	0.195^{de}±0.012
B. longum	0	0.265^{b}±0.022
	5	0.279^{b}±0.015
	10	0.218^{cd}±0.015
L. casei	0	0.233^{c}±0.015
	5	0.264^{b}±0.009
	10	0.206^{cde}±0.014
Unfermented milk (control)		0.231^{c}±0.017
Ascorbic acid (standard)		0.692^{a}±0.024

-Values are means of three replicates ± SD. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

with storage period. The milk fermented with *B. longum* had a highest reducing power value (0.279^{b}±0.015) followed by *L. casei* (0.264^{b}±0.009) on the 5th day of storage period. The lowest reducing power value of fermented milk was recorded with *L. acidophilus* (0.185^{e}±0.005) on the 5th day of storage period compared with unfermented milk (0.231^{c}±0.017).

DPPH radical scavenging activity. The DPPH radical has been widely used for assessment of the radical scavenging activity because of facility and convenience. DPPH is a compound that possesses a proton free radical. This feature of DPPH is used to determine proton-radical scavenging actions. The DPPH radical exhibits a characteristic absorption at 517 nm and the purple color fades upon encountering proton radical scavengers [20]. In this study, the DPPH radical scavenging activity of three fermented milks during different refrigerated storage periods (0–10 days) was investigated. Results obtained from DPPH radical scavenging activities for three fermented milks are shown in Table 4. The scavenging effect of each fermented milk on the 10th day of refrigerated storage and ascorbic acid standard solution with the DPPH radical was in the following order: milk fermented with *B. longum* (99.66%) > milk fermented with *L. acidophilus* (96.72%) > ascorbic acid (93.10%) > milk fermented with *L. casei* (86.73%). Data also revealed that the scavenging activity of each fermented milk sample was increased with increasing the storage period.

TABLE 4
Scavenging activity of three different fermented milks during refrigerated storage periods against DPPH radical.

Probiotic strain (fermented milk)	Storage period (days)	Scavenging activity (%)
L. acidophilus	0	68.69^{e}±4.49
	5	84.52^{d}±0.95
	10	96.72^{b}±0.80
B. longum	0	95.76^{bc}±0.61
	5	97.83^{ab}±0.52
	10	99.66^{a}±0.22
L. casei	0	71.11^{e}±0.36
	5	85.14^{d}±0.55
	10	86.73^{d}±0.67
Unfermented milk (control)		38.49^{f}±2.13
Ascorbic acid (standard)		93.10^{c}±0.36

-Values are means of three replicates ± SD. Numbers in the same column followed by the same letter are not significantly different at $P<0.05$.

In general, our data obtained demonstrated that the three fermented milks possessed antioxidant and scavenging activity of radicals, including superoxide anion radical and hydroxyl radical, and nitric oxide radical. The antioxidative activity of fermented milk was associated with its content and type of amino acids (Table 5). The antioxidant activity of milk protein hydrolysates and individual peptides released after hydrolysis has been reported by several studies [21,22]. The antioxidant activity has been attributed to certain amino acid sequences and high concentrations of histidine and some hydrophobic amino acids [23,24]. Hernandez-Ledesma *et al*. [22] identified one peptide (Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ser-Asp-Ile) which possessed radical scavenging activity higher than that of butylated hydroxyanisole. The antioxidant peptides have been identified in fermented milk with lactic acid bacteria and these peptides had an important role in the oxidative stability of yoghurt [8,25]. Nishino *et al.* [26] suggested that the casein-derived peptides may be one of the factors enhancing radical-scavenging activity. In addition, Lin and Chang [27] found that both intact cells and intracellular cell-free extracts of *Bifidobacterium longum* and *L. acidophilus* demonstrated the ability to scavenge DPPH free radicals.

Proteolysis activity. The degree of protein hydrolysis activity was measured and recorded as percentage of bonds cleaved (Fig. 1). The protein hydrolysis activity in casei fermented milk was significantly ($P < 0.05$) higher than acidophilus and bifidus fermented milks indicating generating higher amounts of different peptides including bioactive peptides in casei fermented milks than acidophilus and bifidus fermented milks. Our observation supports findings reported by Sah *et al*. [28] that yoghurt contains *L. casei* or *L. paracasei* had higher degree of protein hydrolysis.

Amino acid profiles. The changes in percentages (%) of amino acids in three different fermented milks during refrigerated storage period are presented in Table (5). In general, lysine, phenylalanine, valine, alanine, tyrosine, proline, arginine and glutamic acid were all found in three different fermented milks during ten days of refrigerated storage. The results revealed that the each fermented milk varied in its content of amino acids during refrigerated storage period. On the 10th day of refrigerated storage, fermented milk with *L. casei* was highest in its content of essential amino acids while fermented milk with *B. longum* had the lowest content of essential amino acids. On contrary, fermented milk with *B. longum* had the highest content of nonessential amino acids compared with others. The three fermented milks had a well balanced amino acid composition. In the same trend, Muradyan *et al*. [29] reported that fermentation of milk by thermophilic lactic streptococci or acidophilic rods enriched the final products with at least 4 amino acids (cysteine, valine, proline and arginine).

FIGURE 1

Degree of protein hydrolysis (%) of three different fermented milks during different refrigerated storage periods.

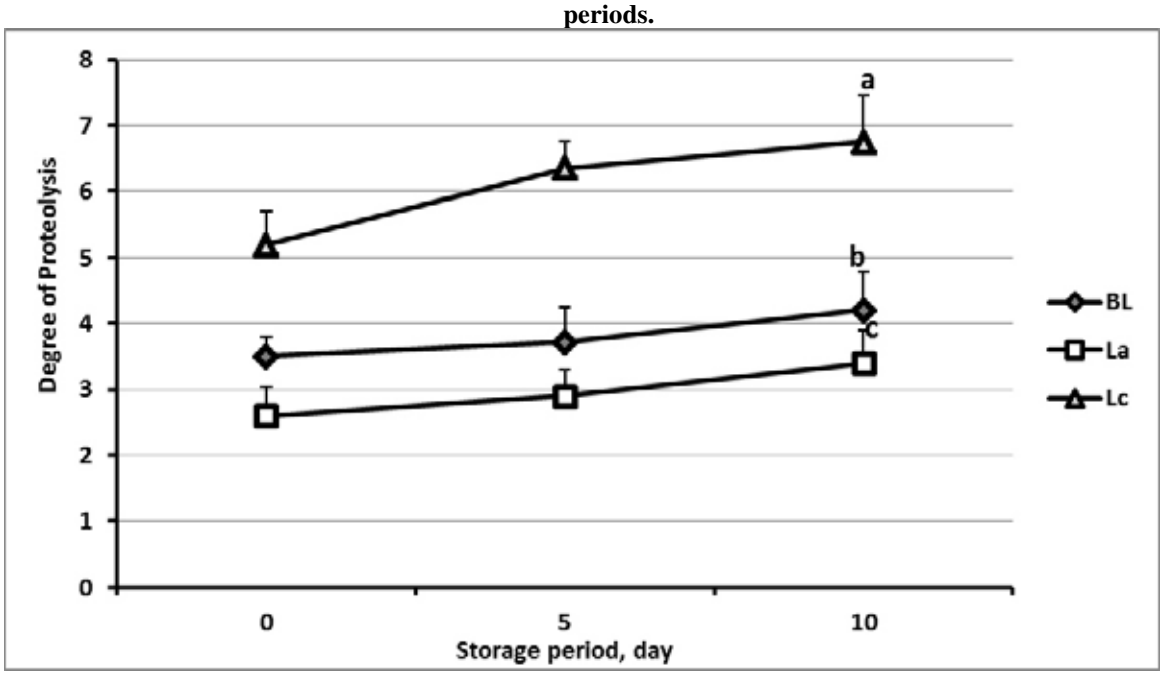

TABLE 5
Amino acid profiles (%) of three fermented milks during refrigerated storage period.

Amino acid	Probiotic strain (fermented milk)						Unfermented milk (Control)
	L. acidophilus		*B. longum*		*L. casei*		
	Storage period (days)						
	0	10	0	10	0	10	
Essential amino acids							
Isoleucine	-	-	-	-	-	-	0.61
Leucine	-	0.04	0.83	0.02	0.20	0.20	-
Lysine	87.22	89.04	87.18	84.75	84.22	80.86	40.30
Phenylalanine	1.82	1.94	1.99	1.81	1.51	2.41	6.05
Valine	0.32	0.12	0.65	0.92	0.38	0.24	-
Hisitidine	0.07	0.14	0.39	-	0.28	0.33	0.63
Threonine	0.45	-	-	-	-	0.25	-
Nonessential amino acids							
Alanine	0.85	1.59	1.53	3.12	0.68	1.03	-
Glycine	0.23	-	0.61	0.13	-	-	2.35
Serine	-	0.31	1.41	0.35	0.39	-	-
Tyrosine	7.08	5.21	4.89	5.76	6.54	11.22	46.50
Proline	0.11	0.09	0.13	0.21	0.03	0.02	-
Arginine	1.59	1.30	0.01	2.04	3.17	1.13	-
Aspartic acid	-	-	-	-	-	0.09	0.11
Glutamic acid	0.26	0.22	0.39	0.87	2.17	2.24	3.45

Organic acid profiles. Data in Table (6) show changes in concentration of different organic acids (lactic, acetic, formic, orotic and propionic acids) in different fermented milks compared with non-fermented milk during cold storage. It could be noticed that levels of lactic and acetic acids were increased in comparison with non-fermented milk during ten days of cold storage. However, levels of formic and orotic acid are relative stable between all treatments during cold storage period. No amounts of propionic acid could be detected by our HPLC system. Lactic acid is the major metabolites produced in acidophilus and casei fermented milks. While lactic and acetic acids were the major two metabolites produced in bifidus fermented milk. *L. acidophilus* NRRL-B-4495 and *L. casei* subsp *casei* NRRL-B- 1922 were able to ferment lactose and produced lactic acid as major metabolite through glycolysis [30]. The ability of lactobacilli to ferment lactose is due to presence of β-galactosidase, an inducible enzyme in *L. acidophilus* and *L. casei* [19, 31]. Co-culture of bifidobacteria with yoghurt cultures resulted produced more amounts of acetic acid than lactic acid in yoghurt [32]. *B. longum* catabolized sugars via the bifid shunt, with lactate and acetate as main metabolites [Table 6; 33,34]. With regard to amounts of orotic and formic acids, levels of both acids were relative stable between all treatments

TABLE 6
Concentration of organic acids (µg/ml) in three different fermented milks during cold storage

Fermented milks	Storage period, days	Concentration of organic acids (µg/ml)				
		Lactic Acid	Acetic acid	Formic acid	Orotic acid	Propionic acids
*Control	0	ND	ND	2.05±0.85	3.06±0.82	ND
	10	ND	ND	1.85±0.72	2.86±0.50	ND
Acidophilus	0	70.65±1.70	3.52±0.45	2.01±0.22	3.01±1.21	ND
	10	130.90±0.45	4.50±0.50	1.95±0.15	2.65±0.92	ND
Bifidus	0	100.65±0.60	290±0.60	1.95±0.23	2.71±0.62	ND
	10	180.30±0.54	475±1.06	1.80±0.20	2.60±0.56	ND
Casei	0	71.35±0.45	3.22±0.30	1.92±0.30	3.05±0.82	ND
	10	131.40±0.35	3.80±0.45	1.78±0.26	2.81±0.90	ND

*: Non-fermented milk, ND: Not detected

indicating that tested bacterial strains did not use them during fermentation period or during cold storage period.

CONCLUSION

Our data show that fermented milk with *L. casei* had the highest antimicrobial, antioxidant and proteolytic activities during cold storage period. This effect attributes to accumulation of different metabolites e.g. organic and amino acids during cold storage period. Our results suggest that crude extract of fermented milk made with *L. casei* could be used as nature bio-preservatives in different milk products.

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Discussion and Conclusion: This part should interpret the results in reference to the problem outlined in the introduction and of relatedobservations by the author/s or others. Implications for further studies or application may be discussed. A conclusion should be added if results and discussion are combined.

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