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Research Article

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Influence of Mixing Peanut Milk and Honey with Cow Milk on the Nutritional and Health Properties of Bio-Rayeb Milk

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Abstract

In Egypt and other Arab countries, stirred yogurt prepared using probiotic starter is usually called Rayeb milk. In this study, six treatments of Rayeb milk were manufactured from cow milk, peanut milk or cow and peanut milk mixture (1:1) with or without adding 4% honey. Cow milk Rayeb had the highest levels of acidity and total solids. Conversely, peanut milk Rayeb possessed the highest fat content. Blending peanut milk with cow milk and adding honey decreased saturated fatty acids level by 44.67% and inversely increased unsaturated fatty acids value by 73.29% in Rayeb milk. Also, Rayeb milk prepared from cow milk, peanut milk and honey mixture contained higher levels omega-6 (21.51%), omega-9 (38.80%), and antioxidant activity (46.31%) as compared with Rayeb made from cow milk (1.94, 27.21, and 30.60% respectively). The recommended count of bifidobacteria as a probiotic (10^7 cfu.g^{-1}) was achieved for Rayeb contained peanut milk and honey. So it is recommended that bio-Rayeb milk with high nutritional and health values can be successfully made from peanut and cow milk mixture (50%:50%) with blending 4% honey and using ABT culture.

Keywords

Peanut milk, Rayeb milk, Omega-6, Bifidobacteria, Honey

Introduction

The advantageous impacts of food contained probiotics on human health, and in particular of milk foods on children, are being increasingly cleared by health specialists [1]. Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'[1]. Recently, this definition was revised to 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [2]. Research evidence for numerous probiotic benefits such as obesity-related disorders, antibiotic-associated diarrhea, infections, and cancer was described [3]. Corsello et al. [4] stated that dietary supplementation with cow's skim milk fermented with *Lactobacillus paracasei* CBA L74 is an efficient strategy in preventing common infectious diseases in children.

Probiotic bacteria not only are used in fermented milk production but also in cheese preparation. Felicio et al. [5] investigated the influence of using potassium chloride instead of sodium chloride and adding arginine in Minas cheese manufacture on probiotic activity. They concluded that reducing of sodium level and adding arginine can be developed from of probiotic dairy foods industry. Silva et al. [6] showed that Prato cheese can be an adequate carrier of probiotics and the addition of different flavor enhancers can contribute developing this functional product in the cheese industry.

Rayeb milk is one of the most prevalent fermented dairy products in Middle East. In many villages, the used method of Rayeb milk preparation depends on activity of microorganisms in raw milk to induce natural fermentation. It is traditionally made by pouring raw whole milk into clay pots and allows the fat to rise at room temperature. The top cream layer is removed, and partially skimmed milk is allowed to undergo spontaneous fermentation. The organisms responsible for fermentation are thermophilic lactobacilli in the summer and mesophilic lactococci in the winter [7]. Newly, standardized and sterilized Rayeb milk is made in dairy products plants using whole milk and ABT culture. In the same context, Hamad et al. [8] manufactured bio-Rayeb milk from goat's milk supplemented with Tamr (dried dates). They cleared that mixing Tamr with bio-Rayeb milk increased nutritional value by decreasing saturated fatty acids and increasing α-linolenic, linoleic, oleic acids, and antioxidant activity levels. The highest populations of *Bifidobacterium* and *Lactobacillus acidophilus* were observed in Tamr Rayeb milk.

Since the early 1950s, various reports have been published clearing that peanut milk and its products can be made by different methods. The chemical composition of peanut milk depends on the producer desire, but generally, peanut milk has high protein level [9]. Low-cost edible products with high nutritional value could be successfully made from peanut milk [9]. In this regard, researchers have interested in fermented peanut milk products like yoghurt, buttermilk and ripened cheese analogs [9-11]. Also, peanuts can act as an activated substrate for probiotic bacteria [12]. Thus, the aim of this study was to investigate the production of Rayeb milk using peanut milk and probiotic culture (ABT-5) with the addition of 4% honey to improve sensory properties.

Materials and Methods

Material

Raw cow milk used in Rayeb manufacture was obtained from El-Serw Animal Production Research Station Egypt. Peanut (*Arachis hypogaea* L.) and honey were purchased from a local grocery in Damietta Governorate. ABT-5 culture which consists of *Streptococcus thermophiles*, *Lactobacillus acidophilus* + *Bifidobacterium lactis* Bb12 (Chr. Hansen's Lab A/S Copenhagen, Denmark) was used in Rayeb production. Starter cultures were in freeze-dried direct-to-vat set form and stored at -18 ˚C until used.

Methods

Peanut milk manufacture

Peanut milk was prepared according to a method cleared by Bensmira and Jiang [13].

Rayeb milk manufacture

Six samples of Rayeb were prepared from cow and peanut milk and honey as follows:

Sample A: Rayeb manufactured from cow milk.

Sample B: Rayeb prepared from peanut milk.

Sample C: Rayeb made from 50% cow milk + 50% peanut milk.

Sample D: Rayeb manufactured from cow milk and honey (4%) mixture.

Sample E: Rayeb prepared from peanut milk and honey (4%) mixture.

Sample F: Rayeb manufactured from cow milk, peanut milk (1:1) and honey (4%) mixture.

After pasteurization at 85 ˚C for 10 min, cow milk was mixed with peanut milk. Immediately, milk of various treatments was cooled to 40 ˚C, inoculated with starter (0.1 g/L of milk mix), incubated at 40 ˚C for fully coagulation (~300 min), and stored at 4 ˚C overnight. Then, the samples were blended for five min. Finally, Rayeb milk was filled in sterilized jars which kept at 4 ˚C for 14 days. Rayeb milk treatments were tested at the beginning of storage period and after 7 and 14 days.

Chemical analysis

Total nitrogen (TN), fat, total solids, and ash values of samples were measured according to AOAC [14]. Titratable acidity (as lactic acid percentage) was determined by titrating 10 g of sample mixed with 10 ml of boiling distilled water against 0.1 N NaOH using a 0.5% phenolphthalein indicator to an end point of faint pink color. pH of the samples was tested using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Redox potential was determined with a platinum electrode [model P14805-SC-DPAS-K8S/325; Ingold (now Mettler Toledo), Urdorf, Switzerland]. Water soluble nitrogen (WSN) content was measured according to Ling [15]. The method of Kosikowiski [16] was followed to determine total volatile fatty acids (TVFA). The antioxidant activity of Rayeb milk samples was estimated as hydrogen donating or radical scavenging ability, using the stable radical DPPH [17].

Measuring of fatty acids composition

Fatty acids composition of Rayeb milk samples was tested as cleared by Jahreis et al. [18].

Determination of amino acids composition

The method of Walsh and Brown [19] was followed in determining of amino acids contents of Rayeb milk samples.

Microbial tests

Streptococcus thermophiles and *Lactobacillus acidophilus* populations of Rayeb milk treatments were estimated as mentioned by Tharmaraj and Shah [20]. The numbers of *Bifidobacterium lactis* Bb12 was measured using the method of Dinakar and Mistry [21].

Sensory attributes evaluation

The sensory characteristics of Raybe milk treatments were measured by a panel of judges who were familiar with the product using the hedonic scale where 1-10 represents dislike extremely to like extremely [22]. The panelists analyzed the samples for color, smell, texture, taste, appearance, and mouth feel.

Statistical analysis

The obtained results were statistically analyzed using a software package [23] based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan [24] for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

Results and Discussion

Chemical composition of Rayeb milk made from cow and peanut milk

As shown in table 1, the values of titratable acidity and E_h in different Rayeb milk treatments gradually increased during the storage period. These results may be attributed to the lactose fermentation by starter bacteria, which produced lactic acid [25].

The findings of table 1 indicate significant differences between control and peanut Rayeb milk in acidity, pH, E_h, total solids, fat ash, and total volatile fatty acids. The acidity, E_h, total solids and ash levels of sample A were greater than those of Rayeb prepared from peanut milk or mixture of cow and peanut milk (treatment B and C respectively). On the contrary, peanut Rayeb milk samples were characterized by high-fat content as compared with control. The values of total solids and fat for samples A, B, and C at the beginning of storage period were 13.41-3.6, 12.07-4.6 and 12.78-4.2% respectively. These results may be attributed to low acidity, total solids and ash contents and high-fat value in peanut milk used in Rayeb manufacture. In our previous study [26], the total solids and fat contents of cow milk and peanut milk were 12.62-3.6 and 11.80-4.5% respectively.

On the other side, the development rates of acidity during storage were greater in cow milk Rayeb (control) than that observed in peanut milk one. These phenomena may result from absence of lactose in peanut milk. Giyarto et al. [27] reported that *Lactobacillus acidophilus* SNP-2 can grow well in plain peanut milk, but the production of acid is very low. It means that sugar content in the peanut milk supports the growth of that particular bacteria but the amount of fermentable sugar is not sufficient for the production of acid. Thus sugar must be added to support the production of acid.

No significant differences in the contents of TN and WSN were detected between Rayeb manufactured from cow milk or peanut milk or their mixture. Total volatile fatty acids (TVFA) levels of Rayeb contained peanut milk were higher than of control Rayeb. Moreover, blending peanut milk with cow milk increased TVFA contents in Rayeb milk as compared with that made from cow milk or peanut milk. These outcomes refer to the combined effect of cow and peanut milk on culture bacteria stimulation. The results of this study are close to the ones cleared by Isanga and Zhang [10] who showed that protein, fat, and water holding capacity values were higher in yoghurt made from peanut milk (~12 g/100 g total solids) supplemented with 4 g/100 g skimmed milk powder (PMY) than those of yoghurt made from cow milk (CMY). Levels of mineral and essential amino acids were high in both types of yoghurt.

On the other side, mixing 4% honey with milk used in Rayeb preparation increased acidity while lowered pH values in the end product which could be attributed to fructooligosacchrides in honey [28]. Giyarto [29] reported the significant increase in titratable acidity of sugar supplemented fermented peanut milk during storage, but not for the plain fermented peanut milk. High concentration of sugar supports the metabolism activity of lactic acid bacteria during storage. Acidity of fermented peanut milk drink at day 28 was twice of that at the day 0. This increase in titratable acidity was presumably attributed to lactose fermentation by lactic acid bacteria during storage.

Results of fat, ash, and TN were similar in Rayeb samples with or without honey whereas total solids, WSN and TVFA values of honey Rayeb were higher than those of control which may refer to the stimulation effect of fructooligosaccharides in honey on bifidobacteria [30]. These findings are in line with those reported by Chick et al. [31], they reported that the production of organic acids by bifidobacteria was improved by adding honey which may be due to the stimulation effect of oligosaccharides.

Free fatty acids profile of Rayeb milk

The free fatty acids content of fresh Rayeb milk was showed in table 2.

Saturated (SFA) and unsaturated fatty acids (USFA) levels

The concentrations of SFA were higher than USFA in treatments A and D (cow milk Rayeb). In other samples which contained peanut milk, the trend was quite the opposite, where the contents of USFA were considerably greater than SFA. The results indicated that replacement of 50% cow milk with 50% peanut milk markedly lowered the amount of SFA and inversely increased the amount of USFA of the produced Rayeb. Isanga and Zhang [10] reported that yoghurt made from peanut milk supplemented with 4 g/100 g skimmed milk powder (PMY) had higher levels of USFA than SFA comparing with cow milk yoghurt (CMY).

Supplementation of Rayeb milk with 4% honey reduced the concentrations of SFA and increased the amounts of USFA. Lowering of SFA and increasing USFA contents in the functional dairy product prepared in this study increase the healthful features of this food.

Monounsaturated (MUSFA) and polyunsaturated (PUSFA) fatty acids

Using peanut milk in the manufacture of Rayeb markedly affected the contents of MUSFA and PUSFA. Peanut milk Rayeb had higher contents of MUSFA than that made from cow milk. Concentrations of PUSFA in peanut milk Rayeb nearly doubled seven times from those found in Rayeb prepared from cow milk. Consequently, mixing 50% peanut milk with 50% cow milk greatly raised the amounts of MUSFA and PUSFA of Rayeb.

Table 1: Physicochemical properties of Rayeb milk.

Properties	**Treatments**	**Storage period (days)**			**Means ± SD**
		Fresh	7	14	
Acidity %	A	0.62	0.81	0.94	$0.79^{ab} \pm 0.05$
	B	0.38	0.50	0.57	$0.48^{d} \pm 0.07$
	C	0.54	0.69	0.78	$0.67^{bc} \pm 0.06$
	D	0.70	0.91	1.06	$0.89^{a} \pm 0.05$
	E	0.44	0.56	0.65	$0.55^{cd} \pm 0.04$
	F	0.60	0.77	0.88	$0.75^{ab} \pm 0.08$
	Means ± SD	$0.55^{B} \pm 0.04$	$0.71^{A} \pm 0.07$	$0.81^{A} \pm 0.06$	
pH values	A	4.91	4.74	4.60	$4.75^{d} \pm 0.42$
	B	5.68	5.57	5.48	$5.58^{a} \pm 0.28$
	C	5.36	5.33	5.22	$5.30^{b} \pm 0.36$
	D	4.80	4.60	4.43	$4.61^{d} \pm 0.47$
	E	5.60	5.45	5.32	$5.46^{ab} \pm 0.55$
	F	5.13	4.96	4.82	$4.97^{c} \pm 0.69$
	Means ± SD	$5.25^{A} \pm 0.25$	$5.11^{B} \pm 0.31$	$4.98^{C} \pm 0.22$	
E_h mV	A	136.4	155.7	165.8	$152.63^{b} \pm 2.01$
	B	84.4	99.1	106.7	$96.73^{f} \pm 3.11$
	C	108.5	126.3	134.2	$123.00^{d} \pm 2.65$
	D	149.2	170.1	183.6	$167.63^{a} \pm 2.47$
	E	101.7	118.9	127.6	$116.07^{e} \pm 4.10$
	F	123.6	142.0	152.6	$139.40^{c} \pm 2.98$
	Means ± SD	$117.30^{C} \pm 2.10$	$135.35^{B} \pm 2.36$	$145.08^{A} \pm 2.45$	
Total solids (TS)%	A	13.41	13.48	13.45	$13.45^{d} \pm 1.25$
	B	12.07	12.10	12.11	$12.09^{f} \pm 1.20$
	C	12.78	12.75	12.80	$12.78^{e} \pm 0.99$
	D	16.51	16.48	16.60	$16.53^{a} \pm 1.31$
	E	15.24	15.20	15.21	$15.22^{c} \pm 1.22$
	F	15.69	15.70	15.72	$15.70^{b} \pm 1.75$
	Means ± SD	$14.28^{A} \pm 1.04$	$14.28^{A} \pm 1.10$	$14.32^{A} \pm 1.12$	
Fat %	A	3.6	3.7	3.7	$3.67^{d} \pm 0.41$
	B	4.6	4.6	4.7	$4.63^{a} \pm 0.36$
	C	4.2	4.3	4.3	$4.27^{b} \pm 0.51$
	D	3.5	3.6	3.6	$3.57^{d} \pm 0.78$
	E	4.4	4.6	4.5	$4.50^{a} \pm 0.91$
	F	4.1	4.0	4.1	$4.07^{c} \pm 0.54$
	Means ± SD	$4.07^{A} \pm 0.55$	$4.13^{A} \pm 0.46$	$4.15^{A} \pm 0.61$	
Ash %	A	0.85	0.87	0.86	$0.86^{a} \pm 0.07$
	B	0.17	0.20	0.18	$0.18^{c} \pm 0.04$
	C	0.56	0.53	0.54	$0.54^{b} \pm 0.08$
	D	0.90	0.92	0.88	$0.90^{a} \pm 0.06$
	E	0.23	0.21	0.22	$0.22^{c} \pm 0.04$
	F	0.60	0.61	0.64	$0.62^{b} \pm 0.07$
	Means ± SD	$0.55^{A} \pm 0.08$	$0.56^{A} \pm 0.07$	$0.55^{A} \pm 0.09$	
Total nitrogen (TN)%	A	0.520	0.535	0.531	$0.529^{a} \pm 0.025$
	B	0.530	0.534	0.532	$0.532^{a} \pm 0.055$
	C	0.519	0.527	0.529	$0.525^{a} \pm 0.067$
	D	0.505	0.508	0.510	$0.508^{a} \pm 0.078$
	E	0.501	0.504	0.503	$0.503^{a} \pm 0.098$
	F	0.504	0.500	0.502	$0.502^{a} \pm 0.075$
	Means ± SD	$0.513^{A} \pm 0.077$	$0.518^{A} \pm 0.065$	$0.518^{A} \pm 0.082$	
Water soluble nitrogen (WSN)%	A	0.091	0.133	0.146	$0.123^{a} \pm 0.011$
	B	0.090	0.131	0.144	$0.122^{a} \pm 0.014$
	C	0.088	0.134	0.148	$0.123^{a} \pm 0.016$
	D	0.097	0.142	0.160	$0.133^{a} \pm 0.017$
	E	0.095	0.140	0.157	$0.131^{a} \pm 0.018$
	F	0.098	0.144	0.159	$0.134^{a} \pm 0.012$
	Means ± SD	$0.093^{C} \pm 0.011$	$0.137^{B} \pm 0.013$	$0.152^{A} \pm 0.019$	
Total volatile fatty acids (TVFA)*	A	6.6	9.2	10.6	$8.80^{e} \pm 0.94$
	B	7.0	9.4	11.4	$9.27^{d} \pm 0.75$
	C	7.3	9.8	11.8	$9.63^{c} \pm 0.69$
	D	7.3	10.2	11.9	$9.80^{c} \pm 0.84$
	E	7.6	10.5	12.4	$10.17^{b} \pm 0.77$
	F	8.0	11.1	12.9	$10.73^{a} \pm 0.65$
	Means ± SD	$7.3^{C} \pm 0.68$	$10.1^{B} \pm 0.54$	$11.8^{A} \pm 0.71$	

* expressed as ml 0.1 NaOH 100 g^{-1} Rayeb milk.

Table 2: Free fatty acids (%) content of fresh Rayeb milk.

Fatty acids	**C**	**Treatments**					
		A	**B**	**C**	**D**	**E**	**F**
		Saturated fatty acids (SFA)%					
Caprylic	8:0	0.45	-	0.25	0.30	-	0.20
Capric	10:0	2.33	0.52	1.53	2.04	0.40	1.45
Undecanoic	11:0	0.20	-	0.15	0.11	-	0.20
Lauric	12:0	2.87	0.71	1.85	2.32	0.64	1.26
Tridecanoic	13:0	0.27	-	0.15	0.15	-	0.15
Myristic	14:0	10.10	0.23	4.41	9.54	0.25	3.23
Pentadecanoic	15:0	3.18	-	1.61	3.03	-	0.90
Palmitic	16:0	27.80	11.00	18.40	27.30	9.80	17.50
Heptadecanoic	17:0	2.98	-	1.43	2.86	-	1.02
Stearic	18:0	10.00	2.84	6.24	10.10	2.09	5.12
Arachidic	20:0	0.10	1.23	0.81	0.10	1.20	0.93
Behenic acid	22:0	0.28	2.30	1.57	0.16	1.95	1.55
Total		60.56	18.83	38.40	58.01	16.33	33.51
Unsaturated fatty acids (USFA)%							
	12:1 ω5	0.42	0.21	0.19	0.48	0.24	0.20
5-Tetradecenoic (phytosteric)	14:1 ω5	0.48	-	0.20	0.52	-	0.25
	14:1 ω7	0.35	-	0.20	0.40	-	0.25
Myristioleic acid	14:1 ω9	0.31	-	0.12	0.25	-	0.22
	16:1 ω5	0.17	-	0.11	0.20	-	0.15
Palmitioleic	16:1 ω7	2.51	0.35	1.15	2.90	0.45	1.17
	16:2 ω4	0.29	-	0.16	0.33	-	0.21
Hexagonic	16:3 ω4	0.58	-	0.21	0.51	-	0.20
	18:1 ω4	0.14	-	-	0.15	-	-
Octadecosaenoic	18:1 ω5	0.44	0.15	0.33	0.49	0.25	0.31
Vaccienic	18:1 ω7	1.09	-	0.80	1.66	-	0.91
Oleic	18:1 ω9	27.21	46.10	37.20	27.98	46.20	38.80
	18:2 ω4	0.69	-	0.27	0.55	-	0.31
	18:2 ω5	0.40	-	0.25	0.45	-	0.34
Linoleic	18:2 ω6	1.94	34.22	18.60	2.44	35.50	21.51
	18:2 ω7	0.25	-	0.21	0.26	-	0.23
α-Linolenic	18:3 ω3	0.71	0.10	0.42	0.81	0.15	0.25
	18:3 ω4	-	-	0.10	0.10	-	0.15
Gamma linolenic	18:3 ω6	-	-	0.10	-	-	-
Octadecatetraenoic	18:4 ω3	0.39	-	0.25	0.60	-	0.27
Gadoleic acid	20:1 ω9	-	-	0.62	-	0.75	0.76
Eicosaenoic	20:1 ω11	-	0.10	-	0.10	0.13	-
Eicosatrienoic	20:3 ω6	-	-	0.11	0.12	-	-
Total		38.37	81.08	61.60	41.30	83.67	66.49
Total MUSFA		33.12	46.81	40.30	35.13	47.14	42.26
Total PUSFA		5.25	34.27	21.30	6.17	36.53	24.23
Non identified fatty acid		1.07	0.09	0.45	0.69	0	0.66

The addition of honey to milk utilized in Rayeb production raised the amounts of MUSFA and PUSFA. The contents of MUSFA of A, B, C, D, E, and F treatments were 33.12, 46.80, 40.30, 35.13, 47.14 and 42.26% respectively. In all Rayeb treatments, monounsaturated fatty acids contents were greater than polyunsaturated fatty acids. Among MUSFA, oleic acid recoded the highest level in different Rayeb treatments. The prevailing fatty acid of PUSFA was linoleic acid.

Lots of literature showed that omega fatty acids are very important for human health. Rayeb made from peanut milk individually or mixed with cow milk distinguished by very high concentrations of omega-6 (linoleic acid) and omega-9 (oleic acid) comparing with cow milk Rayeb. Levels of linoleic acid in samples A, B and C were 1.94, 34.22 and 18.60% respectively. Unfortunately, peanut milk just contained traces of α-linolenic acid (omega-3); therefore the level of this acid was very low in Rayeb contained peanut milk. Similar outcomes were found by Giyarto et al. [27] who reported that the nutritional benefits of peanut milk and its products are very high because of very high contents of minerals, protein and essential fatty acids like linoleic and oleic acids.

Free amino acids content (FAA) of Rayeb milk

Data in table 3 present the impact of adding peanut milk honey on the composition of FAA of fresh Rayeb milk.

Table 3: Free amino acids content (g/100 ml) of fresh Rayeb milk.

Amino acids	**Treatments**					
	A	**B**	**C**	**D**	**E**	**F**
Aspartic (ASP)	0.60^{c}	1.09^{a}	0.84^{b}	0.64^{c}	1.17^{a}	0.88^{b}
Threonine (THR)	0.42^{a}	0.35^{b}	0.37^{b}	0.45^{a}	0.39	0.40^{a}
Serine (SER)	0.65^{a}	0.60^{b}	0.61^{b}	0.70^{a}	0.64^{a}	0.62^{b}
Glutamic acid (GLU)	0.84^{b}	0.95^{a}	0.93^{a}	0.87^{b}	1.03^{a}	0.94^{a}
Proline (PRO)	0.71^{a}	0.25^{c}	0.51^{b}	0.72^{a}	0.28^{c}	0.55^{b}
Glycine (GLY)	0.09^{b}	0.17^{a}	0.13^{a}	0.09^{b}	0.19^{a}	0.13^{a}
Alanine (ALA)	0.22^{a}	0.24^{a}	0.22^{a}	0.23^{a}	0.22^{a}	0.22^{a}
Valine (VAL)	0.47^{a}	0.39^{b}	0.44^{a}	0.49^{a}	0.42^{a}	0.45^{a}
Methionine	0.13^{a}	0.08^{b}	0.12^{a}	0.15^{a}	0.08^{b}	0.11^{a}
Isoleucine (ILE)	0.29^{a}	0.24^{a}	0.25^{a}	0.30^{a}	0.25^{a}	0.27^{a}
Leucine (LEU)	0.58^{a}	0.47^{b}	0.55^{a}	0.60^{a}	0.50^{b}	0.57^{a}
Tyrosine (TYR)	0.30^{a}	0.23^{b}	0.27^{a}	0.30^{a}	0.23^{b}	0.28^{a}
Phenylalanine (PHE)	0.45^{b}	0.61^{a}	0.57^{a}	0.47^{b}	0.60^{a}	0.59^{a}
Histidine (HIS)	0.28^{a}	0.25^{a}	0.25^{a}	0.28^{a}	0.26^{a}	0.24^{a}
Lysine (LYS)	0.50^{a}	0.33^{c}	0.40^{b}	0.51^{a}	0.35^{b}	0.41^{b}
Arginine (ARG)	0.30^{c}	0.94^{a}	0.69^{b}	0.30^{c}	0.98^{a}	0.74^{b}
Cystine (CYS)	0.11^{c}	0.27^{a}	0.20^{b}	0.13^{c}	0.29^{a}	0.22^{b}
Total amino acids	6.94^{c}	7.46^{b}	7.35^{b}	7.23^{b}	7.88^{a}	7.62^{a}
Total EAA	3.12^{a}	2.72^{c}	2.95^{b}	3.25^{a}	2.85^{b}	3.04^{a}
Total Non-EAA	3.82^{c}	4.74^{b}	4.40^{b}	3.98^{c}	5.03^{a}	4.58^{b}
E/T (%)	44.96^{a}	36.46^{c}	40.14^{b}	44.95^{a}	36.17^{c}	39.89^{b}

Total free amino acids

All Rayeb samples contained peanut milk had the highest levels of the total free amino acids as compared with to that prepared from cow milk. Rayeb milk manufactured from 50% peanut milk and 50% cow milk mixture possessed lower total free amino acids content than those made from peanut milk. On the other side, mixing honey with Rayeb milk slightly raised the total free amino acids content which can be explained on the basis that honey caused bacteria stimulation to secrete proteinases which increased the content of amino acids in Rayeb milk.

Essential amino acids (EAA)

The levels of EAA were slightly higher in control Rayeb (made from cow milk) than those detected in peanut milk Rayeb. Using 50% cow milk + 50% peanut milk mixture in Rayeb preparation slightly increased EAA levels comparing with peanut Rayeb. Also, Rayeb made from peanut milk had low values of essential amino acids to total amino acids (E/T) among the different treatments. Honey Rayeb had a little increasing of EAA than that of control. Settaluri et al. [32] stated that a diet including peanuts in any form could provide essential amino acids that are not synthesized in the body but are essential to building blocks of proteins.

Nonessential amino acids (Non-EAA)

In contrast to the trend of EAA, peanut milk Rayeb had the highest concentration of nonessential amino acids among various treatments. Fortification of milk used in Rayeb preparation with honey also raised the content of non-EAA. Generally, glutamic and aspartic acids were predominant in different Rayeb treatments.

Branched-chain amino acids (BCAA)

Rayeb made from cow milk had slightly higher amounts of total BCAA than that made from peanut milk. Thus, incorporation of cow milk with peanut milk increased the Rayeb content of total BCAA as compared with that made from only peanut milk. On the other side, very slight increase in BCAA values were noticed when 4% honey was added to Rayeb milk.

Antioxidants activity of Rayeb milk

Peanut milk Rayeb possessed higher antioxidant activity values than those detected in Rayeb prepared from cow milk (table 4). Unlikely expected, Rayeb made from cow and peanut mixture (50:50) possessed the highest levels of antioxidant activity. Duncan et al. [33] stated that peanut is a good source for antioxidant which may be contributing factors to potential health benefits of their consumption.

Table 4: Antioxidant activity of fresh Rayeb milk.

Treatments	*Antioxidant activity (%)*
A	$30.60^{e} \pm 1.11$
B	$36.24^{c} \pm 1.54$
C	$37.40^{c} \pm 2.04$
D	$35.24^{d} \pm 1.48$
E	$44.86^{b} \pm 1.77$
F	$46.31^{a} \pm 1.82$

A: Rayeb manufactured from cow milk.
B: Rayeb prepared from peanut milk.
C: Rayeb made from 50% cow milk + 50% peanut milk.
D: Rayeb manufactured from cow milk and honey (4%) mixture.
E: Rayeb prepared from peanut milk and honey (4%) mixture.
F: Rayeb manufactured from cow milk, peanut milk (1:1) and honey (4%) mixture.

Rayeb milk contained honey especially that manufactured from cow and peanut milk mixture had the greatest levels of antioxidant activity. The levels of antioxidant activity of A, B, C, D, E, and F treatments were 30.60, 36.24, 37.40, 35.24, 44.86 and 46.31% respectively. Aljadi and Kamaruddin [34] reported that the phenolic compounds and flavonoids are chiefly responsible for the antioxidant capacity of honey.

Population of starter bacteria in Rayeb milk during storage

Peanut milk Rayeb had higher counts of *Streptococcus thermophiles* and *Lactobacillus acidophilus* than those of control (table 5). In contrast to expected, more increases in the number of mentioned bacteria were detected when Rayeb prepared from 50% cow milk and 50% peanut milk. Furthermore, loss of viability ratios of *Streptococcus thermophiles* or *Lactobacillus acidophilus* during storage period highly decreased in mixed milk Rayeb. Giyarto et al. [27] cleared that lactic acid bacteria activity in peanut milk depends on a number of factors like the strain of lactic acid bacteria, availability of nutrition, and fermentation temperature and time. Bansal et al. [35] reported that inoculum level of 1.9%, temperature of incubation at 38 °C and incubation time for 12 h was found optimum conditions for probiotic peanut yoghurt manufacture.

Table 5: Starter bacteria counts of Rayeb milk.

Properties	Treatments	Storage period (days)			Means ± SD
		Fresh	7	14	
Streptococcus thermophiles (CFU x10^7/ml)	A	36	28	21	$28.33^{e} \pm 2.12$
	B	38	32	26	$32.00^{d} \pm 2.78$
	C	40	35	29	$34.67^{c} \pm 3.14$
	D	40	36	30	$35.33^{c} \pm 3.65$
	E	44	40	35	$39.67^{b} \pm 3.79$
	F	45	42	38	$41.67^{a} \pm 2.99$
	Means ± SD	$40.5^{A} \pm 2.11$	$35.5^{B} \pm 2.75$	$29.8^{C} \pm 3.10$	
Lactobacillus acidophilus (CFU x10^5/ml)	A	10	7	4	$7.00^{e} \pm 1.02$
	B	18	16	12	$15.33^{c} \pm 1.42$
	C	21	18	14	$17.67^{b} \pm 1.56$
	D	16	13	9	$12.67^{d} \pm 1.35$
	E	22	19	16	$19.00^{b} \pm 1.74$
	F	27	25	21	$24.33^{a} \pm 2.03$
	Means ± SD	$19.00^{A} \pm 1.20$	$16.30^{B} \pm 1.11$	$12.70^{C} \pm 1.24$	
Bifidobacterium (CFU x10^5/ml)	A	21	17	11	$16.33^{f} \pm 1.25$
	B	25	22	16	$21.00^{e} \pm 1.87$
	C	31	29	24	$28.00^{c} \pm 1.36$
	D	30	25	18	$24.33^{d} \pm 2.04$
	E	39	35	30	$34.67^{b} \pm 2.63$
	F	42	39	33	$38.00^{a} \pm 2.35$
	Means ± SD	$31.30^{A} \pm 1.45$	$27.80^{B} \pm 2.01$	$22.00^{C} \pm 1.56$	

^{abcde}Letters indicate significant differences between Rayeb milk treatments.
^{ABCD}Letters indicate significant differences between storage times.

The counts of *Streptococcus thermophiles* and *Lactobacillus acidophilus* raised in honey Rayeb milk as compared with control. The highest populations were recorded in Rayeb made from mixture of cow milk, peanut milk and 4% honey (sample F). Losses of viability values of *Streptococcus thermophiles* and *Lactobacillus acidophilus* throughout storage period were lower in Rayeb milk contained honey than those of other treatments. Loss of viability levels of *Streptococcus thermophiles* in samples A, B, C, D, E, and F were 41.67, 31.58, 27.50, 25.00, 20.45 and 15.56% respectively. Nagpal and Kaur [36] cleared that adding honey to the level of 5% improved the viability of lactobacilli pure cultures after 5 weeks storage and that improvement might be strain dependent.

As *Streptococcus thermophiles* and *Lactobacillus acidophilus* counts increased in Rayeb milk manufactured from peanut milk individually or mixed with cow milk, also *Bifidobacterium lactis* Bb12 numbers increased. This may be resulted from two reasons; the first is high acid formation and accumulation in Rayeb manufactured from cow milk and second provides sufficient amounts of nutrients in peanut milk Rayeb. The differences in viability were explained by the metabolic activity of *Bifidobacterium* in various fermented foods, which might be affected by the availability and composition of carbon sources and nitrogen in growth media as stated by Chou and Hou [37]. Ibraheem et al. [38] referred to fermented peanut milk is the suitable carrier to *Bifidobacterium longum* BB536 for at least one-week storage.

The blending of 4% honey with cow or peanut milk or their mixture increased counts while decreased loss of viability of *Bifidobacterium lactis* Bb12 in Rayeb milk. Within 14 days of storage, viability loss rates of treatments A, B, C, D, E, and F were 47.62, 36.00, 22.58, 40.00, 23.08 and 21.43% respectively. Ustunol and Gandhi [39] found that the honey promotes of *Bifidobacterium bifidium* growth.

In all Rayeb milk treatments, there were significant ($p < 0.05$) decrease in the numbers of *Streptococcus thermophiles*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* Bb12 which attributed to the acids accumulation or lowering of availability of nutrient required for the growth and activity as showed by Kabeir et al. [40]. However, lowering of bifidobacteria populations during storage, but the recommended number of bifidobacteria as a probiotic (10^7 cfu.g^{-1}) was carried out in various Rayeb milk treatments and remained above 10^7 cfu g^{-1} to the end of storage stage especially in peanut milk Rayeb treatments. These outcomes are similar to those detected by Kabeir et al. [40] who showed that fermented peanut milk drinks contained greater than 10^7 CFU/ml of viable lactic acid bacteria at the end of 28-day shelf life. Thus, at the end of storage time, the fermented peanut milk drink retained recommended level of probiotic product. The count of probiotic bacteria in dairy products should be at least 10^7 cfu/ml or per g at the time of consumption in order to give health effects in the host.

From microbial analysis data plus results of free fatty acids, free amino acids and antioxidants activity, it can be stated that utilization of probiotics in cow milk fermentation, peanut milk and honey mixture produced very healthy fermented dairy product. It is a new trend that can be used to develop the dairy industry by producing innovative functional dairy food contains the nutritional and health benefits of animal milk, vegetarian milk, honey and probiotic. Modern consumers are increasingly interested in their personal health and expect the food that they eat to be healthy or even capable of preventing illness.

Scores of sensory attributes evaluation of Rayeb milk

Table 6 shows the organoleptic properties of various Rayeb milk samples. Color and appearance properties scores evaluated at the beginning and during storage were slightly lower in peanut milk Rayeb than of Rayeb prepared from cow milk. The effect of supplementation Rayeb milk with 4% honey was not so much pronounced in color and appearance.

As it is expected, the beany flavor of peanut milk decreased smell, taste and mouth feel scores of Rayeb milk. Thus, blending of cow milk with peanut milk improved these properties in the resulted Rayeb milk. Elsamani and Ahmed [41] stated that the fresh yoghurt prepared by adding different levels of skimmed milk powder was significantly ($p \leq 0.05$) higher than that of pure peanut milk with regard to overall acceptability, taste and flavor. Anyway, fermentation of peanut milk highly optimizes its sensory attributes as mentioned by Lee and Beuchat [42] who stated that hexanal completely disappeared as a result of peanut fermentation. Hexanal is one of the compounds which cause the undesirable green/beany aroma in peanut milk. Changes in levels of these compounds were associated with organoleptic properties which showed that a significant reduce in green/beany flavor and a significant increase in creamy flavor as a result of fermentation.

Table 6: Sensory evaluation of Rayeb milk.

Properties	Treatments	Storage period (days)			Means ± SD
		Fresh	7	14	
Color	A	9	9	8	$8.67^{a} \pm 0.44$
	B	8	8	7	$7.67^{a} \pm 0.35$
	C	8	8	8	$8^{a}.00 \pm 0.71$
	D	9	9	9	$9^{a}.00 \pm 0.80$
	E	8	8	8	$8^{a}.00 \pm 0.57$
	F	9	9	8	$8.67^{a} \pm 0.49$
	Means ± SD	$8.5^{A} \pm 0.25$	$8.5^{A} \pm 0.34$	$8.0^{A} \pm 0.47$	
Appearance	A	9	9	8	$8.67^{a} \pm 0.47$
	B	8	8	7	$7.67^{a} \pm 0.26$
	C	9	8	8	$8.33^{a} \pm 0.33$
	D	9	9	9	$9.00^{a} \pm 0.54$
	E	8	8	7	$7.67^{a} \pm 0.81$
	F	9	9	8	$8.67^{a} \pm 0.90$
	Means ± SD	$8.67^{A} \pm 0.56$	$8.50^{A} \pm 0.74$	$7.80^{A} \pm 0.58$	
Smell	A	9	9	8	$8.67^{a} \pm 0.26$
	B	8	8	7	$7.67^{a} \pm 0.33$
	C	8	8	7	$7.67^{a} \pm 0.57$
	D	9	9	9	$9.00^{a} \pm 0.91$
	E	8	8	7	$7.67^{a} \pm 0.82$
	F	9	9	9	$9.00^{a} \pm 0.64$
	Means ± SD	$8.5^{A} \pm 0.22$	$8.5^{A} \pm 0.41$	$7.8^{A} \pm 0.35$	
Taste	A	9	9	8	$8.67^{ab} \pm 0.23$
	B	7	7	6	$6.67^{c} \pm 0.31$
	C	8	8	7	$7.67^{bc} \pm 0.42$
	D	10	10	9	$9.67^{a} \pm 0.50$
	E	9	9	8	$8.67^{ab} \pm 0.24$
	F	10	10	10	$10.00^{a} \pm 0.61$
	Means ± SD	$8.8^{A} \pm 0.11$	$8.8^{A} \pm 0.43$	$8.0^{A} \pm 0.23$	
Mouth feel	A	9	9	8	$8.67^{ab} \pm 0.25$
	B	7	7	6	$6.67^{c} \pm 0.35$
	C	8	8	7	$7.67^{bc} \pm 0.41$
	D	10	10	9	$9.67^{a} \pm 0.62$
	E	8	8	7	$7.67^{bc} \pm 0.40$
	F	10	10	10	$10.00^{a} \pm 0.33$
	Means ± SD	$8.67^{A} \pm 0.44$	$8.67^{A} \pm 0.25$	$7.83^{A} \pm 0.50$	
Texture & Body	A	8	8	7	$7.67^{b} \pm 0.20$
	B	8	8	7	$7.67^{b} \pm 0.31$
	C	8	8	7	$7.67^{b} \pm 0.43$
	D	10	10	9	$9.67^{a} \pm 0.25$
	E	10	10	9	$9.67^{a} \pm 0.12$
	F	10	10	9	$9.67^{a} \pm 0.22$
	Means ± SD	$9.0^{A} \pm 0.12$	$9.0^{A} \pm 0.32$	$8.0^{A} \pm 0.24$	

^{abcde}Letters indicate significant differences between Rayeb milk treatments.
^{ABCD}Letters indicate significant differences between storage times.

Next to fermentation, supplementation of peanut milk Rayeb with honey improved the smell, taste, and mouth feel evaluation scores. Adding honey also improved these properties in cow milk Rayeb. Honey Rayeb treatments were favorite for the panelists which attributed that to the sweet taste of honey. In similar trend to our present work, Amiri et al. [43] found that the adding honey improved sensory properties of sweetened synbiotic acidophilus milk. Mixing 7% honey to acidophilus milk raised the sensory rates of texture, colour, flavour and overall acceptability of the product. They also mentioned that incorporation of *Bifidobacterium* increased the flavour of synbiotic acidophilus milk when compared to *Lactobacillus acidophilus* as the control.

Texture and body scores were similar in Rayeb milk made from cow milk or peanut milk or mixture of cow milk and peanut milk. Addition honey increased the scores of texture and body in the produced Rayeb which may be due to the increasing of TS content. Adebanke et al. [44] made yoghurt from cow milk partially replaced by bambara groundnut milk in various levels (0, 10, 20, 30 and 40%). Sensory results showed that there was no significant difference ($p > 0.05$) in the aroma and consistency while there was significant difference ($p < 0.05$) in the taste, color and overall acceptability as influenced by varying proportions of added bambara groundnut milk.

Among all treatments, mixed milk Rayeb fortified with 4% honey represented the highest scores of all sensory attributes and remains superior in these regards in fresh and during the storage period.

Conclusion

It can be concluded that incorporation of 50% peanut milk with 50% cow milk and adding 4% honey and utilization of ABT culture could be manufactured bio-Rayeb milk with high nutritional value due to the increasing of unsaturated fatty acids, omega-6, omega-9, the total free amino acids, antioxidant activity levels and *Bifidobacterium lactis* Bb12 populations. Also, this fermented milk product characterized by acceptable in properties of mouth feel, texture, body, color, appearance, taste and smell.

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