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Effect of Using Vegetarian Milk and Adding Different Sweeteners on Probiotic Activity of Rayeb Milk

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Abstract
Rayeb milk (stirred yogurt made using probiotic culture) is one of the most popular functional fermented milk products in Middle East regions. To create novel bio-Rayeb milk, the effects of using mixtures of cow milk with oat milk, barley milk or sesame milk (50%:50%) sweetened with 5% sucrose, 5% honey, 2.5% fructose, or 1.5% sorbitol on the activity of starter bacteria (*S. thermophilus, L. acidophilus* and *Bifidobacterium*) were studied. The obtained results showed that the populations of the above mentioned bacteria were higher in Rayeb milk made from oat milk, barley milk or sesame milk than those detected in Rayeb made from cow milk. Also, the numbers of starter bacteria increased in Rayeb prepared using mixtures of cow milk with oat milk, barley milk or sesame milk. Adding the sweeteners especially fructose and honey to mixed milk led to pronounced increasing in starter bacteria populations of Rayeb milk. Utilization of vegetarian milk individually or mixed with cow milk as well as addition sweeteners lowered the loss of viability levels of *S. thermophilus, L. acidophilus* and *Bifidobacterium* during the storage period of bio-Rayeb milk.

1. Introduction

Probiotics, as non-digestible substances, have an important physiological effect on the host by provoking the growth or activity of the limited indigenous bacteria. Species of *Lactobacillus* and *Bifidobacterium* are the most frequently utilized probiotics [1]. Probiotics are considered as living drugs that can reduce antibiotic consumption and increase human health development. The application of probiotics in preventing and managing gastrointestinal (GI) disorders has gained considerable interest over the past two decades. The consumption of these products is increasing worldwide because probiotics are generally regarded as safe. Now probiotics may be used as anti-carcinogenic, anti-diabetic, anti-allergic and anti-inflammatory [2].

Rayeb milk which is also called Laban rayeb is one of the most produced and consumed fermented dairy products in Egypt and Arab countries. In rural areas, the traditional method of Rayeb milk manufacture applies continues natural fermentation. Raw whole buffalo's, cow's or goat's milk is poured into earthenware pots called mattered and left undisturbed at 25-30°C. After two days, the top aggregated cream layer is removed and used in butter making whereas the remaining sour milk (partially skimmed milk) is warmed to coagulate and consumed as Rayeb milk. Now, in specialized dairy plants in Arab world, Rayeb milk is made from pasteurized cow's milk with using probiotic cultures, resembling bio-stirred yoghurt in the developed countries.

On the other side, a growing number of consumers opt for vegetarian milk (plant based milk substitutes) for medical reasons or as a lifestyle choice. Vegetarian milk is often perceived as healthy, possibly due to negative perceptions about the nutritional properties of cow's milk. Also, vegetarian milk can be fermented to produce dairy free yoghurt type products while rendering the raw material into a more palatable form. For example, the levels of hexanal responsible for the undesired beany flavour in peanut milk were efficiently reduced with fermentation [3]. In order to produce fermented products, the starter cultures must be able to grow and dominate the microflora in the plant medium and produce a desired texture. Lactic acid bacteria have been used for cereal fermentations for centuries and many cereals known to support their growth [4]. Therefore, the objective of this work was to study the impact of adding different types of sweeteners to cow, oat, barley, and sesame milk on the counts, activity, and loss of viability of *S. thermophilus, L. acidophilus* and *Bifidobacterium* during storage of Rayeb milk.

2. Materials and Methods

2.1. Materials

Fresh cow's milk was obtained from El-Serw Animal Production Research Station, Animal Production Research Institute, Agriculture Research Center. Sugar, honey, rolled oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and sesame (*Sesamum indicum*) were purchased from a local grocery in Damiette Governorate. Fructose and sorbitol were obtained from El-Gomhouria Chemical Company, Egypt. ABT-5 culture which consists of *S. thermophilus*, *L. acidophilus* + *Bifidobacterium* was obtained d from Chr. Hansen's Lab A/S Copenhagen, Denmark. Starter cultures were in freeze-dried direct-to-vat set form and stored at –18°C until used.

2.2. Methods

2.2.1. Preparation of Oat Milk

Rolled oats were soaked in water (1:1) at 75°C for 10 minutes. Then the soaked oats and water were blended in a blender at high speed for 5 min with adding water again to final ratio 1:3. The liquid solution obtained was filtered by using a cotton cloth. The solution obtained was the oat milk. Finally, oat milk was cooled to room temperature.

2.2.2. Preparation of Barley Milk

Barley grains were soaked in water (1:1) at 75°C for 6 hours. Additional water (2 parts) was added to the soaked barley and blended in a blender at high speed for 5 min. The plant-based milk (barley milk) was filtered through cotton cloth and separated from the spent barley grain. Finally, barley milk was cooled to room temperature.

2.2.3. Preparation of Sesame Milk

The decorticated sesame seed was firstly grinded then hot water (75°C) was added (1 sesame: 2 water). The mixture was blended in a blender at high speed for 10 min. The resulted sesame milk was filtered through cheesecloth to separate coarse particles. For pasteurization, sesame milk was filled in a beaker and heated to 90°C for 5 min in boiling water bath with manual stirring. After finishing the pasteurization process, sesame milk was cooled to room temperature.

2.2.4. Microbial Analysis

Rayeb milk samples were analyzed for *Streptococcus thermophilus* and *Lactobacillus acidophilus* counts according to the methods described by Tharmaraj and Shah [5]. The count of bifidobacteria was determined according to Dinakar and Mistry [6]. 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.

2.2.5. Statistical Analysis

The obtained results were statistically analyzed using a software package [7] based on analysis of variance. When F-test was significant ($P<0.05$), least significant difference (LSD) was calculated according to Duncan [8] for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

3. Results and Discussion

3.1. Effect of Adding Various Sweeteners on Starter Bacteria Counts of Rayeb Milk Made from Cow and Oat Milk

Data presented in Table 1 clear the populations of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* through storage period of Rayeb milk made from cow milk, oat milk and their mixture (50%:50%) with or without adding sweeteners.

Rayeb made from cow milk (sample A) had the lowest numbers of *S. thermophilus* among different treatments. The counts of the mentioned bacteria increased in oat milk Rayeb. Unexpectedly, blending 50% cow milk with 50% oat milk significantly ($P<0.05$) increased *S. thermophilus* counts in fresh Rayeb milk and during storage period. Furthermore, loss of survival rates of *S. thermophilus* through storage were low for mixed milk Rayeb while they doubled in Rayeb made from cow milk. In supplementary, Mårtensson et al. [9] studied the growth and product characteristics of an oat milk medium fermented with a range of starter cultures. They found out, that strains of *Leuconostoc mesenteriodes*, *Leuc. dextranicum*, *Pediococcus damnosus* and *Lactobacillus kefiri* produced the highest levels of lactic acid, resulting in a pleasant flavour. In addition, an EPS producing strain of *L. delbrueckii ssp. Bulgaricus* yielded a viscosity comparable to dairy yoghurts after 72 h fermentation at 25°C when glucose was used as a carbon source.

Numbers of *S. thermophilus* were significantly ($P<0.05$) higher in treatments of Rayeb milk contained 5% sucrose, 5% honey, 2.5% fructose, and 1.5% sorbitol. The greatest effect was observed in fructose sample followed by honey, sucrose and sorbitol samples. On the other hand, loss of viability levels were lower in sweetened treatments than those of unsweetened and also the lowest level was recoded in fructose sample. Values of loss of viability for samples A, B, C, D, E, F and G were 35.59, 22.86, 18.52, 16.28, 14.13, 12.37 and 18.07%, respectively. These results are in agreement with those of Ayad et al. [10] who mentioned that viable count were significantly higher in yoghurt like product supplemented with honey than control. However, Oliveira et al. [11] stated that the viable *S. thermophilus* counts were not significantly influenced by fortification of milk with oligofructosein a symbiotic low fat milk manufacture.

It could be seen from outcomes of Table 1 that Rayeb milk made from oat milk possessed higher counts of *L. acidophilus* than those found in cow milk one. Mixing of oat milk with cow milk led to another increase in populations of *L. acidophilus* in Rayeb milk. On the contrary, values of the loss of survival for these bacteria were lower in Rayeb manufactured from mixed milk as compared with that made from cow milk or oat milk. This is in close agreement with the report of Dinkçiet al. [12] who showed that the lactococci and lactobacilli viable cell counts differed among the kefir samples made from cow-oat milk mixtures (20, 40 and 60% of oat milk). The highest count was detected in sample with the highest amount of oat milk.

Improvement of sensory attributes of Rayeb milk by addition sweeteners increased the activity and numbers of *L. Acidophilus* while decreased viability loss rates. The clearest influences were noticed in fructose and honey Rayeb milk. Loss of viability values during storage time for treatments A, B, C, D, E, F and G were 36.36, 28.00, 26.67, 21.21, 18.42, 11.90 and 25.00%, respectively.

In similar trend to *S. thermophilus* and *L. acidophilus*, counts of *Bifidobacterium* were higher in Rayeb made from oat milk individually or mixed with cow milk (50:50) than those of cow milk Rayeb. Losses of viability levels of *Bifidobacterium* were low in oat milk treatments comparing with those of cow milk samples. Activation of bifidobacteria in oat milk treatments indicates that oat milk acted as prebiotic of bifidobacteria. These results are in agreement with those of Martensson et al. [13] who cleared that non-dairy, oat-based products are suitable substrates and can support high cell viability during cold storage for 30 days for the probiotic strains *Lactobacillus reuteri* ATCC 55730, *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20456. Lower viability was seen when the strains were grown in the presence of the yoghurt culture compared to when they were grown as pure cultures. These products also exhibited a lower pH value in comparison to products fermented with the pure cultures. The utilization of the main fermentable carbohydrates in the products varied across the bacterial strains. A decrease in β-glucan content was seen for the products fermented by the *B. bifidum* DSM 20456 strain.

Regardless of milk type used in Rayeb preparation, adding sweeteners had positive effect on the numbers of bifidobacteria (Table 1). Raybe contained fructose possessed the highest counts of probiotic whereas honey Rayeb milk came in the second class. Loss of viability levels through storage period lowered in sweetened Rayeb milk. Samples A, B, C, D, E, F and G had 40.00, 24.32, 22.22, 18.00, 14.2810.61 and 18.75% viability loss values respectively. Based on these results, the used sweeteners in this study should not only be used as sweeteners, but also utilized as prebiotic. The stimulation effect of sweeteners on probiotic may be attributed to some components. Oligosaccharides in honey were found to enhance the viability of starter culture as prebiotics [14]. Ustunol [15] cleared that dairy products are the favored food for introducing lactic acid bacteria and bifidobacteria into the human digestive tract. The purpose for doing this is to improve the microbial balance of the intestine. Bifidobacteria, however, are fastidious microorganisms. Keeping their numbers large enough to be meaningful can be a challenge to food manufacturers. Honey contains a small percentage of oligosaccharides that could serve as a food source for these beneficial bacteria, thereby, making honey a “prebiotic” for the “probiotic” dairy food. Mehanna et al. [16] and Sanz et al. [17] found that as a prebiotic, honey contains carbohydrates called oligosaccharides, which may improve gastrointestinal health by stimulating the growth of good bacteria in the colon. Honey has been shown to enhance growth, activity of *Bifidobacteria* in fermented dairy food. Oliveira et al. [11] reported that significant differences were observed for viable counts of the probiotic bacteria (*L. rhamnosus*, *L. acidophilus*, and *Bifidobacterium* spp), which were strongly influenced by the presence of oligofructose. The mean probiotic viable counts were 7.8% higher than in the control, in the presence of oligofructose.

During storage period, numbers of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* reduced in all Rayeb milk samples. This decrease could be evidently attributed to the increase in titratable acidity which controlled the rate of bacterial growth or acted as bactericidal agent [18]. However, the bifidobacteria counts remained above 10^6 $cfu.g^{-1}$ in all Rayeb milk treatments within 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 [19]. Ouwehand and Salminen [20] stated that in order to exhibit positive health effects of probiotics, they have to deliver in certain numbers. As a guide, the International Dairy Federation (IDF) suggested a minimum of 10^7 cfu of probiotics/g product should be alive at the time of consumption. Similar results and recommendations were reported by Moreno et al. [21] and Jayamanne and Adams [22].

Table 1. *Starter bacteria counts of Rayeb milk made from cow and oat milk with adding various sweeteners.*

Properties	Treatments	Storage period (days)			Means
		Fresh	7	14	
	A	59	50	38	49.00^f
	B	70	63	54	62.33^e
	C	81	75	66	74.00^d
Streptococcus thermophilus	D	86	80	72	79.33^c
(cfu x10^7/g)	E	92	86	79	85.67^b
	F	97	92	85	91.33^a
	G	83^A	76^B	68^C	75.67^d
	Means	81.14	74.57	66.00	
	A	22	19	14	18.33^f
	B	25	22	18	21.67^e
	C	30	26	22	26.00^d
Lactobacillus acidophilus	D	33	30	26	29.67^c
(cfu x 10^5/g)	E	38	34	31	34.33^b
	F	42	40	37	39.67^a
	G	32	28	25	28.33^c
	Means	31.71^A	28.43^B	24.71^C	
	A	30	25	18	24.33^g
	B	37	33	28	32.67^f
	C	45	40	35	40.00^e
Bifidobacterium	D	50	46	41	45.67^c
(cfu x 10^5/g)	E	56	53	48	52.33^b
	F	66	63	59	62.67^a
	G	48	44	39	43.67^d
	Means	47.43^A	43.43^B	38.28^C	

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

^{ABCD} Letters indicate significant differences between storage times

A: Rayeb milk made from cow milk; B: Rayeb milk made from oat milk; C: Rayeb milk made from 50% cow milk + 50% oat milk; D: Rayeb milk made from 50% cow milk + 50% oat milk + 5% sucrose; E: Rayeb milk made from 50% cow milk + 50% oat milk + 5% honey, F: Rayeb milk made from 50% cow milk + 50% oat milk + 2.5% fructose; G: Rayeb milk made from 50% cow milk + 50% oat milk + 1.5% sorbitol

3.2. Effect of Adding Various Sweeteners on Starter Bacteria Counts of Rayeb Milk Made from Cow and Barley Milk

Table 2 clears the numbers of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* of Rayeb milk made from cow and barley milk with adding different sweeteners.

Rayeb manufactured from barley milk had higher numbers of *S. thermophilus* than those of control. In contrast to expected, more increases in the populations of mentioned bacteria were found when Rayeb prepared from mixture of 50% cow milk and 50% barley milk. On the other side, loss of viability levels of *S. thermophilus* during storage period highly decreased in mixed milk Rayeb. These results were consistent with that reported by Gouda et al. [23] who found that adding whole barley flour increased the log 10 cfu/gm in bio-yoghurt. Also, the intensive growth of bacteria lactobacillus spp. and bifidobacterium spp. in mixtures of milk with barley or oat malt extracts in the ratio 95:5 was proved by Chapel and Koshova [24].

It is of interest to note that the numbers of *S. thermophilus* increased in Rayeb milk samples sweetened with sucrose, honey, fructose and sorbitol as compared with control. The greatest count was noticed in Rayeb made from cow and barley milk mixture with adding 2.5% fructose (treatment L). Inversely, losses of viability levels of *S. thermophilus* during storage were lower in Rayeb milk contained sweeteners. Values of loss of viability for samples A, H, I, J, K, L, and M were 35.59, 24.32, 16.87, 14.44, 13.68, 11.11 and 17.04%, respectively. Nagpal and Kaur [25] reported that honey added at the level of 5% improved the viability of lactobacilli pure cultures after 5 weeks storage and that improvement might be strain dependent.

L. acidophilus, which generally attained a lower counts during storage compared to *S. thermophilus*, recorded a significantly higher populations in the Rayeb milk made from barley milk. Blending cow milk (50%) with barley milk (50%) added a further increase in *L. acidophilus* numbers of Rayeb milk. Conversely, levels of survival loss during storage period were lower in mixed milk Rayeb than that of Rayeb made from cow or barley milk. Patel et al. [26] showed that malt, wheat and barley extracts demonstrated to have a good influence in increasing bile tolerance and viability of *L. acidophilus* and *L. reuteri*.

Adding sweeteners to Rayeb milk especially fructose and honey caused significantly increasing in *L. acidophilus* counts and significantly decreasing in survival loss rates. Values of loss of survival through storage were 36.36, 27.59, 26.47, 19.44, 17.50, 12.50 and 22.68% for samples A, H, I, J, K, L, and M, respectively. The influence of fructooligosaccharides (FOS) on probiotic survival is consistent with the observations of several authors, who observed a clear beneficial action of these prebiotics on the viability of *L. rhamnosus*, *L. acidophilus*, and *Bifidobacterium* spp. in fermented milk and other dairy products [27, 28, 29].

The numbers of *Bifidobacterium* also increased in Rayeb milk prepared from barley milk. Incorporation of cow milk with barley milk raised the probiotic bacteria counts in fresh Rayeb milk and during storage period. This may be attributed to provide a lot of nutrients in barley milk Rayeb. These results were in agreement with Vasiljevic et al. [30], Rosburg et al. [31], and Elsanhoty et al. [32], who reported an increase in probiotic bacteria growth in yoghurts supplemented with β-glucan. The highest viable numbers of *B. bifidum* were observed in the yogurt made with barley based β-glucan. Ozcan and Kurtuldu [33] reported that *Bifidobacterium* growth and viability were greatly enhanced by β-glucan supplementation. The viability proportion index of *B. bifidum* in probiotic yoghurt at the end of fermentation was significantly higher in yoghurt contained barley-based β-glucan than that of yoghurt contained oat-based β- glucan or control.

Adding different sweeteners to Rayeb milk not only improved the sensory properties (results not shown) but also increased counts and decreased loss of viability of *Bifidobacterium*. Fructose and honey showed the best effects comparing with sucrose and sorbitol. Loss of viability rates during 14 days of storage for samples A, H, I, J, K, L, and Mwere 40.00, 23.08, 20.83, 16.67, 13.56, 10.00, 18.00%, respectively. According to Angus et al. [34], both inulin and oligofructose promote the growth of bifidobacteria in absolute terms. Briefly, fructose released from their partial hydrolysis catalyzed by inulinase may be metabolized as an additional carbon and energy source [35]. In the presence of external oligosaccharides and polysaccharides like inulin or oligofructose, bifidobacteria are, in fact, able to uptake the monomers from their hydrolysis through the fructose-6-phosphate shunt [5, 36]. Ustunol and Gandhi [37] found that the honey promotes of *Bifidobacterium bifidium* growth.

In various Rayeb milk samples, there were significant ($p<0.05$) lowering in viable counts of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* through storage period which can be attributed to the accumulation of acids or reduction of availability of nutrient required for the growth [38]. Giyarto et al. [39] reported that during storage of fermented peanut milk, the viable cells counts of *L. acidophilus* SNP-2 decreased from 9.4×10^8 CFU/ml at day 0 to 6.9×10^8 CFU/ml and 4.3×10^8 CFU/ml at day 28 for samples put in sealed plastic cup or in sterile Erlenmeyer respectively. The most important contributing factors for loss of cell viability are decreasing pH during storage (post-acidification) and the accumulation of organic acids as a result of growth and fermentation [40]. Akalin et al. [41] noted a significant reduction on *B. longum* BB46 in yoghurt after only 1 week refrigeration. This indicates that the viability of *Bifidobacterium* in fermented products was dependent on the carrier type and pH of the fermented products during storage. The statement of Vinderola et al.[42] is in support to this point, where the pH of 4.5 and below was found to jeopardize the viability of probiotics in yoghurt stored at lower temperature of 5°C.

In spite of reducing bifidobacteria counts during storage of Rayeb milk, but the recommended level of 10^7 cfu.g^{-1} of bifidobacteria as a probiotic was exceeded for various Rayeb milk samples and remained above 10^7 cfu g^{-1} until the end of storage period especially in barley milk treatments. The number of probiotic bacteria in the food should be at least 10^7 cfu/ml or per g at the time of consumption in order to exert beneficial effects in the host [20].

Table 2. *Starter bacteria counts of Rayeb milk made from cow and barley milk with adding various sweeteners.*

Properties	Treatments	Storage period (days)			Means
		Fresh	7	14	
Streptococcus thermophilus (cfu x10^7/g)	A	59	50	38	49.00^g
	H	74	66	56	65.33^f
	I	83	78	69	67.67^e
	J	90	84	77	83.67^c
	K	95	91	82	89.33^b
	L	99	95	88	94.00^a
	M	88	81	73	80.67^d
	Means	84.00^A	77.86^B	69.00^C	
Lactobacillus acidophilus (cfu x 10^5/g)	A	22	19	14	18.33^f
	H	29	25	21	25.00^e
	I	34	29	25	29.33^d
	J	36	33	29	32.67^c
	K	40	37	33	36.67^b
	L	45	43	40	42.67^a
	M	35	31	27	31.00^c
	Means	34.43^A	31.00^B	27.00^C	
Bifidobacterium (cfu x 10^5/g)	A	30	25	18	24.33^g
	H	39	34	30	34.33^f
	I	48	44	38	43.33^e

Properties	Treatments	Storage period (days)			Means
		Fresh	7	14	
	J	54	50	45	49.67^{c}
	K	59	56	51	55.33^{b}
	L	70	67	63	66.67^{a}
	M	50	45	41	45.33^{d}
	Means	50.00^{A}	45.86^{B}	40.86^{C}	

^{abcde} Letters indicate significant differences between Rayeb milk treatments
^{ABCD} Letters indicate significant differences between storage times
A: Rayeb milk made from cow milk; H: Rayeb milk made from barley milk; I: Rayeb milk made from 50% cow milk + 50% barley milk; J: Rayeb milk made from 50% cow milk + 50% barley milk + 5% sucrose; K: Rayeb milk made from 50% cow milk + 50% barley milk + 5% honey, L: Rayeb milk made from 50% cow milk + 50% barley milk + 2.5% fructose; M: Rayeb milk made from 50% cow milk + 50% barley milk + 1.5% sorbitol

3.3. Effect of Adding Various Sweeteners on Starter Bacteria Counts of Rayeb Milk Made from Cow and Sesame Milk

The findings in Table 3 show the changes in populations of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* of Raybe milk prepared from cow milk, sesame milk and their mixture (50%:50%) with or without mixing sweeteners.

Numbers of *S. thermophilus* significantly (P< 0.05) increased in Raybe made from sesame milk. However cow milk Raybe (sample A) possessed the lowest count of *S. thermophilus*, but blending cow milk with sesame milk (50:50) highly raised the numbers of this microorganism in the produced Rayeb (sample O). Moreover, the loss of survival levels of *S. thermophilus* during storage stage reduced in mixed milk Rayeb comparing with those observed in Raybe milk made from cow or sesame milk. Researches demonstrated the need for vegetable milk supplementation to enhance the activity and acid production of inoculated yoghurt cultures. This is due to the low concentration of sugars [43] and other substrates needed by the yoghurt cultures [44]. These results contradicted with Afaneh et al. [45] who reported that the highest lactic acid bacteria count was found in the formula based on cow's milk, while the lowest one was for that based on sesame milk with added glucose. Addition of dairy products to sesame milk significantly increased the count of lactic acid bacteria but with varying degrees. The most effective dairy products in this regard were nonfat dry milk followed by dried whey and then by sesame milk extended with skim cow's milk (in a ratio of 1:1) or sesame milk with added lactose.

Incorporation of Raybe milk with sucrose, honey, fructose and sorbitol significantly (P< 0.05) increased the numbers of *S. thermophilus*. This increasing was the highest in Raybe made from mixed milk contained fructose (sample R). On the other hand, the loss of viability values of *S. thermophilus* during storage of Rayeb milk were lower in sweetened treatments than those of unsweetened. Ratios of loss of viability for samples A, N, O, P, Q, R and S were 35.59, 12.82, 12.50, 11.76, 10.58, 11.01, and 11.83%, respectively. Rao et al. [46] not all yoghurt cultures can ferment sucrose; therefore the selection of sugar is very important. On the other hand, Hyvonen and Slotte [47] concluded that sorbitol, 15%, is not suitable for use as the only sweetener in pre-sweetened yoghurt retarding the growth of yoghurt culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*).

Also the counts of *L. acidophilus* increased in Raybe made from sesame milk. Mixing cow milk with sesame milk led to more increasing in numbers of *L. acidophilus*. Loss of viability values reduced when Raybe was manufactured from sesame milk individually or mixed with cow milk.

Using various sweeteners especially fructose and honey in improvement of Raybe milk properties increased the numbers of *L. acidophilus* and at the same time decreased the loss of survival during cold preservation. Values of loss of survival through storage were 36.36, 26.47, 24.32, 19.51, 15.55, 12.00, and 22.22% for samples A, N, O, P, Q, R and S, respectively. Rossi et al.[48] stated that the lactobacilli in the small intestine, e.g. *Lactobacillus acidophilus*, could well be affected advantageously by the sugars and/or other components in honey. Hedberg et al. [49] stated that the metabolic capacity to form acid from dietary sugars such as sucrose, maltose, arabinose, and sorbitol differed significantly between the various probiotic strains. Contrary to the results obtained in our study, Mousa et al. [50] found that total viable counts and lactic acid bacteria numbers were higher in yoghurt sweetened with sucrose than those sweetened with fructose.

Regarding the effect of milk type on the counts of *Bifidobacterium*, Raybe made from mixture of cow and sesame milk (sample O) recorded the highest counts of the mentioned bacteria followed by treatment prepared from sesame milk (sample N) and in the end of the order sample made from cow milk (sample A). The loss of viability rates during storage followed the opposite trend.

Mixing the sweeteners with mixture of cow and sesame milk had stimulated effect on *Bifidobacterium*. The highest count of probiotic bacteria was detected in Rayeb milk supplemented with fructose. On the other side, loss of viability percentages of *Bifidobacterium* during storage considerably reduced in fructose Raybe samples. Loss of viability levels within 14 days of storage for samples A, N, O, P, Q, R and S were 40.00, 22.73, 18.87, 15.00, 13.85, 9.33, and 16.67%, respectively. It has been established that different honeys contain specific oligosaccharides, e.g. isomaltose and melezitose in New Zealand honey [51] and raffinose in Italian honey [52] and it is likely that one or more of these compounds would prove stimulatory to *Bifidobacterium spp.*[53]. Haddadin et al.[54] 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. Pricopeet al. [55] cleared that experiments confirmed the possibility of obtaining fermented milk from milk with added sweeteners. The presence of saccharin 0.05‰ and sorbitol 1.5%, had no significant influence on the fermentative capacity of BB-12® (*Bifidobacterium lactis*) due to the fact that the fermentative process ended at the same time and all samples reached closes pH values at the same time even if the presence of sorbitol reduces the lag period from 8 to 2 hours due to its prebiotic character. The presence of saccharin 0.05‰ and sorbitol 1.5% do not significantly affect the bacteria viability after 14 days refrigeration of the fermented milk samples.

In all Raybe milk treatments, there were significant ($p<0.05$) reducing in the counts of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* during storage (Table 3). 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. [56], 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 [20].

However reducing in viable count of bifidobacteria in different Raybe milk treatments during storage period, the count still above the number required to presence in probiotic food which is at least 6 log cfu/ml fermented products.

By comparing the results illustrated in Tables 1, 2 and 3, it is clear that sesame milk had the greatest positive effect on *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* followed by barley milk then oat milk.

Table 3. *Starter bacteria counts of Rayeb milk made from cow and sesame milk with adding various sweeteners.*

Properties	Treatments	Storage period (days)			Means
		Fresh	7	14	
Streptococcus thermophilus (cfu x10^7/g)	A	59	50	38	49.00^f
	N	78	75	68	73.67^e
	O	88	84	77	83.00^d
	P	95	92	85	90.67^c
	Q	104	100	93	99.00^b
	R	109	103	97	103.00^a
	S	93	90	82	88.33^c
	Means	89.43^A	84.86^B	77.14^C	
Lactobacillus acidophilus (cfu x 10^5/g)	A	22	19	14	18.33^f
	N	34	30	25	29.67^e
	O	37	32	28	37.33^c
	P	41	37	33	37.00^c
	Q	45	43	38	42.00^b
	R	50	48	44	47.33^a
	S	36	32	28	32.00^d
	Means	37.86^A	34.43^B	30.00^C	
Bifidobacterium (cfu x 10^5/g)	A	30	25	18	24.33^f
	N	44	39	34	39.00^e
	O	53	49	43	48.33^d
	P	60	56	51	55.67^c
	Q	65	60	56	60.33^b
	R	75	71	68	71.33^a
	S	54	49	45	49.33^d
	Means	54.43^A	49.86^B	45.00^C	

^{abcde} Letters indicate significant differences between Rayeb milk treatments
^{ABCD} Letters indicate significant differences between storage times
A: Rayeb milk made from cow milk; N: Rayeb milk made from sesame milk; O: Rayeb milk made from 50% cow milk + 50% sesame milk; P: Rayeb milk made from 50% cow milk + 50% sesame milk + 5% sucrose; Q: Rayeb milk made from 50% cow milk + 50% sesame milk + 5% honey, R: Rayeb milk made from 50% cow milk + 50% sesame milk + 2.5% fructose; S: Rayeb milk made from 50% cow milk + 50% sesame milk + 1.5% sorbitol

4. Conclusions

Bio-Rayeb milk could be successfully manufactured from mixtures of cow milk with oat milk (50%:50%), cow milk with barley milk (50%:50%) or cow milk with sesame milk (50%:50%) with adding 5% sucrose, 5% honey, 2.5% fructose, and 1.5% sorbitol to improve the sensory attributes. The mixed milk and sweetened with fructose or honey had considerably activated effects on *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* in bio-Raybe milk.

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