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Functional Rice Rayeb Milk: Chemical, Microbiological and Sensory Properties

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Abstract

Stirred yoghurt made using probiotic bacteria (called Rayeb milk) is one of the most important functional fermented milk products in Arab countries. Six treatments of Rayeb milk were made from cow and rice milk and cow and rice milk mixture (50:50) with or without adding 4% honey. An ABT-5 culture was used as a probiotic starter. Cow milk Rayeb had higher acidity, total solids, fat, ash, total nitrogen values than those of Rayeb milk manufactured from rice milk. Concentrations of saturated fatty acids were lower whereas unsaturated fatty acids (USFA) were higher in rice milk Rayeb than that of Rayeb made from cow's milk. Omega-6, omega-3, omega-9 acids and antioxidant activity greatly rose in Rayeb made from cow and rice milk mixture in comparison with cow milk Rayeb. The amounts of total free amino acids were lower in rice milk Rayeb than those detected in Rayeb made from cow milk. Counts of *Str. thermophilus, L. acidophilus*, and *Bifidobacterium* increased in Rayeb made from rice milk. The count of bifidobacteria was above the number required to a presence in probiotic food. Supplementation of the cow or rice milk Rayeb with 4% honey increased USFA, omega-6, omega-3, omega-9 acids, free amino acids and antioxidant activity. Honey Rayeb milk contained the highest numbers of *Str. thermophilus, L. acidophilus* and *Bifidobacterium* and recorded the highest sensory evaluation scores. Functional Rayeb milk with high nutritional and health values can be successfully made from cow and rice milk mixture (50%: 50%) with adding 4% honey and using ABT-5 culture.

Keywords: Rice milk; ABT; Bifidobacteria; Rayeb milk; Honey

Introduction

Non-dairy probiotic products represent a huge growth potential for the food industry and may be widely explored through the development of new ingredients, processes, and products. There are a wide variety of traditional non-dairy fermented beverages produced around the world. Much of them are non-alcoholic beverages manufactured with cereals as the main raw material [1].

Lactic acid fermentation of cereals has been studied extensively in the past few decades. Yoghurt like products have been produced from various kinds of cereals such as liquefied starch (starch treated with enzymatic hydrolysis) [2]; prefermented and extruded rice flour [3], and cooked maize meal mixture [4]. A product, so-called Risogurt, was produced from a mixture of fermented rice and soy protein isolates (the most concentrated form of soy protein available, low-fat, 90% protein content) [5]. Method for producing a highly concentrated lactic product from rice with improved quality by a secondary enzymatic treatment during fermentation was further developed by Mok *et al.* [6]. Tominaga and Sato [7] reported the production of fermented beverage from rice flour using enzyme hydrolysis followed by lactic fermentation by *L. mesenteroides*. Other lactic acid bacteria used for developing a fermented rice product was amylolytic bifidobacterium spp. [8].

On the other hand, Rayeb milk is traditional fermented dairy products in various Arab countries. In rural areas, the method of Rayeb milk manufacture depends on natural fermentation of raw milk by the activity of microorganisms and their enzymes. Now, safe and standardized Rayeb milk is prepared on large scale in dairy products plants which use ABT culture (*Str. thermophilus, Lactobacillus acidophilus+Bifidobacterium*) in manufacture. Therefore, the objective of this study was to develop new probiotic Rayeb milk using ABT culture, cow and rice milk mixtures and honey to improve sensory properties of the end product.

Materials and Methods

Materials

Fresh cow's milk was obtained from El-Serw Animal Production Research Station, Animal Production Research Institute,

Agricultural Research Center. Rice (*Oryza sativa*) and honey were purchased from a local grocery in Damiette Governorate. An ABT-5 culture which consists of *Str. thermophilus, Lactobacillus acidophilus+Bifidobacterium* (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

Preparation of Rice Milk: The rice (one cup) was washed thoroughly and kept in a big pot. About eight cups of water were added and the whole content was placed on the heating mantle. The mixture of rice and water was boiled (103 °C) for three hours and a soupy rice pudding was obtained. The content was later blended twice till a smooth content was obtained. The blended rice was sieved twice so as to have good texture [9].

Preparation of Rayeb Milk: Six treatments of Rayeb milk were made from cow and rice milk and honey as follows:

A: Rayeb milk made from cow milk
B: Rayeb milk made from rice milk
C: Rayeb milk made from 50% cow milk+50% rice milk
D: Rayeb milk made from cow milk+4% honey
E: Rayeb milk made from rice milk+4% honey
F: Rayeb milk made from 50% cow milk+50% rice milk+4% honey

After pasteurization at 80 °C for 10min, cow milk was mixed with rice milk. Immediately, milk of various treatments was cooled to 40 °C, inoculated with cultures (0.1 g/L of milk mix), incubated at 40 °C for full coagulation (~300min), and stored at 4 °C overnight. Once blended for five min and divided into three parts transferred to three jars which preserved at 4 °C for 14 days. Rayeb milk samples were analyzed when fresh and after 7 and 14 days of refrigerated storage.

Chemical Analysis: Total solids, fat, total nitrogen and ash contents of samples were determined according to AOAC [10]. Titratable acidity in terms of % lactic acid was measured by titrating 10g of sample mixed with 10ml of boiling distilled water against 0.1 N NaOH using a 0.5% phenolphthalein indicator to an endpoint of faint pink color. pH of the sample was measured at 17 to 20 °C using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Redox potential was measured with a platinum electrode [model P14805-SC-DPAS-K8S/325; Ingold (now Mettler Toledo), Urdorf, Switzerland] connected to a pH meter (model H 18418; Hanna Instruments, Padova, Italy). Water-soluble nitrogen (WSN) of Rayeb milk was estimated according to Ling [11]. Total volatile fatty acids (TVFA) were determined according to Kosikowiski [12]. The antioxidant activity of Rayeb milk was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH as described by Olivera *et al.* [13].

Determination of Fatty Acids Composition: The extraction of milk fat was done using the method of Rose-Gottlieb using diethyl ether and petroleum ether. After that, the solvents were evaporated on a vacuum rotary evaporator. For obtaining methyl esters of the fatty acids, sodium methylate (CH_3ONa) was used [14]. The fatty acid composition of Rayeb milk was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC™- WAX, 30 m, ID 0.25 mm, Film:0,25 μm.

Determination of Amino Acids Composition: Amino acid profile of fresh Rayeb milk was performed following the protocol of Walsh and Brown [15]. Hydrochloric acid (6 M) was added to the sample vial for a final concentration of 5 mg of protein/mL of HCl. Hydrolysis vial was placed in an ultrasonic cleaner and flushed with nitrogen gas before sealing under vacuum. A sample was placed in a heating block for 4 hr. at 145 °C. Afterward, the sample was removed from the heating block and allowed to cool before filtration through 0.2 μm filter. A sample was dried with nitrogen gas and dissolved in a dilution buffer. The prepared sample was analyzed for amino acid profile by running through Automated Amino Acid Analyzer (Model: L-8500 A, Hitachi, Japan). Areas of amino acid standards were used to quantify each amino acid in the representative sample.

Microbial Analysis: Rayeb milk samples were analyzed for *Streptococcus thermophilus* and *Lactobacillus acidophilus* counts according to the methods described by Tharmaraj and Shah [16]. The count of bifidobacteria was determined according to Dinakar and Mistry [17]. A mixture of antibiotics, including 2 g of neomycin sulfate, 4 g of paromomycin sulfate, 0.3 g of nalidixic acid, and 60 g of lithium chloride (NPNL, Sigma Chemical Co.), was prepared in 1 L of distilled water, filter-sterilized, and stored at 4 °C until use. The mixture of antibiotics (5 ml) was added to 100 ml of MRS agar medium. Cysteine-HCl was added at the rate of 0.05% to decrease the redox potential of the medium. Plates were incubated at 37 °C for 48 to 72 h under anaerobic condition. The colony morphology was 1 mm, white, shiny and smooth.

Sensory Properties Judging: The sensory properties of Rayeb milk samples were determined by a panel of judges who were familiar with the product using the hedonic scale where 1-10 represents dislike extremely to like extremely [18].

Statistical Analysis: The obtained results were statistically analyzed using a software package [19] based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan [20] 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 Rice Milk

During the storage period, titratable acidity and E_h values of the control and other Rayeb milk treatments tended to increase while pH values tended to decrease (Table 1). These results may be due to acid production in the Rayeb milk during storage as a result of lactose fermentation [21].

Properties	Treatments	Storage period (days)			Means±SD
		Fresh	7	14	
Acidity %	A	0.62	0.81	0.94	0.79^{ab}±0.17
	B	0.49	0.62	0.72	0.61^{c}±0.12
	C	0.57	0.73	0.82	0.71^{bc}±0.15
	D	0.70	0.91	1.06	0.89^{a}±0.18
	E	0.54	0.71	0.81	0.69^{bc}±0.17
	F	0.60	0.78	0.89	0.76^{abc}±0.15
	Means±SD	0.59^{g}±0.15	0.76^{g}±0.10	0.87^{g}±0.11	
pH values	A	4.91	4.74	4.60	4.75^{c}±0.12
	B	5.35	5.20	5.07	5.21^{a}±0.11
	C	5.15	4.99	4.77	4.97^{b}±0.15
	D	4.80	4.60	4.43	4.61^{c}±0.16
	E	5.29	5.11	4.95	5.12^{ab}±0.14
	F	5.15	4.97	4.80	4.97^{b}±0.16
	Means±SD	5.11^{g}±0.14	4.94^{h}±0.16	4.77^{i}±0.15	
E_h mV	A	136.4	155.7	165.8	152.63^{b}±5.1
	B	98.7	117.9	125.6	114.07^{f}±4.8
	C	117.2	137.9	146.4	133.83^{d}±6.2
	D	149.2	170.1	183.6	167.63^{a}±4.3
	E	108.5	127.5	136.1	124.03^{e}±7.1
	F	129.1	150.1	151.1	143.43^{c}±6.2
	Means±SD	123.18^{i}5.5	143.20^{h}6.0	151.43^{g}±7.3	
TS %	A	13.41	13.48	13.45	13.45^{d}±0.15
	B	12.88	12.93	12.90	12.90^{f}±0.12
	C	13.21	13.18	13.30	13.23^{e}±0.11
	D	16.51	16.48	16.60	16.53^{a}±0.14
	E	16.32	16.33	16.30	16.32^{b}±0.12
	F	16.10	16.14	16.11	16.12^{c}±0.13
	Means±SD	14.74^{g}±0.12	14.76^{g}±0.14	14.78^{g}±0.16	
Fat %	A	3.6	3.7	3.7	3.67^{a}0.10±
	B	0.3	0.3	0.4	0.33^{c}±0.10
	C	2.2	2.3	2.3	2.27^{b}±0.11
	D	3.5	3.6	3.6	3.57^{a}±0.12
	E	0.3	0.3	0.3	0.30^{c}±0.10
	F	2.1	2.2	2.1	2.13^{b}±0.10
	Means±SD	2.00^{g}±0.10	2.07^{g}±0.11	2.07^{g}±0.12	

^{abcde}Letters indicate significant differences between Rayeb milk treatments

^{ghi}Letters indicate significant differences between storage times

Table 1: Physicochemical properties of Rayeb milk during storage period

Rayeb made from cow milk had higher acidity, E_h, total solids (TS), total nitrogen (TN), WSN and TVFA values than those of Rayeb milk manufactured from rice milk (Tables 1 and 2). Utilization cow and rice milk mixture (50:50) in production of Rayeb milk gave intermediate values between Rayeb made from cow or rice milk. Also, cow milk Rayeb possessed the highest values of

the acidity development rates during storage as compared with other samples. Similar results were found by Wongkhalaung and Boonyaratanakornkit [22] who showed that the acidity values were 0.86 and 1.02% (as lactic acid) for fresh rice-based yogurt and commercial dairy yogurt respectively. However, ABT culture used in Rayeb milk preparation contains *Str. thermophilus* which produces higher acid amount than that of bifidobacteria, but the acidity values of rice milk Rayeb obtained in this study were lower than those found in the study of El Tahir [23]. He mentioned that the acidity ratios of rice milk fermented with *B. infantis* 20088 were 0.29 and 0.99% at the beginning and after two weeks of storage respectively.

Properties	Treatments	Storage period (days)			Means±SD
		Fresh	7	14	
Ash %	A	0.85	0.87	0.86	0.86^{ab}±0.05
	B	0.40	0.43	0.42	0.42^{e}±0.04
	C	0.61	0.63	0.60	0.61^{cd}±0.05
	D	0.90	0.92	0.88	0.90^{a}±0.03
	E	0.46	0.45	0.47	0.46^{ed}±0.06
	F	0.72	0.70	0.73	0.72^{bc}±0.04
	Means±SD	0.66^{g}±0.04	0.67^{g}±0.05	0.66^{g}±0.05	
TN %	A	0.520	0.535	0.531	0.559^{a}±0.004
	B	0.251	0.259	0.250	0.253^{b}±0.005
	C	0.387	0.382	0.390	0.386^{ab}±0.010
	D	0.505	0.508	0.510	0.508^{a}±0.004
	E	0.240	0.246	0.248	0.245^{b}±0.006
	F	0.369	0.364	0.360	0.364^{ab}±0.004
	Means±SD	0.379^{g}±0.007	0.382^{g}±0.005	0.381^{g}±0.008	
WSN %	A	0.091	0.133	0.146	0.123^{a}±0.005
	B	0.070	0.097	0.105	0.091^{a}±0.006
	C	0.081	0.117	0.127	0.108^{a}±0.005
	D	0.097	0.142	0.160	0.133^{a}±0.004
	E	0.081	0.113	0.125	0.106^{a}±0.005
	F	0.090	0.130	0.145	0.122^{a}±0.007
	Means±SD	0.085^{g}±0.007	0.122^{g}±0.005	0.135^{g}±0.006	
TVFA*	A	6.6	9.2	10.6	8.80^{b}±0.6
	B	1.2	1.6	1.8	1.53^{f}±0.5
	C	4.2	5.3	6.5	5.33^{d}±0.6
	D	7.3	10.2	11.9	9.80^{a}±0.6
	E	1.6	2.1	2.5	2.07^{e}±0.4
	F	4.6	5.9	7.4	5.97^{c}±0.3
	Means±SD	4.25^{i}±0.5	5.72^{h}±0.6	6.78^{g}±0.5	

^{abcde}Letters indicate significant differences between Rayeb milk treatments

^{ghi}Letters indicate significant differences between storage times

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

Table 2: Chemical properties of Rayeb milk during storage period

Supplementation of Rayeb milk with 4% honey (samples D, E, and F) increased titratable acidity and E_h and lowered pH values which could be attributed to fructooligosacchrides in honey [24].

Because of a very low-fat level of rice milk, Rayeb manufactured from it also contained a very little amount of fat that did not exceed ~ 0.3%. Therefore, rice milk Rayeb can be consumed as a low-fat and cholesterol free product. Ash concentration of rice milk Rayeb was half that of cow milk one. Mixing of 50% cow milk with 50% rice milk raised the fat and ash values of Rayeb as compared with that made from rice milk.

Significant increases in TS, WSN, and TVFA levels were found with blinding of 4% honey in Rayeb milk. Fat values were similar in both samples with or without honey while ash contents were slightly higher in Rayeb milk treatments contained honey.

Free Fatty Acids Content (FFA) of Rayeb Milk

FFA contents were measured in fresh Rayeb milk treatments. Results are presented in Table 3.

Fatty acids	C	Treatments					
		A	B	C	D	E	F
		Saturated fatty acids (SFA) %					
Caprylic	8:0	0.45	-	0.40	0.30	-	0.25
Capric	10:0	2.33	-	1.27	2.04	-	1.45
Undecanoic	11:0	0.20	-	0.15	0.11	-	0.10
Lauric	12:0	2.87	0.22	1.46	2.32	-	1.20
Tridecanoic	13:0	0.27	-	0.16	0.15	-	0.10
Myristic	14:0	10.1	7.10	9.01	9.54	6.75	7.33
Pentadecanoic	15:0	3.18	2.20	2.93	3.03	2.40	2.80
Palmitic	16:0	27.80	28.18	27.85	27.30	27.20	27.61
Heptadecanoic	17:0	2.98	2.20	2.85	2.86	2.00	2.62
Stearic	18:0	10.0	7.90	9.60	10.1	7.00	8.76
Arachidic	20:0	0.10	-	-	0.10	-	0.20
Behenic acid	22:0	0.28	-	0.21	0.16	-	0.14
Total		60.56	47.80	55.89	58.01	45.35	52.56
Unsaturated fatty acids (USFA) %							
	12:1 ω5	0.42	-	0.31	0.48	-	0.35
5-Tetradecenoic (phytosteric)	14:1 ω5	0.48	-	0.25	0.52	-	0.28
	14:1 ω7	0.35	-	0.15	0.40	-	0.19
Myristioleic acid	14:1 ω9	0.31	-	0.10	0.25	-	0.15
	16:1 ω5	0.17	0.21	0.19	0.20	0.25	0.21
Palmitioleic	16:1 ω7	2.51	2.42	2.48	2.90	2.54	2.53
	16:2 ω4	0.29	-	-	0.33	-	0.14
Hexagonic	16:3 ω4	0.58	-	0.26	0.51	-	0.61
	18:1 ω4	0.14	-	-	0.15	-	-
Octadecosaenoic	18:1 ω5	0.44	-	0.30	0.49	-	0.27
Vaccienic	18:1 ω7	1.09	0.48	0.95	1.66	0.50	1.07
Oleic	18:1 ω9	27.21	32.36	29.15	27.98	32.90	30.80
	18:2 ω4	0.69	-	0.34	0.55	-	0.27
	18:2 ω5	0.40	-	0.18	0.45	-	0.15
Linoleic	18:2 ω6	1.94	15.01	7.55	2.44	15.86	8.32
	18:2 ω7	0.25	-	-	0.26	-	-
α-Linolenic	18:3 ω3	0.71	0.80	0.76	0.81	0.85	0.82
	18:3 ω4	-	-	-	0.10	-	-
Gamma linolenic	18:3 ω6	-	-	-	-	0.85	-
Octadecatetraenoic	18:4 ω3	0.39	0.92	0.42	0.60	0.90	0.43
Gadoleic acid	20:1 ω9	-	-	0.11	-	-	-
Eicosaenoic	20:1 ω11		-	-	0.10	-	-
Eicosatrienoic	20:3 ω6	-	-	-	0.12	-	-
Total		38.37	52.24	43.20	41.30	54.65	46.57
Total MUSFA		33.12	35.47	33.88	35.13	36.19	35.85
Total PUSFA		2.25	16.77	9.32	6.17	18.46	10.72
Non identified fatty acid		1.07	0	0.91	0.69	0	0.87

Table 3: Free fatty acids (%) content of fresh Rayeb milk

Saturated and Unsaturated Fatty Acids

Pronounced differences were observed in the levels of saturated and unsaturated fatty acids between various Rayeb milk samples. The concentration of saturated fatty acids (SFA) was lower in rice milk Rayeb than that of Rayeb made from cow's milk. In contrast, the value of unsaturated fatty acids (USFA) was higher in the former than the latter. Values of SFA and USFA of Rayeb milk made from mixture of cow and rice milk (50:50) were at an intermediate position between those of cow or rice milk Rayeb. Perezgonzalez [25] cleared that rice milk is low in saturated fat (0.1%).

The effect of fortification of Rayeb milk with 4% honey on SFA and USFA levels was similar to that of rice milk but to a lesser extent. Addition of honey decreased SFA by 4.21, 5.12 and 5.96% while increased USFA of Rayeb milk by 7.64, 4.61 and 7.80% for samples D, E and F respectively. Decreasing of SFA and increasing of USFA by mixing rice milk and honey with cow milk used in Rayeb manufacturing provide very health aspect. As it is well known, unsaturated fatty acids are now a nutritional hot topic, and their presence in foods has attracted both public and industrial interest. There are undeniable benefits, in terms of cardiovascular disease prevention, that can be obtained if dietary SFA are reduced, and some of the remainder substituted with both USFA. There are also a number of new and emerging areas where dietary unsaturated fatty acids may play a role in affecting an individual's risk of developing other diseases, including diabetes, inflammatory conditions such as rheumatoid arthritis or asthma, and perhaps even cancer [26].

Monounsaturated (MUSFA) and Polyunsaturated Fatty Acids (PUSFA) Fatty Acids

Data found in Table 3 cleared that Rayeb made from rice milk (samples B and E) possessed higher amounts of MUSFA than those determined in cow milk Rayeb. Therefore, mixing rice milk with cow milk slightly increased levels these acids in Rayeb milk produced as compared with those of cow milk Rayeb. Values of PUSFA took the same trend of MUSFA where their contents were greatly high in Rayeb milk prepared from rice milk individually or mixed with cow milk compared with those of cow milk Rayeb.

Not only rice milk but also addition 4% honey to milk used in Rayeb preparation increased both MUSFA and PUSFA in the end product. Generally speaking, values of MUSFA were higher than those of PUSFA in all Rayeb milk samples. Increasing of MUSFA content in Rayeb milk has a positive effect on a public health.

It is remarkable to note that essential fatty acids, linoleic acid (omega-6), α-linolenic acid (omega-3) and oleic acid (omega-9) which have special importance in healthy nutrition greatly raised in mixed milk Rayeb in comparison with that made from cow milk. Increasing rates in these threes acids in sample F were 328.86, 15.49 and 13.19% respectively. This means that utilization of probiotic starter (ABT-5), cow and rice milk mixture (50:50) and 4% honey in Rayeb milk production succeeded in production of very healthy fermented milk product which combined between advantages of animal and vegetarian milk. Mangels *et al.* [27] stated that two polyunsaturated fatty acids (PUFAs) that cannot be made in the body are linoleic acid and alpha-linolenic acid. They must be provided by diet and are known as essential fatty acids. Within the body, both can be converted to other PUFAs such as arachidonic acid.

Free Amino Acids Content (FAA) of Rayeb Milk

Results in Table 4 show the free amino acid content of fresh Rayeb milk made from cow, or rice milk or their mixture with and without addition honey.

Amino acids	Treatments					
	A	B	C	D	E	F
Aspartic (ASP)	0.60^{a}	0.39^{d}	0.51^{b}	0.64^{a}	0.44^{c}	0.55^{b}
Threonine (THR)	0.42a	0.33^{c}	0.38^{b}	0.45^{a}	0.38^{b}	0.42^{a}
Serine (SER)	0.65^{a}	0.55^{c}	0.61^{b}	0.70^{a}	0.61^{b}	0.65^{a}
Glutamic acid (GLU)	0.84^{a}	0.56^{c}	0.72^{b}	0.87^{a}	0.60^{c}	0.75^{b}
Proline (PRO)	0.71^{a}	0.45^{d}	0.59^{c}	0.72^{a}	0.48^{d}	0.62^{b}
Glycine (GLY)	0.09^{a}	0.08^{a}	0.08^{a}	0.09^{a}	0.09^{a}	0.09^{a}
Alanine (ALA)	0.22^{a}	0.17^{b}	0.18^{b}	0.23^{a}	0.17^{b}	0.19^{b}
Valine (VAL)	0.47^{a}	0.38^{c}	0.43^{b}	0.49^{a}	0.40^{b}	0.44^{b}
Methionine	0.13^{a}	0.06^{c}	0.10^{a}	0.15^{a}	0.09^{b}	0.11^{a}
Isoleucine (ILE)	0.29^{a}	0.19^{c}	0.25^{b}	0.30^{a}	0.21^{c}	0.26^{b}
Leucine (LEU)	0.58^{a}	0.45^{c}	0.54^{b}	0.60^{a}	0.46^{c}	0.57^{a}
Tyrosine (TYR)	0.30^{a}	0.21^{b}	0.25^{b}	0.30^{a}	0.22^{b}	0.26^{b}
Phenylalanine (PHE)	0.45^{a}	0.37^{c}	0.40^{b}	0.47^{a}	0.40^{b}	0.44^{a}

Amino acids	Treatments					
	A	B	C	D	E	F
Histidine (HIS)	0.28^{a}	0.22^{b}	0.26^{a}	0.28^{a}	0.24^{a}	0.27^{a}
Lysine (LYS)	0.50^{a}	0.35^{c}	0.44^{b}	0.51^{a}	0.37^{c}	0.47^{a}
Arginine (ARG)	0.30^{a}	0.28^{a}	0.30^{a}	0.30^{a}	0.29^{a}	0.31^{a}
Cystine (CYS)	0.11^{a}	0.08^{b}	0.09^{b}	0.13^{a}	0.09^{b}	0.10^{a}
Total amino acids	6.94^{a}	5.12^{b}	6.13^{a}	7.23^{a}	5.54^{b}	6.50^{a}
Total EAA	3.12^{a}	2.35^{b}	2.80^{b}	3.25^{a}	2.79^{b}	2.98^{a}
Total Non-EAA	3.82^{a}	2.77^{b}	3.33^{a}	3.98^{a}	2.75^{b}	3.52^{a}
E/T (%)	44.96^{b}	45.90^{b}	45.68^{b}	44.95^{b}	50.36^{a}	45.85^{b}

Table 4: Free amino acids content (g/100mL) of fresh Rayeb milk

Total Free Amino Acids

Unfortunately, the amounts of total FAA were lower in rice milk Rayeb than those detected in Rayeb made from cow milk. So, substitution of 50% rice milk with 50% cow milk increased the concentrations of total FAA in resulted Rayeb. On the other side, fortification of Rayeb milk with 4% honey slightly raised the levels of total FAA which may be attributed to the content of FAA in honey or to the stimulant effect of honey on lactic acid bacteria to degrade milk protein. Bogdanov et al. [28] cleared that honey contains roughly 0.5% proteins, mainly enzymes, and free amino acids. Chick *et al.* [29] stated that 5% (w/w) honey supported growth of *Str. thermophilus, L. acidophilus, L. bulgaricus*, or *Bifidobacterium* in nonfat dry milk.

Essential Amino Acids (EAA)

Like total free amino acids, values of essential amino acids (EAA) of rice milk Rayeb were lower than those of cow milk one. Despite this, the ratios of EAA to total FAA were higher in the former than that of the latter which grants rice milk special nutritional importance. Mixing of honey with milk used in Rayeb manufacturing slightly increased the amounts of EAA. Barka *et al.* [30] showed that rice contains a better balance of essential amino acids. Umadevi *et al.* [31] reported that rice protein, which comprises up to eight percent of the grain, has a special benefit as it has eight of the essential amino acids in delicately balanced proportions.

Nonessential Amino Acids (Non-EAA)

In the same trend of essential amino acid results, rice milk Rayeb possessed lower levels of nonessential amino acids (Non-EAA) than those determined in Rayeb made from cow milk. Preparation of Rayeb from cow and rice milk mixture (50:50) increased Non-EAA values as compared with those in rice milk Rayeb. Supplementation of Rayeb milk with honey slightly increased Non-EAA contents especially in that made from cow or mixed milk. Umadevi *et al.* [31] stated that the diet contains rice and milk, characterized by high nutrition and health values.

Antioxidants Activity of Rayeb Milk

Data presented in Table 5 summarize the antioxidants activity of fresh Rayeb milk made from cow or rice milk or their mixture with and without fortification of honey. Utilization of rice milk in Rayeb production increased the ratio of DPPH inhibition as compared with that made from cow's milk. Surprisingly, mixing of cow milk with rice milk raised the antioxidants activity levels of Rayeb to record the highest value. Sirirat and Jelena [32] reported that the increase of antioxidant activity of the rice milk-kefir may be correlated to phenolic compounds. Phenolic compounds are plant-based materials; phytochemicals. Due to their scavenging abilities on free radicals, rice milk-kefir might possess good antioxidant properties. These findings have suggested that rice milk-kefir is potential candidates for the role of useful natural antioxidant supplements for the human food.

Treatments	Antioxidant activity (DPPH inhibition %)
A	30.60^{e}
B	34.95^{d}
C	36.01^{c}
D	35.24^{c}
E	41.91^{b}
F	44.06^{a}

Table 5: Effect of using rice milk and addition honey on antioxidant activity of fresh Rayeb milk

As it is expected, incorporation of honey with Rayeb milk increased the values of antioxidants activity and the increase was more

pronounced in mixed milk Rayeb. The antioxidants activity levels raised by 15.16, 19.91 and 22.35% for treatments D, E and F respectively. The results of Erejuwa *et al.* [33] suggested that combination of honey with metformin or glibenclamide might offer additional antioxidant effect to these drugs. This might reduce oxidative stress-mediated damage in diabetic kidneys.

Changes in Microbial Counts of Rayeb Milk during Storage

To measure the health effect of bio-Rayeb milk, probiotic bacteria were determined in fresh samples and during the storage period. It could be noticed from Table 6 that the counts of *Str. thermophilus* and *L. acidophilus* significantly (P<0.05) increased in Rayeb made from rice milk. However, cow's milk Rayeb contained the lowest numbers of *Str. thermophilus*, but mixing cow milk with rice milk (50:50) highly raised their numbers in the resulted Rayeb. Add to that, the loss of survival rates of *Str. thermophilus* during storage lowered in mixed milk comparing with those observed in cow or rice milk Rayeb. Study of Subhasree *et al.* [34] supports the use of brown rice as an excellent substrate which contains the essential nutrients to support the growth of lactobacilli and can directly be used as substrates for fermentation of probiotic bacteria.

Supplementation of Rayeb milk with 4% honey significantly (P<0.05) increased the counts of *Str. thermophilus* and *L. acidophilus*. This increase was greater in Rayeb prepared from mixed milk which obtained the highest numbers and at the same time achieved the lowest loss of viability value during storage. Ratios of loss of viability for *Str. thermophilus* in samples A, B, C, D, E, and F were 41.67, 27.50, 25.58, 25.00, 22.22 and 19.15% respectively.

Regarding the counts of *Bifidobacterium* in different Rayeb milk treatments, samples made from cow and rice mixture scored the highest counts of the mentioned bacteria followed by samples prepared from rice milk and at the end of the order samples made from cow milk. The loss of viability rates during storage followed the opposite order. Charalampopoulos *et al.* [35] reported that rice is one of the most promising alternatives to milk due to its ability to support the growth of probiotic bacteria and their protective bile resistance effect. Rice, rice bran, and broths have been found to support the growth of probiotic bacteria.

Addition honey had a positive effect on the numbers of *Bifidobacterium* in fresh Rayeb and during storage. On the other side, loss of viability percentages of *Bifidobacterium* during storage considerably reduced in honey Rayeb samples. Loss of viability rates during 14 days of storage for treatments A, B, C, D, E, and F were 47.62, 25.81, 20.00, 40.00, 28.57 and 21.62% respectively. It has been established that different kinds of honey contain specific oligosaccharides, e.g. isomaltose and melezitose in New Zealand honey [35] and raffinose in Italian honey [36] and it is likely that one or more of these compounds would prove stimulatory to *Bifidobacterium spp.* [37]. *Haddadin et al.* [38] showed that adding honey to skim-milk increased the viable cell numbers of *B. infantis* and *L. acidophilus* because honey contains certain components that flavour the growth of these probiotic bacteria.

In different Rayeb milk samples, there were significant (p<0.05) lowering in viable counts of *Str. thermophilus, L. acidophilus*, and *Bifidobacterium*. The main metabolic products of carbohydrate fermentation by probiotics activity are organic acids substantiated by a drop in pH of the surrounding environment. This statement was approved by the study of McMaster *et al.* [39], who noted a great loss in viability of *Bifidobacterium* due to acidic injury, which justified by its lower survivability in fermented milk than in control without fermentation [40]. Kabeir *et al.* [41] reported that survivability of *B. longum* BB536 under refrigeration storage of fermented Sudanese Media beverages was not affected for a period of 2 weeks. While Alkalin *et al.* [42] noted a significant reduction of *B. longum* BB46 in yoghurt after only one week refrigeration. This indicates that the viability of *Bifidobacteriu*m in fermented products was dependent on the carrier type and pH of the fermented products during the storage.

In spite of decreased in viable count of bifidobacteria in all types of Rayeb milk during storage period, the count still above the number required to a presence in probiotic food which is at least 6 log cfu/ml fermented products, [43].

Properties	Treatments	Storage period (days)			Means±SD
		Fresh	7	14	
Streptococcus thermophilus (cfu x10^7/g)	A	36	28	21	28.33^{e}±2.0
	B	40	34	29	34.33^{d}±3.0
	C	43	37	32	37.33^{c}±2.0
	D	40	36	30	35.33^{d}±4.0
	E	45	39	35	39.67^{b}±5.0
	F	47	43	38	42.67^{a}±3.0
	Means±SD	41.83^{g}±3.0	36.17^{h}±2.0	30.83^{i}±4.0	
Lactobacillus acidophilus (cfu x10^5/g)	A	10	7	4	7.0^{d}±1.0
	B	11	7	5	7.7^{d}±2.0
	C	13	9	7	9.7^{c}±1.0
	D	16	13	9	12.7^{b}±3.0
	E	19	17	13	16.3^{a}±2.0
	F	21	18	15	18.0^{a}±4.0
	Means±SD	15.0^{g}±2.0	11.8^{h}±1.0	8.8^{i}±2.0	

Properties	Treatments	Storage period (days)			Means±SD
		Fresh	7	14	
Bifidobacterium (cfu x10^5/g)	A	21	17	11	16.3^{e}±2.0
	B	31	27	23	27.0^{c}±3.0
	C	35	32	28	31.7^{ab}±2.0
	D	30	25	18	24.3^{d}±4.0
	E	35	32	25	30.7^{b}±3.0
	F	37	33	29	33.0^{a}±2.0
	Means±SD	31.5^{g}±2.0	27.7^{h}±3.0	22.3^{i}±1.0	

^{abcde}Letters indicate significant differences between Rayeb milk treatments
^{ghi}Letters indicate significant differences between storage times
Table 6: Starter bacteria counts of Rayeb milk during cold storage

From the findings of Table 6, plus outcomes of FFA, FAA, and antioxidants activity, it can be said that using of probiotic culture in fermentation of cow and rice milk mixture contained 4% honey produced very health fermented dairy product.

Changes in Sensory Evaluation of Rayeb Milk during Storage

Fermentation processes have been utilized to improve the sensorial attributes and also to decrease the properties of undesirable compounds in products [44].

As can be seen from Table 7, results of organoleptic properties revealed that scores of color and appearance properties of Rayeb made from rice milk were higher than those of Rayeb prepared from cow or mixed milk. Of course, this due to the white bright color of rice milk which preferred for Egyptians in dairy products. Color and appearance attributes of Rayeb milk didn't change by the addition of honey. In contrast to results of color and appearance, scores of smell, taste, mouthfeel, texture and body of rice milk Rayeb were lower than those made from cow milk. The blending of cow milk with rice milk improved the mentioned previous properties for fresh Rayeb and during storage. These results are in line with those reported by Wongkhalaung and Boonyaratanakornkit [22], they reported that commercial yogurt was slightly preferred than rice-based yogurt in all categories tested (color, odor, flavor, texture, and acceptability) but the differences were not significant at 95% confidential level.

Properties	Treatments	Storage period (days)			Means±SD
		Fresh	7	14	
Color	A	9	9	8	8.67^{a}±1.0
	B	10	10	9	9.67^{a}±1.0
	C	9	9	9	9.00^{a}±2.0
	D	9	9	9	9.00^{a}±1.0
	E	10	10	9	9.67^{a}±2.0
	F	9	9	8	8.67^{a}±1.0
	Means±SD	9.33^{g}±1.0	9.33^{g}±1.0	8.67^{g}±2.0	
Appearance	A	9	9	8	8.67^{a}±2.0
	B	10	10	10	10.00^{a}±3.0
	C	9	9	9	9.00^{a}±1.0
	D	9	9	9	9.00^{a}±2.0
	E	10	10	10	10.00^{a}±2.0
	F	9	9	9	9.00^{a}±1.0
	Means±SD	9.33^{g}±2.0	9.33^{g}±2.0	9.17^{g}±1.0	
Smell	A	9	9	8	8.67^{ab}±1.0
	B	8	7	6	7.00^{b}±2.0
	C	9	9	8	8.67^{ab}±2.0
	D	9	9	9	9.00^{a}±3.0
	E	8	8	7	7.67^{ab}±2.0
	F	8	8	7	7.67^{ab}±1.0
	Means ± SD	8.5^{g}±1.0	8.3^{g}±2.0	7.5^{g}±2.0	

Properties	Treatments	Storage period (days)			Means±SD
		Fresh	7	14	
Taste	A	9	9	8	8.67^{ab}±1.0
	B	7	6	6	6.33^{c}±1.0
	C	8	8	7	7.67^{bc}±2.0
	D	10	10	9	9.67^{a}±1.0
	E	8	7	6	7.00^{bc}±3.0
	F	9	8	7	8.00^{abc}±2.0
	Means±SD	8.5^{g}±1.0	$8g^{h}$±2.0	7.17^{h}±2.0	
Mouth feel	A	9	9	8	8.67^{ab}±1.0
	B	7	7	6	6.67^{c}±2.0
	C	8	7	7	7.33^{bc}±3.0
	D	10	10	9	9.67^{a}±1.0
	E	8	8	7	7.67^{bc}±2.0
	F	9	9	8	8.67^{ab}±1.0
	Means±SD	8.5^{g}±1.0	8.3^{g}±2.0	7.5^{g}±3.0	
Texture & Body	A	8	8	7	7.67^{bc}±1.0
	B	6	6	5	5.67^{d}±2.0
	C	7	7	6	6.67^{cd}±2.0
	D	10	10	9	9.67^{a}±3.0
	E	8	8	7	7.67^{bc}±2.0
	F	9	9	8	8.67^{ab}±1.0
	Means±SD	8.0^{g}±2.0	8.0^{g}±1.0	7.0^{g}±2.0	

^{abcde}Letters indicate significant differences between Rayeb milk treatments

^{ghi}Letters indicate significant differences between storage times

Table 7: Effect of adding of rice milk and honey on sensory evaluation of Rayeb milk

Rayeb treatments with the inclusion of 4% honey gained the highest scores of smell, taste, mouthfeel, texture, and body which probably due to the sweet taste of honey which enhanced the mood of the panelists. The obtained results for samples enriched with honey are in agreement with the results of Sert *et al.* [45]. They found that sensory characteristic of yoghurt samples significantly changed according to honey addition. Taste intensity of yoghurt samples significantly increased according to honey addition. The odour and appearance values of yoghurt samples were not affected by honey addition and cold storage process. The consistency of honey incorporated samples was higher than the consistency of control.

Sample of Rayeb milk made from cow and rice milk mixture with 4% honey was preferred by the panelists as probiotic fermented dairy product.

Conclusion

Bio-Rayeb milk was successfully manufactured from mixtures of cow and rice milk (1:1) with adding 4% honey and using ABT-5 culture as a bio-starter. Blending rice milk with cow milk increased omega-6, omega-3, omega-9 acids and antioxidant activity in the produced Rayeb milk.

Rice milk and honey acted as prebiotic in Rayeb milk production where bifidobacteria counts increased in Rayeb sample made from cow milk, rice milk, and honey mixture. Incorporation of honey highly improved the sensory evaluation scores of bio-Rayeb milk.

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References

1. Prado FC, Parada JL, Pandey A, Soccol CR (2008) Trends in non-dairy probiotic beverages. Food Res Int J 41: 111-23.

2. Shin DH (1998) A yogurt like product development from rice by lactic acid bacteria. Korean J Food Sci and Technol 21: 686-90.

3. Lee CH, Min KC, Souane M, Chung MJ, Mathiasen TE, et al. (1992) Fermentation of prefermented and extruded rice flour by the lactic acid bacteria from Sikhae. Food Biotech 6: 239-55.

4. Zulu RM, Dillon VM, Owens JD (1997) Munkoyo beverage, a traditional Zambian fermented maize gruel using Rhynchosia root as amylase source. Int J Food Microbiol 34: 249-58.

5. Mok C, Han J, Kim YJ, Kim N, Kwon DY, et al. (1991) Risogurt, a mixture of lactic acid fermented rice and soybean protein: development and properties. Korean J Food Sci and Technol 23: 745-9.

6. Mok C, Han J, Nam YJ, Kim YJ (1993) Method for producing highly concentrated, lactic-acid fermented product utilizing unground grainy rice and improving qualities thereof by the secondary, enzymatic treatment at fermentation. USA.

7. Tominaga M, Sato K (1996) Lactic acid fermentation of saccharified solution from rice flour. J Food Sci 61:627-31.

8. Park JH, Song HK, Ahn JB, Ji GE, Mok C (1997) Rice fermentation by Korean amylolytic Bifidobacterium spp. Korean J Food Sci and Technol 29: 581-7.

9. Belewu MA, Abdulsalam KO, Belewu K, Belewu N (2013) Rice- Coconut Yoghurt: Preparation, Nutritional and Sensory Qualities. Asian J Agri and Rural Develo 3: 924-8.

10. AOAC Official Methods (2000) Association of Official Analytical Chemists. 17th Edn. Washington, USA.

11. Ling ER (1963) A Text-Book of Dairy Chemistry. 3rd Edn. London, England, UK.

12. Kosikowski FV (1978) Cheese and Fermented Milk Foods. 2nd Edn. New York, USA.

13. Olivera P, Mila J, Mladen M (2006) Chemical composition and antioxidant activity of essential oils of twelve spice plants. Croatica Chemica Acta J 79: 545-52.

14. Jahreis GJ, Fritsche MSC, Steinhart H (1997) Conjugated linoleic acid in milk fat: High variation depending on production system. Nutr Res 17: 1479- 84.

15. Walsh MK, Brown RJ (2000) Use of amino acid analysis for estimating the individual concentrations of proteins in mixtures. J Chromatography A 891: 355- 60.

16. Tharmaraj N, Shah NP (2003) Selective Enumeration of Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and Propionibacteria. J Dairy Sci 86: 2288-96.

17. Dinakar P, Mistry VV (1994) Growth and viability of Bifidobacterium bifidum in Cheddar cheese. J Dairy Sci 77: 2854-64.

18. Tunde-Akintunde TY, Souley A (2009) Effect of processing methods on quality of soymilk. Pakistan J Nut 8: 1156-8.

19. SAS (1991) SAS User's guide: statistics. SAS Inst Inc Cary NC, USA.

20. Duncan DB (1955) Multiple Range and Multiple F-test. Biometrics 11: 1-42.

21. Cakmakci S, Cetin B, Turgut T, Gurses M, Erdoğan A (2012) Probiotic properties, sensory qualities, and storage stability of probiotic banana yogurts. Turk J Vet Anim Sci 36: 231-7.

22. Wongkhalaung C, Boonyaratanakornkit M (2000) Development of a Yogurt-type Product from Saccharified Rice. Kasetsart J Nat Sci 34: 107-16.

23. El Tahir Limia HM (2015) Microbiological and physiochemical study on Bifidobacterium infantis 20088 fermented peanut milk and rice milk and their blends. Sudan: College of Graduate Studies, Sudan University of Science & Technology, Sudan.

24. Akalin AS, Gonc S, Unal G, Fenderya S (2007) Effects of fructoooligo-saccharide and whey protein concentrate on the viability of starter culture in reduced-fat probiotic yoghurt during storage. J Food Sci 72: 222-7.

25. Perezgonzalez JD (2008) Nutritional balance of rice milk. J Knowledge Advancement & Integration 87-90.

26. Lunn J, Theobald HE (2006) The health effects of dietary unsaturated fatty acids. British Nutrition Foundation Nutrition Bulletin 31: 178-224.

27. Mangels R, Messina V, Messina M (2011) The dietitian's guide to vegetarian diets: issues and applications. 3rd Edn. Sudbury, Canada.

28. Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for Nutrition and Health: a Review. Am J College Nutr 27: 677-89.

29. Chick H, Shin HS, Ustunol Z (2001) Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey. J Food Sci 66: 4878-86.

30. Barka MK, Mustafa S, Kharidah M, Suraini AA, Abdul MY (2004) A nutritious medida (sudanese cereal thin porridge) prepared by fermenting malted brown rice flour with Bifidobacterium longum BB 536. Malaysian J Nut 10: 183-193.

31. Umadevi M, Pushpa R, Sampathkumar KP, Bhowmik D (2012) Rice-Traditional Medicinal Plant in India. J Pharmacog Phytochem 1: 6-12.

32. Sirirat D, Jelena P (2010) Bacterial inhibition and antioxidant activity of kefir produced from Thai jasmine rice milk. Biotechnol 9: 332-7.

33. Erejuwa OO, Sulaiman SA, Wahab MS, Salam SKN, Md Salleh M et al. (2011) Comparison of Antioxidant Effects of Honey, Glibenclamide, Metformin, and Their Combinations in the Kidneys of Streptozotocin-Induced Diabetic Rats. Int J Mol Sci 12: 829-43.

34. Subhasree RS, Bhakyaraj R, Dinesh Babu P (2013) Evaluation of brown rice and germinated brown rice as an alternative substrate for probiotic food formulation using Lactobacillus spp. isolated from goat milk. Int Food Res J 20: 2967-71.

35. Charalampopoulos D, Wang R, Pandiella SS, Webb C (2002) Application of cereals and cereal components in functional foods: A review Int J Food Microbiol 79: 131-41.

36. Oddo LP, Piazza MG, Sabatini AG, Accorti M (1995) Characterization of unifloral honeys. Apidologie J 26: 453-65.

37. Itsaranuwat P, Al-Haddad KHS, Robinson RK (2003) The potential therapeutic benefits of consuming health-promoting fermented dairy products. Int J Dairy Technol 56: 203-10.

38. Haddadin MSY, Nazer I, Sara' Jamal A, Robinson RK (2007) Effect of honey on the growth and metabolism of two bacterial species of intestinal origin. Pakistan J Nut 6: 693-7.

39. McMaster LD, Kokott SA, Reid SJ, Abratt VR (2005) Use of traditional African fermented beverages as delivery vehicles for Bifidobacterium lactis DSM 10140. Int J Food Microbiol 102: 231- 7.

40. Ouwehand AC, Salminen SJ (1998) The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 8: 749 -58.

41. Kabeir BM, Abd Aziz S, Muhamed M, Yazid AM (2005) Growth of Bifidobacterium longum BB536 in Media (fermented cereal porridge) and their survival during storage. Let App Microbiol J l41: 125-31.

42. Akalin AS, Gönç S, Dinkçi N (2004) Liquid chromatographic determination of thiamin in dairy products. Int J Food Sci Nutr 55: 345-9.

43. Vinderola CG, Bailo N, Reinheimer JA Survival of probiotic microflora in Argentinean yoghurts during refrigeration storage. Food Res Int J 33: 97-102.

44. Kpodo KFM, Afoakwa EO, Amoa BB, Budu AS, Saalia FK (2014) Effect of Ingredient Variation on Microbial Acidification, Susceptibility to Syneresis, Water Holding Capacity and Viscosity of Soy-Peanut-Cow Milk Yoghurt. J Nutr Health Food Eng 1: 1-6.

45. Sert D, Akin N, Dertli E (2011) Effects of sunflower honey on the physicochemical, microbiological and sensory characteristics in set type yoghurt during refrigerated storage. Int J Dairy Technol 64: 99-107.