RESEARCH ARTICLE

Diabetes Management

Preparation of functional fermented dairy product containing high levels of omega-6, omega-9, antioxidants activity and probiotic

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ABSTRACT

Background and objective: The consumption of fermented milk was shown to have an inverse association with incident diabetes risk. Stirred yoghurt is one of the most popular fermented milk products in the world. In Arab countries, stirred yoghurt made using probiotic bacteria is usually called Rayeb milk. In this study, soy milk and honey were utilized to improve nutritional properties of Rayeb milk. Methodology: Rayeb milk were prepared from cow milk, soy milk or cow and soy milk mixture (1:1) with or without adding 4% honey and using ABT-5 culture. All samples were stored at 4°C for 14 days and tested when fresh and after 7 and 14 days. Results: Manufacture of Rayeb milk from cow and soy milk and 4% honey admixture increased the levels of unsaturated fatty acids. The values of omega-3, omega-6 fatty acids and antioxidants activity were considerably higher in Rayeb milk supplemented with soy milk and honey. *Bifidobacterium* population exceeded the recommended level of 106 cfu.g^{-1} of *Bifidobacteria* as a probiotic in Rayeb milk possessed soy milk and honey. Rayeb milk manufactured from cow and soy milk and honey mixture gained the highest scores of sensory evaluation. Conclusion: Functional Rayeb milk with high nutritional and health benefits could be successfully prepared from mixture of cow and soy milk (1:1) with adding 4% honey and using ABT-5 culture. The outcomes suggest that consumption of this functional Rayeb milk was associated with a reduce risk of diabetes.

Introduction

Dairy product intake has been correlated with a lower risk of type 2 diabetes in three meta-analyses of large prospective epidemiological studies [1]. Furthermore, using objective measures, evidence is emerging that dairy derived fatty acids may have a protective effect on the risk of diabetes [2]. Fermented dairy product intake was associated with an inverse risk of diabetes in a study of eight European countries in the European Prospective Investigation of Cancer (EPIC)-Interact study [3]. Cheese and fermented dairy products were shown to have an inverse association with glucose regulation measures but not with incident diabetes risk in a study of Danish adults [4].

On the other hand, Rayeb milk is a traditional fermented milk product popular in Arab world. Rayeb milk is traditionally manufactured from raw buffalo milk by spontaneously fermentation [5]. In a modern technique for Rayeb milk manufacture, especial cultures contained bifido and lactic-acid-producing bacteria are added to pasteurized milk at 40°C.

Soy foods have been part of the human diet for millennia, but more recently considerable attention has been given to the associated health benefits of soy. The hypocholesterolemic effect of soy is well-established which led to the regulatory approval of a health claim relating soy protein to a lowered risk of cardiovascular disease [6]. Functional soy milk

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KEYWORDS

- rayeb milk
- soy milk
- honey
- antioxidants activity
- omega-3
- omega-6
- *bifidobacteria*

is soy milk that has a lot of bioactive components and may help to improve health or reduce risk of diseases [7]. Fermenting soymilk by lactic acid bacteria greatly raises its health benefit. Because of greater anti-oxidative actions [8], fermented soy milk is considered healthier than pure soymilk. Fermented soy milk products range from yoghurt [9], bio-yoghurt [10], kefir [11] and fermented soy milk beverage [12].

Utilization of natural honey as food and medicine by mankind has been in existence from time immemorial. Number of oligosaccharides found in honey might play a role in the anti-diabetic impact of honey [13]. In dairy products industries, honey is usually used in production of fermented dairy foods such as yoghurt [14].

Using soy milk in Rayeb milk preparation was not previously studied. Therefore, the aim of this study was to develop innovative Rayeb milk made from mixture of cow milk, soy milk, honey and ABT-5 culture. The nutritional and healthy quality of bio-Rayeb milk was evaluated by means of chemical and microbiological analyses. Moreover, acceptability of the products to the consumer was studied by sensory attributes evaluation.

Materials and methods

■ Materials

Fresh cow's milk was obtained from Animal Production Research Institute, Agricultural Research Center, Egypt. Yellow soybean (*Glycine max* (L.) and honey were purchased from a local grocery in Damietta Governorate. ABT-5 culture which consists of *S. thermophilus, L. acidophilus* and *Bifidobacterium* BB-12 (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

Manufacture of Soymilk

Soymilk was prepared as described by Ikya *et al.* [15].

Manufacture of Rayeb milk

Six treatments of Rayeb milk were made from cow and soy milk and honey as follows:

A: Rayeb milk prepared from cow milk.

B: Rayeb milk prepared from soymilk.

C: Rayeb milk prepared from cow milk+soymilk (1:1).

D: Rayeb milk made from cow milk+4% honey.

E: Rayeb milk made from soymilk+4% honey.

F: Rayeb milk manufactured from cow milk+soymilk (1:1)+4% honey.

After pasteurization, cow milk was mixed with soy milk. Immediately, milk of all samples was cooled to 40°C, inoculated with cultures (0.1 g/L of milk mix), incubated at 40°C for fully coagulation, and stored at 4°C overnight. Once blended for five min and divided to three parts transferred to three jars which stored at 4°C for 14 days. Rayeb milk samples were tested when fresh and after 7 and 14 days of storage.

■ Chemical analysis

Total solids, fat, total nitrogen and ash contents of samples were measured according to AOAC [16]. Titratable acidity in terms of% lactic acid was measured 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 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). The methods of Ling [17] and Kosikowski [18] were used to determine Water Soluble Nitrogen (WSN) and Total Volatile Fatty Acids (TVFA) respectively. 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.* [19].

■ 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 [20]. The fatty acid composition of Rayeb milk was measured 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

The method of Walsh and Brown [21] was followed to measure the content of amino acid of fresh Rayeb milk.

■ Microbial analysis

Rayeb milk treatments were tested for *S. thermophilus* and *L. acidophilus* populations according to the method cleared by Tharmaraj and Shah [22]. The number of *Bifidobacteria* was detected according to Dinakar and Mistry [23].

■ Sensory properties judging

The sensory attributes of Rayeb milk treatments were measured by a panel of judges who were familiar with the product using the hedonic scale [24].

■ Statistical analysis

The obtained results were statistically analyzed using a software package [25] based on analysis of variance. One-factor Analysis of Variance (ANOVA) was carried out with SPSS software (SPSS Inc., Chicago, Illinois, USA) version 17. When F-test was significant, Least Significant Difference (LSD) was calculated according to Duncan [26] for the comparison between means. Significance was set at $p < .05$. The data presented, in the tables, are the mean of 3 experiments.

Results

■ Chemical composition of Rayeb milk manufacture from cow milk or soy milk

Using soy milk in Rayeb milk preparation lowered acidity and E_h and raised pH values as compared with that prepared from cow milk **(TABLE 1)**. The levels of acidity development during storage were lower in Rayeb manufactured from soymilk than that prepared from cow milk. In the same trend, TS, fat, ash and TVFA contents were low in soy milk Rayeb. On the contrary, levels of TN and WSN were higher in soy milk Rayeb

Table 1. Physicochemical properties of Rayeb milk.

Properties	Treatments	Storage period (days)			Means
		Fresh	7	14	
Acidity %	A	0.62	0.81	0.94	0.79^{a}
	B	0.59	0.76	0.86	0.74^{a}
	C	0.60	0.78	0.89	0.76^{a}
	D	0.70	0.91	1.06	0.89^{a}
	E	0.68	0.87	0.99	0.85^{a}
	F	0.68	0.88	1.01	0.86^{a}
	Means	0.65^{C}	0.84^{B}	0.96^{A}	
pH values	A	4.91	4.74	4.60	4.75^{ab}
	B	4.97	4.82	4.71	4.83^{a}
	C	4.93	4.77	4.65	4.78^{a}
	D	4.80	4.60	4.43	4.61^{b}
	E	4.85	4.68	4.52	4.68^{ab}
	F	4.84	4.66	4.49	4.66^{ab}
	Means	4.88^{A}	4.71^{B}	4.57^{C}	
E_h mV	A	136.4	155.7	165.8	152.63^{d}
	B	131.9	150.5	160.1	147.50^{f}
	C	134.1	152.7	161.9	149.57^{e}
	D	149.2	170.1	183.6	167.63^{a}
	E	145.6	164.2	175.3	161.70^{c}
	F	146.9	166.3	178.2	163.80^{b}
	Means	140.68^{C}	159.92^{B}	170.82^{A}	
Total solids (TS) %	A	13.41	13.48	13.45	13.45^{d}
	B	11.67	11.60	11.64	11.64^{f}
	C	12.60	12.56	12.58	12.58^{e}
	D	16.51	16.48	16.60	16.53^{a}
	E	14.83	14.90	14.86	14.86^{c}
	F	15.70	15.67	15.73	15.70^{b}
	Means	14.12^{A}	14.12^{A}	14.14^{A}	
Fat %	A	3.6	3.7	3.7	3.67a
	B	2.6	2.6	2.7	2.63^{c}
	C	3.2	3.2	3.1	3.17^{b}
	D	3.5	3.6	3.6	3.57^{a}
	E	2.4	2.3	2.4	2.37^{d}
	F	3.0	3.1	3.0	3.03^{b}
	Means	3.05^{A}	3.08^{A}	3.08^{A}	

Ash %	A	0.85	0.87	0.86	0.86^{ab}
	B	0.66	0.65	0.68	0.66^{c}
	C	0.77	0.74	0.75	0.75^{abc}
	D	0.90	0.92	0.88	0.90^{a}
	E	0.69	0.70	0.71	0.70^{bc}
	F	0.80	0.79	0.82	0.80^{abc}
	Means	0.78^{A}	0.78^{A}	0.78^{A}	
Total nitrogen (TN) %	A	0.520	0.535	0.531	0.53^{a}
	B	0.604	0.600	0.609	0.60^{a}
	C	0.573	0.580	0.576	0.58^{a}
	D	0.505	0.508	0.510	0.51^{a}
	E	0.591	0.590	0.595	0.59^{a}
	F	0.560	0.565	0.562	0.56^{a}
	Means	0.56^{A}	0.56^{A}	0.56^{A}	
Water soluble nitrogen (WSN) %	A	0.091	0.133	0.146	0.123^{a}
	B	0.113	0.168	0.184	0.155^{a}
	C	0.099	0.146	0.161	0.135^{a}
	D	0.097	0.142	0.160	0.133^{a}
	E	0.117	0.179	0.197	0.164^{a}
	F	0.105	0.160	0.178	0.148^{a}
	Means	0.10^{A}	0.15^{A}	0.17^{A}	
Total volatile fatty acids (TVFA)*	A	6.6	9.2	10.6	8.80^{b}
	B	5.2	7.4	8.6	7.07^{e}
	C	5.6	8.4	9.4	7.80^{d}
	D	7.3	10.2	11.9	9.80^{a}
	E	5.8	8.4	9.8	8.00^{c}
	F	6.5	9.2	11.0	8.90^{b}
	Means	6.17^{C}	8.80^{B}	10.22^{A}	

*Expressed as ml 0.1 NaOH 100 g^{-1} Rayeb milk; Significance was set at $p<0.05$.

comparing with control (sample A). Outcomes of Rayeb manufactured from cow and soy milk mixture (1:1) were intermediate between those of cow milk and soy milk Rayeb.

Addition 4% honey to Rayeb milk increased titratable acidity, E_h, total solids, ash, WSN and TVFA while decreased pH, fat and TN ratios values. Regardless of utilization soy milk or honey addition, acidity and E_h values of different Rayeb milk samples increased within storage period.

■ Free fatty acids content (FFA) of Rayeb milk

The content of FFA in fresh Rayeb milk was illustrated in **TABLE 2**.

■ Saturated and unsaturated fatty acids

Soymilk Rayeb contained the lowest levels of Saturated Fatty Acids (SFA) and the highest contents of Unsaturated Fatty Acids (USFA) as compared with control. Mixing 50% soy milk with 50% cow milk lowered SFA and raised USFA of Rayeb than those of cow milk one. Generally, SFA concentrations were higher than USFA for treatments A and D (cow milk). In samples B, C, E and F the opposite trend was noticed. Adding 4% honey to milk utilized in Rayeb preparation also decreased SFA and increased USFA values.

■ Monounsaturated (MUSFA) and polyunsaturated fatty acids (PUSFA) fatty acids

The total monounsaturated fatty acids levels of the soymilk Rayeb were 25.86 and 27.43% (samples A and F respectively) that were lower than those of Rayeb manufactured from cow milk. As a consequence, mixing of 50% soymilk with cow milk lowered the levels of MUSFA in the Rayeb resulted.

The MUSFA and PUSFA values of Rayeb samples contained 4% honey were higher than those plain Rayeb. The levels of MUSFA were higher than those of PUSFA in various Rayeb treatments except for samples B and E (soymilk) which possessed the high content from the later than the former.

The concentrations of most important essential fatty acids for human health–linoleic acid (omega-6) and α-linolenic acid (omega-3)–were considerably higher in soymilk Rayeb than those determined in Rayeb made from cow milk. Thus, blending of soy milk with cow milk greatly compensated the shortage of these acids in cow milk Rayeb. Concerning to oleic acid (omega-9), Rayeb manufactured from mixture of cow milk and soymilk contained lower values than that of cow milk Rayeb. Mixing honey with Rayeb milk

Table 2. Effect of using soy milk and addition honey on 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	0.21	0.39	0.30	0.15	0.19
Capric	10:0	2.33	1.11	1.98	2.04	0.95	1.89
Undecanoic	11:0	0.20	-	0.15	0.11	-	-
Lauric	12:0	2.87	1.54	2.14	2.32	0.74	2.90
Tridecanoic	13:0	0.27	-	0.20	0.15	-	-
Myristic	14:0	10.1	2.56	6.83	9.54	1.70	4.52
Pentadecanoic	15:0	3.18	0.85	1.51	3.03	0.62	1.42
Palmitic	16:0	27.80	16.00	21.60	27.30	15.14	21.64
Heptadecanoic	17:0	2.98	-	1.45	2.86	-	1.58
Stearic	18:0	10.0	5.12	7.33	10.10	4.94	6.95
Arachidic	20:0	0.10	0.34	0.30	0.10	0.33	0.25
Behenic acid	22:0	0.28	0.36	0.38	0.16	0.34	0.22
Total		60.56	28.09	44.26	58.01	24.91	41.56
Unsaturated fatty acids (USFA) %							
	12:1 ω5	0.42	0.62	0.37	0.48	0.65	0.50
5-Tetradecenoic (phytosteric)	14:1 ω5	0.48	-	0.22	0.52	-	0.16
	14:1 ω7	0.35	0.41	0.38	0.40	0.48	0.42
Myristioleic acid	14:1 ω9	0.31	0.59	0.41	0.25	0.61	0.40
	16:1 ω5	0.17	-	0.15	0.20	-	0.15
Palmitioleic	16:1 ω7	2.51	0.37	1.16	2.90	0.40	1.28
	16:2 ω4	0.29	-	0.20	0.33	-	0.15
Hexagonic	16:3 ω4	0.58	-	0.30	0.51	-	0.29
	18:1 ω4	0.14	-	-	0.15	-	-
Octadecosaenoic	18:1 ω5	0.44	-	0.22	0.49	0.18	0.23
Vaccienic	18:1 ω7	1.09	1.17	1.11	1.66	2.04	1.81
Oleic	18:1 ω9	27.21	22.70	24.20	27.98	23.07	25.16
	18:2 ω4	0.69	-	0.22	0.55	-	0.20
	18:2 ω5	0.40	-	0.17	0.45	-	0.15
Linoleic	18:2 ω6	1.94	40.70	22.40	2.44	41.93	23.10
	18:2 ω7	0.25	-	0.15	0.26	-	-
α-Linolenic	18:3 ω3	0.71	5.02	3.21	0.81	5.38	3.76
	18:3 ω4	-	-	0.10	0.10	-	-
Gamma linolenic	18:3 ω6	-	-	-	-	-	0.10
Octadecatetraenoic	18:4 ω3	0.39	-	0.25	0.60	-	-
Gadoleic acid	20:1 ω9	-	-	0.11	-	-	-
Eicosaenoic	20:1 ω11		-	-	0.10	-	-
Eicosatrienoic	20:3 ω6	-	-	-	0.12	-	0.11
Total	-	38.37	71.58	55.33	41.30	74.74	57.97
Total MUSFA	-	33.12	25.86	28.22	35.13	27.43	30.11
Total PUSFA	-	5.25	45.72	27.11	6.17	47.31	27.86
Non identified fatty acid	-	1.07	0.33	0.41	0.69	0.35	0.47

SFA: Saturated Fatty Acids; USFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids (C:1); PUSFA: Polyunsaturated Fatty Acids (C:2+C:3).

slightly increased oleic, linoleic and α-linolenic acids.

■ Free amino acids content (FAA) of Rayeb milk

Data in **TABLE 3** clear the levels of FAA in fresh Rayeb milk samples.

■ Total free amino acids

Soy milk Rayeb had little low content of total amino acids as compared with that of control. Therefore, blending of 50% soy milk with cow milk (sample C) slightly lowered the contents of amino acids in the produced Rayeb. Fortification of Rayeb milk with 4% honey

increased the total free amino acids content. In various Rayeb samples, glutamic and aspartic acids were the highest levels of total free amino acids. Glutamic acid is responsible for protection from cardiovascular diseases. On the contrary, methionine and cystine acids had the lowest concentrations of total amino acids.

■ Essential Amino Acids (EAA)

Mixing 50% soy milk with 50% cow milk slightly lowered the levels of the essential amino acids in Rayeb milk. Rayeb prepared from cow milk had the highest contents of threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine and lysine. Essential amino acids values slightly raised in honey Rayeb. In various Rayeb samples, the major essential amino acid was leucine followed by lysine. Methionine content was the lowest.

■ Non-Essential Amino Acids (Non-EAA)

Contrary to essential amino acid values, the concentrations of non-essential amino acids were higher in soy milk Rayeb treatments than those of cow milk samples. Mixing soy milk with cow milk increased the amounts of nonessential amino acids by 3.93 and 6.03% for treatments C and F respectively. The soy milk Rayeb, however, is higher in aspartic, glutamic, glycine, alanine and arginine but lower in some of the other nonessential amino acids such as serine, proline, tyrosine and cystine.

Like increasing of essential amino acids content as a result of honey addition to Rayeb milk, the values of nonessential amino acid also increased.

■ Antioxidants activity of Rayeb milk

Ratios of DPPH inhibition as indicator of antioxidant activity were greatly higher in fresh soy milk Rayeb than those measured in Rayeb made from cow milk **(TABLE 4)**. Rayeb prepared from cow and soy milk mixtures possessed higher

Table 3. Effect of using soy milk and addition honey on free amino acids content (g/100mL) of fresh Rayeb milk.

Amino acids	Treatments					
	A	B	C	D	E	F
Aspartic (ASP)	0.60	0.69	0.66	0.64	0.77	0.72
Threonine (THR)	0.42	0.37	0.40	0.45	0.39	0.42
Serine (SER)	0.65	0.59	0.66	0.70	0.63	0.68
Glutamic acid (GLU)	0.84	0.97	0.86	0.87	1.03	0.95
Proline (PRO)	0.71	0.64	0.70	0.72	0.65	0.70
Glycine (GLY)	0.09	0.12	0.10	0.09	0.18	0.12
Alanine (ALA)	0.22	0.27	0.21	0.23	0.32	0.24
Valine (VAL)	0.47	0.43	0.46	0.49	0.46	0.45
Methionine	0.13	0.09	0.10	0.15	0.10	0.11
Isoleucine (ILE)	0.29	0.25	0.23	0.30	0.30	0.28
Leucine (LEU)	0.58	0.50	0.57	0.60	0.52	0.56
Tyrosine (TYR)	0.30	0.24	0.26	0.30	0.28	0.28
Phenylalanine (PHE)	0.45	0.37	0.40	0.47	0.38	0.44
Histidine (HIS)	0.28	0.23	0.27	0.28	0.24	0.25
Lysine (LYS)	0.50	0.40	0.40	0.51	0.45	0.44
Arginine (ARG)	0.30	0.47	0.43	0.30	0.51	0.42
Cystine (CYS)	0.11	0.09	0.09	0.13	0.10	0.11
Total amino acids	6.94	6.72	6.80	7.23	7.31	7.17
Total EAA	3.12	2.64	2.83	3.25	2.84	2.95
Total Non-EAA	3.82	4.08	3.97	3.98	4.47	4.22
E/T (%)	44.96	39.28	41.62	44.95	38.85	41.14

Table 4. Antioxidant activity of fresh Rayeb milk.

Treatments	Antioxidant activity (DPPH inhibition %)
A	30.60^{f}
B	39.6^{d}
C	41.51^{c}
D	35.24^{e}
E	47.35^{b}
F	50.11^{a}

$^{a\text{-}f}$Letters indicate significant differences between treatments. Significance was set at $p < 0.05$.

antioxidant activity than that of cow milk Rayeb but lower than that of soymilk one. Fortification of Rayeb milk with honey considerably increased the antioxidant activity. The highest increasing was observed in Rayeb made from cow and soy milk mixture.

Changes in microbial counts of Rayeb milk during storage

Utilization of mixed milk (50% cow milk with 50% soy milk) in Rayeb milk manufacture increased *S. thermophilus* and *L. acidophilus* counts in fresh product and through storage **(TABLE 5)**. Rayeb made from cow milk had the lowest counts of these microorganisms. Loss of survival rates of *S. thermophilus* through storage were low for mixed milk Rayeb while they doubled in Rayeb made from cow milk.

Numbers of *S. thermophilus* and *L. acidophilus* were significantly higher in Rayeb milk supplemented with honey. The opposite trend was noticed for loss of viability. Values of loss of viability for *S. thermophilus* in samples A, B, C, D, E, and F were 41.67, 28.20, 21.05, 25.00, 22.22, 20.93% respectively.

Because of high values of acidity and acidity development within storage for cow milk Rayeb, counts of *Bifidobacterium* were higher in Rayeb made from soy milk individually or mixed with cow milk (50:50) than those of cow milk Rayeb. Furthermore, losses of viability levels of *Bifidobacterium* were low in soy milk treatments comparing with those of cow milk samples. Losses of viability levels of samples A, B and C were 47.62, 38.46 and 35.71% respectively.

However slight increasing of acidity content of honey treatments which have a negative effect on probiotic cultures as low acid tolerance, *Bifidobacteria* counts of Rayeb samples contained honey were higher than those of control which may be caused by oligosaccharides presence in honey.

Whilst storage time progressive, counts of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* reduced in various Rayeb milk treatments.

Changes in sensory evaluation of Rayeb milk during storage

Influence of using soy milk and adding honey on different sensory properties of Rayeb milk was illustrated in **TABLE 6**. However, Rayeb milk made from cow milk individually or mixed with soy milk (samples A and C respectively) gained high scores of color and appearance (9). Color and appearance of Rayeb contained 4% honey were found to be comparable to those of Rayeb samples manufactured without incorporation of honey. Scores of color for fresh samples A and D were 9 and 9 respectively.

Table 5. Starter bacteria counts of Rayeb milk during cold storage.

Bacteria	Treatments	Storage period (days)			Means
		Fresh	7	14	
Streptococcus thermophilus (cfu × 10^7/g)	A	36	28	21	28.33^{d}
	B	39	34	28	33.67^{c}
	C	38	35	30	34.33^{c}
	D	40	36	30	35.33^{c}
	E	45	42	35	40.67^{a}
	F	43	39	34	38.67^{b}
	Means	**40.17^{A}**	**35.67^{B}**	**29.67^{C}**	-
Lactobacillus acidophilus (cfu × 10^5/g)	A	10	7	4	7.00^{e}
	B	15	13	9	12.33^{d}
	C	17	16	11	14.67^{c}
	D	16	13	9	12.67^{d}
	E	20	17	13	16.67^{b}
	F	21	20	15	18.67^{a}
	Means	**16.50^{A}**	**14.30^{B}**	**10.17^{C}**	-
Bifidobacterium (cfu × 10^5/g)	A	21	17	11	16.33^{d}
	B	26	23	16	21.67^{c}
	C	28	24	18	23.33^{bc}
	D	30	25	18	24.33^{b}
	E	37	33	26	32.00^{a}
	F	36	33	27	32.00^{a}
	Means	**29.67^{A}**	**25.83^{B}**	**19.33^{C}**	-

$^{a-e}$Letters indicate significant differences between Rayeb milk treatments; $^{A-D}$Letters indicate significant differences between storage times; Significance was set at $p<0.05$.

Table 6. Effect of adding of soy milk and honey on sensory evaluation of Rayeb milk.

Properties	Treatments	Storage period (days)			Means
		Fresh	7	14	
Color	A	9	9	8	8.67^{a}
	B	8	8	7	7.67^{a}
	C	9	9	9	9.00^{a}
	D	9	9	9	9.00^{a}
	E	8	8	8	8.00^{a}
	F	9	9	8	8.67^{a}
	Means	**8.67^{A}**	**8.67^{A}**	**8.17^{A}**	-
Appearance	A	9	9	8	8.67^{a}
	B	8	8	8	8.00^{a}
	C	9	9	8	8.67^{a}
	D	9	9	9	9.00^{a}
	E	8	8	7	7.67^{a}
	F	9	9	9	9.00^{a}
	Means	**8.67^{A}**	**8.67^{A}**	**8.17^{A}**	-
Smell	A	9	9	8	8.67^{a}
	B	7	7	6	6.67^{b}
	C	8	8	7	7.67^{ab}
	D	9	9	9	9.00^{a}
	E	8	8	7	7.67^{ab}
	F	9	9	9	9.00^{a}
	Means	**8.33^{A}**	**8.33^{A}**	**7.67^{A}**	-
Taste	A	9	9	8	8.67^{abc}
	B	6	6	5	5.67^{d}
	C	8	8	7	7.67^{c}
	D	10	10	9	9.67^{ab}
	E	8	8	8	8.00^{bc}
	F	10	10	10	10.00^{a}
	Means	**8.50^{A}**	**8.50^{A}**	**7.83^{A}**	-
Mouth feel	A	9	9	8	8.67^{ab}
	B	6	6	5	5.67^{d}
	C	8	8	7	7.67^{bc}
	D	10	10	9	9.67^{a}
	E	7	7	6	6.67^{cd}
	F	10	10	10	10.00^{a}
	Means	**8.33^{A}**	**8.33^{A}**	**7.50^{A}**	-
Texture & Body	A	8	8	7	7.67^{b}
	B	8	8	7	7.67^{b}
	C	8	8	7	7.67^{b}
	D	10	10	9	9.67^{a}
	E	10	9	9	9.33^{ab}
	F	10	10	9	9.67^{a}
	Means	**9.00^{A}**	**8.83^{A}**	**8.00^{A}**	-

$^{a-e}$Letters indicate significant differences between Rayeb milk treatments; $^{A-D}$Letters indicate significant differences between storage times; Significance was set at $p<0.05$.

The smell, taste and mouth feel evaluation tests of Rayeb milk made from soy milk gained the lowest scores as compared with that made from cow milk. Blending cow milk with soymilk markedly improved the above-mentioned tests. The smell, taste and mouth feel evaluation tests of Rayeb supplemented with honey gained the highest scores at zero time and during the storage period.

However, soy milk Rayeb failed to record high scores of color, appearance, smell, taste and mouth feel but the texture and body scores of it were similar to those of cow milk Rayeb. On the other hand, addition honey not only improved smell, taste and mouth feels of Rayeb but also body and texture.

Discussion

Utilization of soymilk in Rayeb milk production caused some changes in the chemical composition which attributed to various reasons. Because soy milk is free from lactose, the basic source of lactic acid, the acidity contents of Rayeb milk made from it reduced comparing with cow milk Rayeb. Soy milk Rayeb also characterized with low fat and TVFA values which may be attributed to

the low fat content of soy milk. Increasing of protein concentrations of soy milk raised the total nitrogen and WSN contents of Rayeb milk. Similar results are reported by Osman and Abdel Razig [9] who showed that the titratable acidity of yoghurt made from soy milk was lower than that of yoghurt made from soy and cow milk mixture (1:2).

Fructooligosaccharides found in honey increased the acidity values in fermented dairy products [27]. So, the acidity of honey Rayeb milk was slightly high. These results agreed with Abd El-Salam *et al.* [28] and disagreed with Varga [29]. Abd El-Salam *et al.* [28] cleared that the pH and titratable acidity of yoghurt supplemented with honey affected slightly compared with that supplemented with *Bifidobacterium lactis* Bb.12. whereas Varga *et al.* [29] reported that honey has the ability to decrease solutions sourness. This property might serve to increase consumer acceptability to acidic products such as yoghurt.

The stimulation effect of honey Fructooligosaccharides on *Bifidobacteria* [30] may be caused more protein and lipid degradations which increased the WSN and TVFA values of Rayeb milk. In supplementary, Chick *et al.* [31] stated that the organic acids production was improved when *Bifidobacteria* were grown in the presence of honey, where various oligosaccharides found in honey may be responsible for enhancing organic acids production by *Bifidobacteria*.

The activity of the starter culture especially within the first week of storage period increased the acidity values in all Rayeb milk treatments. These results agreed with Hamad *et al.* [32] who found that a significant increase in acidity (percent lactic acid) and a decrease in pH were noticed in Rayeb during the storage period.

Saturated fatty acids of fresh Rayeb milk reduced by 26.91% while USFA increased by 44.20% when 50% soymilk was mixed with cow milk (treatment C). Nurliyani *et al.* [33] showed that replacement of 50% of goat milk with soy milk in kefir production can reduce the levels of caproic, heptadecanoic and behenic significantly ($p<0.05$), whereas the substitution of 25% goat milk with soy milk in kefir fermentation can lower the pentadecanoic. Substitution of 50% goat milk with soy milk in kefir fermentation can increase oleic acid concentration.

Unsaturated fatty acids play an important role in human nutrition and health. The fatty acid profile of soymilk, high in unsaturated fatty acids and low in saturated fatty acids, contributes to cholesterol lowering and hence coronary heart disease risk reduction by increasing the High-Density Lipoprotein (HDL) in blood. Also, epidemiological studies indicated the reducing effect of probiotic yoghurt on total cholesterol and LDL levels and the raising effect on HDL [34].

The reduction values of MUSFA of Rayeb milk by adding soymilk were 14.79 and 14.29% for treatments C and F respectively. On the greatly contrary, the levels of PUSFA were very higher in soymilk Rayeb samples than those of other treatments. Replacement of saturated fat with monounsaturated and polyunsaturated fat (especially ω-6 PUFA) decreases the plasma concentration of total and LDL cholesterol [35]. Blending soy milk with cow milk greatly compensated the shortage of linoleic acid (omega-6) and α-linolenic acid (omega-3) in cow milk Rayeb. Generally, outcomes of PUSFA indicate that incorporation of 50% soymilk with 50% cow milk produces very healthy Rayeb because of their content of omega-3, 6 and 9. Rustan and Drevon [36] showed that the essential ω-3 and ω-6 fatty acids are important for fetal growth, in particular for the central nervous system, affecting visual acuity as well as cognitive function. On the other hand, Iwase *et al.* [37] cleared the anti-diabetic effects of omega-3 polyunsaturated fatty acids (n-3 PUFAs) and the role of n-3 PUFAs in the treatment of diabetes mellitus.

The total free amino acids content is slightly affected by the type of milk or adding honey. In agreement with these findings, Nurliyani *et al.* [33] found that goat milk kefir and kefir made from 50% goat milk and 50% soymilk mixture showed not significantly different in amino acid composition.

Treatments of soy milk Rayeb had the lowest amounts of essential amino acids to total amino acids (E/T) among the samples. This may be explained on the basis of a difference in fat content between samples **(TABLE 1)**. Bao *et al.* [38] showed that there was clearly positive relationship between the amount of fat in the milk base and the total FAA contents after fermentation with *L. casei* GBHM-21. With the increase in fat concentration, the concentration of some FAAs, such as Glu, Leu, Trp, Phe, and Lys, were significantly increased. The different changes in the levels of various FAAs could be

attributed to the improved proteolysis and FAA catabolism for *L. casei* GBHM-21 influenced by the increase of fat.

Samples of Rayeb prepared from soymilk had low content of sulfur amino acids (methionine and cysteine), whereas cow milk Rayeb was richer in these amino acids. Chaiwanon *et al.* [39] cleared that the protein content was similar in soymilk and cow milk with close amino acid make up, as for the nine essential amino acids in protein necessary for sustaining life, cow milk and soymilk contain nearly identical amounts except sulfur-containing amino acids which are deficient in soymilk.

Essential amino acids values of Rayeb milk contained honey were slightly higher than control which may be attributed to the activity of bacteria starter by honey components. This special character of honey Rayeb increased the priority for human nutrition.

Results reflect that adding soy milk to Rayeb possessed positive effect on the antioxidant activity which may be due to the protein content of soy bean. Liu *et al.* [40] reported the effect of milk-kefir and soy milk-kefir on the scavenging activity of DPPH radical displayed significant activity than milk and soy milk. They suggested that some components of antioxidant presented in the kefir grains were transferred to milk and soy milk during fermentation. Chen *et al.* [41] reported that the increased scavenging activity of fermented milk may be related to milk protein and soybean protein-derived peptide.

Great antioxidant activity was detected in honey Rayeb milk. This may be due to the phenolic compounds of honey which have high antioxidant activity. Aljadi and Kamaruddin [42] showed that the antioxidant capacity of honey and propolis is due mainly to the phenolic compounds and flavonoids. There is a high degree of correlation between these substances and the antioxidant capacity of honey, although a synergic action between several compounds cannot be discounted. As it is known, foods with high antioxidant activity have negative effects on diabetes. The results of Schumacher *et al.* [43] suggest that the aqueous extract of *Eugenia uniflora* leaves presents antioxidant activity and high total phenols, which were used as a type 1 diabetes mellitus (DM-1) treatment in non-obese diabetic mice. The chronic consumption of aqueous extract reduces the inflammatory infiltrate index in pancreatic islets, maintaining serum insulin levels and hepatic glutathione, and reducing serum lipid peroxidation as well as the risk for diabetes.

The populations of *S. thermophilus* and *L. acidophilus* increased in soy milk Rayeb. This indicates that the components of soy milk activated these bacteria. In supplementary, Sumarna [44] showed that *S. thermophilus,* 001 grew better than *L. casei subsp rhamnosus* FNCC, 098, *L. casei subsp rhamnosus* FNCC, 099*,* and *L. delbrueckii subsp. bulgaricus* FNCC, 0045 and produced higher organic acid than the latter during fermentation of soy milk.

In bio-dairy products special attention should be given to avoid over acidification since this could affect the stability of *Bifidobacteria* during storage [45]. Activation of *Bifidobacteria* in Rayeb samples contained soy milk is not only attributed to low acidity but also to oligosaccharides presence in soy milk. This means that soy milk acted as prebiotic of *Bifidobacteria*. These results are in agreement with those of Scalabrini *et al.* [46] who cleared that soymilk is a good medium for growing *Bifidobacterium* because it contains oligosaccharides that are fermented by most of the strains belonging to this genus.

Bifidobacteria counts increased in honey Rayeb milk treatments which may be caused by oligosaccharides presence in honey. Oligosaccharides were found to enhance the viability of starter culture as prebiotics [47]. Sanz *et al.* [48] showed that honey has been shown to enhance growth, activity of *Bifidobacteria* in fermented dairy food.

The counts of starter bacteria lowered during storage in all Rayeb treatments. This decrease could be evidently attributed to the increase in titratable acidity which controlled the rate of bacterial growth or acted as bactericidal agent [49]. In spite of this decreasing, the *Bifidobacteria* counts remained above 10^6 cfu.g^{-1} in various Rayeb milk treatments during the storage period. This means that the viability of strains after the storage period was sufficient to yield numbers of beneficial organisms that were higher than the accepted threshold (10^6 cfu.g^{-1}) for a probiotic effect [50].

From the results of **TABLE 5**, it appears that the incorporation of probiotics into the mixture of cow milk, soy milk and honey seems to offer additional health-promoting features for diabetic patients. Ostadrahimi *et al.* [51] showed that probiotic fermented milk containing *Lactobacillus casei*, *Lactobacillus*

acidophilus and *Bifidobacteria* can be useful as a complementary or adjuvant therapy in the treatment of diabetes. On the other hand, honey is more tolerable than most common sugars or sweeteners in healthy subjects or patients with impaired glucose tolerance or diabetes mellitus. In animal models of diabetes, there is more compelling evidence in support of honey as a novel anti-diabetic agent [13].

All Rayeb milk samples failed to obtain the greatest score of color and appearance (10). This may be due to the light yellow color which is not preferred for a majority of Egyptian consumers unlike bright white of buffalo milk. Generally, mixing 50% soy milk with 50% cow milk slightly decreased color and appearance grades of the produced Rayeb as compared with that made from 100% cow milk. This is in close agreement with the report of Osman and Abdel Razig [9] who reported that yoghurt sample made from soymilk and cow milk (1:2) significantly ($p<0.05$) secured the best appearance. Samples made from soymilk and cow milk (1:1) or (2:1) were in an intermediate position (3.37 and 2.86, respectively). The worst (2.68) recorded by sample made from soymilk (100%).

The smell, taste and mouth feel attributes of soy milk Rayeb obtained the lowest scores. A beany flavor of soy milk was the main reason for this decreasing. Adding honey improved the smell, taste and mouth feel properties of Rayeb milk because the sweet taste of honey which is preferable for many consumers. The texture and body of Rayeb milk were also improved by addition honey. This is probably attributed to the increase of total solids which improved the viscosity or Rayeb milk. These results are in agreement with those of Riazi and Ziar [52] who stated that as for sensory properties; the product formulation with the highest concentration of honey (that is, 10% w/v) was too sweet and was evaluated as strong in honey flavour. However, the yoghurt samples containing 5% (w/v) of honey were found to have optimum sweetness. The points allocated for colour, body-texture, and taste showed that an increase in honey content brought about an improvement in the texture, flavour and aroma of the products ($P<0.05$). Also, they cleared that the addition of honey had a good effect on sensory properties of fermented milk with *Bifidobacteria* ($P<0.05$), and a particular noticeable yoghurt or probiotic flavour was found.

Conclusion

It can be concluded that blending 50% soymilk with 50% cow milk and adding 4% honey with using of ABT culture produced bio-Rayeb with high nutritional benefits. This fermented dairy product contained high levels of unsaturated fatty acids and antioxidant activity. The recommended level of 10^7 cfu.g^{-1} of *Bifidobacteria* as a probiotic was exceeded for bio-Rayeb. These features have special importance for diabetic patients.

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Conflict of interest

The authors declare that there is no conflict of interest.

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