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Effect of Mixing Soy Milk with Buffalo or Cow Milk on the Chemical Composition, Microbial Properties and Sensory Evaluation of Yoghurt

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Abstract: The effect of blending soy milk with buffalo or cow milk on various yoghurt properties was studied. Five treatments of yoghurt were prepared from soymilk, buffalo milk, cow milk, mixture of 75% buffalo's milk + 25% soymilk and mixture of 75% cow's milk + 25% soymilk. Soy milk yoghurt had the lowest acidity, redox potential, total solids, fat, total nitrogen, ash, total volatile fatty acids, saturated fatty acids and total amino acids contents while buffalo's milk yoghurt had the highest. Mixing of soymilk with buffalo or cow milk lowered these values in the produced yoghurt. Conversely, soymilk yoghurt possessed the highest level of unsaturated fatty acids and linoleic acid and α-linolenic acid. The counts of S. thermophiles were almost similar in fresh yoghurt samples. Using soy milk only or mixed with buffalo or cow milk in yoghurt manufacture clearly decreased the count of L. bulgaricus. Blending buffalo or cow milk with soy milk increased the sensory evaluation scores of yoghurt.
Keywords: Soy milk; Buffalo milk; Linoleic acid; α-linolenic acid; Yoghurt.

1. Introduction

Yoghurt is dairy product obtained by lactic acid fermentation as an action of starter culture contained of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophiles*, and with addition of probiotics, it becomes powerful functional food. It is prepared from fresh whole or skimmed milk, boiled and concentrated by evaporation.

On the other hand, soybean is one of the oldest crops of the Far East and has long been consumed by a significant portion of the world population as one of their most important sources of dietary protein and oil. Soybeans produce the highest amount of protein per unit area and hence are a very important source of non-animal protein. The conversions of the protein present in the soybean to soy protein isolates is highly inefficient and expensive, and thus more direct utilization of soybeans in forms such as soymilk or tofu, provide more efficient means of incorporating soy protein in the human diet [1]. It is important to point out that soybean protein is a high quality protein (meaning fully digestible, with an amino acid composition closely matching the amino acid pattern required for human consumption) but somewhat low in sulfur containing amino acids, with methionine being the limiting amino acid [2].

Functional soy milk can be considered as soy milk that contains extra bioactive components and may help to enhance health or lower risk of diseases. Soybean is a good source of phenolic compounds with antioxidant properties and has an extraordinarily high amount of isoflavones, a group of phytoestrogens that have been reported to possibly lower the risk of hormonal and age-related diseases [3]. Therefore, the aim of this study was the possibility of combining nutritional and health benefits of both yoghurt and soy milk in one fermented dairy product.

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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 whereas fresh buffalo's milk was obtained from private farm in Damiette Governorate, Egypt. Yellow soybeans (*Glycine max* (L.) were purchased from a local grocery in Damiette Governorate.

A commercial classic yoghurt starter containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) (Chr. Hansen's Lab A/S Copenhagen, Denmark) were used. 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 Soymilk

Beans of good quality were carefully selected and soaked overnight 12-18 h, at room temperature in ultrapure water contained 0.5% NaHCO3. Once soaked, water was discarded and the grains were re-soaked in boiling water for 15 min then, hulls were removed under running water by manual rubbing. The peeled soybeans were next rinsed and drained with cold water several times. Of the water to be added to the soaked beans (1:6 beans: water ratios), about half was added at room temperature (23°C) and blended with the beans at high speed for 10 min. The remaining water was heated to 80°C and added to the slurry to enhance protein extraction. This mixture was blended for an additional 3 min. at high speed. The resultant slurry was filtered through 3 layers of cheese-cloth to remove coarse material (okara, which is mainly composed of insoluble fiber material). Thereafter, the isolated soymilk was boiled on a low heat for 5 min. to destroy trypsin inhibitor for improving flavor and cooled down to 25°C.

2.2.2. Yoghurt Preparation

Five treatments of yoghurt were made from soy, buffalo or cow milk mixtures as follows:

A: Yoghurt made from soy milk
B: Yoghurt made from buffalo's milk
C: Yoghurt made from cow's milk
D: Yoghurt made from 75% buffalo's milk + 25% soymilk
E: Yoghurt made from 75% cow's milk + 25% soymilk

Fresh milk of various treatments was tempered to 85°C for 15 min, cooled to 40°C, inoculated with cultures (0.1 g/L of yoghurt mix), transferred to 100-ml plastic cups, incubated at 40°C for fully coagulation, and stored at 4°C for 15 days. Yoghurt samples were analyzed when fresh and after 7 and 15 days of refrigerated storage.

2.2.3. Methods of Analysis

2.2.3.1. Chemical Analysis

Total solids, fat, total nitrogen and ash contents of samples were determined according to AOAC [4]. 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 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). Water soluble nitrogen (WSN) of yoghurt was estimated according to Ling [5]. Total volatile fatty acids (TVFA) were determined according to Kosikowski [6].

2.2.3.2. 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 [7]. The fatty acid composition of yoghurt was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC^{TM}- WAX, 30 m, ID 0.25 mm, Film:0,25 μm.

2.2.3.3. Determination of Amino Acids Composition

Amino acid profile of fresh yoghurt was performed following the protocol of Walsh and Brown [8].

2.2.3.4. Microbial Analysis

Yoghurt samples were analyzed for *Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophiles* and *Lactobacillus acidophilus* counts according to the methods described by Tharmaraj and Shah [9]. The count of bifidobacteria was determined according to Dinakar and Mistry [10].

2.2.3.5. Sensory Properties Judging

The sensory properties of the yoghurt samples were determined according to Tunde-Akintunde and Souley [11].

3. Results and Discussion

3.1. Chemical Composition of Yoghurt As Affected By Milk and Culture Types

As shown in Table 1, acidity, redox potential (E_h), total solids (TS), fat and ash values were lower in yoghurt made from soymilk (sample A) than buffalo or cow milk yoghurt (samples B and C respectively). Also the rates of acidity development during storage period were lower in soymilk yoghurt than those made from buffalo or cow milk. Similar findings are reported by Osman and Abdel Razig [12] who found 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). The same authors showed that TS content was higher for the former than the latter which contrary with our results.

Mixing of soymilk with buffalo or cow milk decreased the acidity, E_h, TS, fat and ash values in the resultant yoghurt.

Regardless of the milk type used, titratable acidity and E_h values of all experimental yoghurt treatments and control increased during storage due to the activity of the starter culture. These results agreed with Vijayalakshmi, *et al.* [13] who found that a significant increase in acidity (per cent lactic acid) and decrease in pH were noticed in low fat yoghurt during the storage period but within the permissible levels. Also, TS, fat and ash contents of all samples increased due to the loss of moisture during storage. Similar observation was reported by Farag, *et al.* [14] and Ammar El-Tahra, *et al.* [15].

As it is expected, buffalo milk yoghurt possessed the highest content of TN comparing with that in cow or soy milk one. Consequently, the contents of WSN were higher in yoghurt made from buffalo milk. However, TS and fat contents were lower in soymilk yoghurt than those of cow milk, but the total nitrogen ratios were higher in the former than the latter. These outcomes are similar to that reported by Opara, *et al.* [16] who stated that soymilk yoghurt contains more proteins and moisture but less fat as compared with commercial yoghurt made from cow milk. The high amount of protein in the soy yoghurt was due to the fact that soybean is a proteinous food.

Manufacturing of yoghurt from buffalo and soy milk mixture (75+25%) reduced the contents of TN and WSN. The opposite trend was found in yoghurt made from cow and soy milk mixture (75+25%). In all yoghurt treatments, WSN contents gradually increased during storage period. The highest increasing rates were noticed in buffalo milk yoghurt followed by cow then soy milk yoghurt. These results suggest some degradation in yoghurt protein during storage as also found by Osman and Ismail [17].

With progressive of storage period, TVFA contents gradually increased in all yoghurt samples. These increases may be due to small degree of lipolysis exhibited by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles*. The increases of TVFA contents also may be due to oxidative deamination and decarboxylation of amino acids, which convert the amino acids into its corresponding volatile fatty acids [18]. Because of low fat content of soymilk, TVFA values of soymilk yoghurt were lower than those of buffalo or cow milk yoghurt and this reflected in yoghurt made from soy, buffalo or cow milk mixtures.

3.2. Free Fatty Acids Content (FFA) of Yoghurt

In our current study, FFA contents were measured in fresh yoghurt samples. Results are presented in Tables 2 and 3.

3.2.1. Saturated and Unsaturated Fatty Acids

The saturated and unsaturated fatty acids composition of all yoghurt samples is quite different. Obviously, soymilk yoghurt had the lowest concentration of saturated fatty acids (SFA) and the highest level of unsaturated fatty acids (USFA) as compared with yoghurt made from buffalo or cow milk. Replacement of 25% buffalo or cow milk with soymilk in yoghurt manufacturing brought less SFA and more USFA after fermentation. Saturated fatty acids reduced by 13.34 and 10.56% while USFA increased by 22.53 and 17.46% with incorporation 25% soymilk to buffalo and cow milk respectively (treatments D and E). In general, SFA percentages were higher than USFA for all yoghurt treatments except sample A (soymilk). Similar observations were found by Boycheva, *et al.* [19] in yoghurt made from cow's milk.

Nurliyani, *et al.* [20] showed that substitution 50% of goat milk with soy milk in kefir fermentation can decrease the concentration of caproic, heptadecanoic and behenic significantly ($p<0.05$), whereas the substitution of 25% goat milk with soy milk in kefir fermentation can decrease the pentadecanoic significantly. Caproic, heptadecanoic, behenic and pentadecanoic acid in soy milk were lower than goat milk kefir. Substitution of 50% goat milk with soy milk in kefir fermentation can increase oleic acid concentration significantly, because oleic acid in soy milk was higher than the goat milk.

Among the saturated fatty acids in various treatments, the most abundant was palmitic acid (C16:0) followed by stearic acid (C18:0), myristic acid (C14:0) and lauric acid (12:0). Palmitic is one of the major SFA's; it raises serum cholesterol while stearic acid does not [21]. For the unsaturated fatty acids, the major acids differed between samples A and F and other treatments. The fatty acid linoleic (18:2 ω6) was the predominant followed by oleic acid (18:1 ω9) and α-linolenic acid (18:3 ω3) in samples A and F (soymilk yoghurt). In other samples, the prevailing acid was oleic acid followed by palmitioleic acid and linoleic acid.

Table-1. Chemical composition of yoghurt during storage period

Treatments	Storage Period (days)	Acidity %	pH values	E_h mV*	TS %	Fat %	Ash %	TN %	WSN %	TVFA**
A	Fresh	0.65	4.86	143.9	11.37	2.4	0.76	0.567	0.114	5.4
	7	0.85	4.72	160.8	11.56	2.4	0.80	0.575	0.130	6.1
	15	0.97	4.61	170.2	11.76	2.5	0.85	0.581	0.139	6.8
B	Fresh	0.79	4.60	165.4	17.85	7.1	1.11	0.770	0.149	12.2
	7	1.08	4.44	184.6	18.05	7.2	1.16	0.778	0.174	13.8
	15	1.27	4.32	197.1	18.17	7.2	1.18	0.788	0.188	15.4
C	Fresh	0.73	4.71	156.1	13.56	3.4	0.88	0.531	0.109	8.1
	7	1.00	4.55	173.4	13.70	3.5	0.91	0.540	0.131	9.3
	15	1.17	4.45	184.4	13.84	3.6	0.94	0.547	0.142	11.0
D	Fresh	0.74	4.71	157.1	14.56	6.7	0.98	0.711	0.135	11.8
	7	0.98	4.59	169.7	14.70	6.8	1.01	0.723	0.156	13.1
	15	1.13	4.47	186.6	14.86	6.9	1.03	0.729	0.167	14.5
E	Fresh	0.67	4.81	146.9	13.36	3.0	0.84	0.542	0.112	7.0
	7	0.87	4.69	163.7	13.52	3.0	0.87	0.550	0.130	8.1
	15	1.02	4.54	174.3	13.66	3.1	0.89	0.552	0.138	9.7

*mV: millivolts
** expressed as ml 0.1 NaOH 100 g^{-1} yoghurt

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

The total monounsaturated fatty acids content of the soymilk yoghurt were 23.61% for sample A that are lower than the contents of yoghurt made from buffalo or cow milk (Tables 2 and 3). As a consequence, addition of 25% soymilk to buffalo or cow milk decreased the levels of MUSFA in yoghurt resulted. The reduction rates of MUSFA by adding soymilk were 1.31 and 4.62% for treatments D and E respectively. On the greatly contrary, the ratios of polyunsaturated fatty acids were very higher in soymilk yoghurt samples than those of other treatments especially that made from buffalo or cow milk. Mixing of soymilk with buffalo or cow milk multiplied the content of yoghurt from the PUSFA.

The levels of most important essential fatty acids for human health – linoleic acid (omega-6) and α-linolenic acid (omega-3) – highly increased in yoghurt made from mixtures of buffalo, cow milk and soy milk in comparison to natural yogurt. Increasing rates were 347.12 and 332.75% for linoleic acid and 163.89 and 113.79% for α-linolenic acid when 25% soymilk was added to buffalo or cow milk (samples D and E) respectively. Also, a very high pronounced increase was observed in linoleic acid and α-linolenic acid in soymilk yogurt sample compared to buffalo or cow milk yoghurt treatments. With regard to oleic acid (omega-9), slight decrease was observed in yoghurt manufactured from mixtures of buffalo and cow milk with soymilk. In all cases, outcomes of PUSFA indicate that incorporation of 25% soymilk with buffalo and cow milk produces very healthy yoghurt because of their content of omega-3, 6 and 9.

3.2.3. Short Chain Fatty Acids (C8 – C12)

During this study, findings showed that the contents of short-chain fatty acids (SCFA) were lower in soymilk yoghurt as compared with that of buffalo or cow milk. Also, substitution of 25% buffalo and cow milk with soymilk lowered the values of SCFA in produced yoghurt. In all yoghurt treatments, the fatty acid lauric (C:12) was the predominant SCFA followed by capric acid (C10:0) and caprylic acid (C8:0).

3.2.4. Medium Chain Fatty Acids (C14 – C16)

The amounts of medium chain fatty acids (MCFA) were greater in yoghurt made from buffalo or cow milk than those of yoghurt made from soymilk only or mixtures of soy, buffalo and cow milk. Generally, palmitic acid (C16:0) was the predominant in medium chain fatty acids followed by myristic acid (C14:0) and pentadecanoic acid (C15:0).

3.2.5. Long Chain fatty Acids (> C16)

Contrary to short and medium chain fatty acids, long chain fatty acids (LCFA) were markedly higher in soymilk yoghurt than those of yoghurt made from buffalo and cow milk. Incorporation of 25% soymilk with two types of animal milk increased LCFA by 13.52 and 10.55% for samples D and E respectively. In sample A, the abundant acid was linoleic (C18:2) followed by oleic acid (18:1 ω9), palmitic acid (16:0) and stearic acid (C18:0). In other treatments, palmitic and oleic acids were the predominant in LCFA followed by stearic acid.

3.3. Free Amino Acids Content (FAA) of Yoghurt

Data in Tables 4 and 5 compare the composition of FAA in fresh yoghurt made from soy, buffalo and cow milk.

3.3.1 Total Free Amino Acids

Results in the mentioned Tables show that the total free amino acids content is slightly affected by the type of milk. Yoghurt prepared from soy milk contained little low amount of total amino acids as compared with that made from buffalo and cow milk. Thus, incorporation of 25% soy milk with both kinds of animal milk slightly decreased the levels of amino acids in the resultant yoghurt. In agreement with these findings, Nurliyani, *et al.* [20] found that goat milk kefir and kefir made from 50% goat milk and 50% soymilk mixture showed not significantly different in amino acid composition. Soy milk could substitute 50% of goat milk in kefir preparation to obtain the similar to goat milk kefir in amino acid composition. Goat milk kefir and kefir made from 50% goat milk and 50% soy milk mixture were composed of a good protein quality.

In all tested yoghurt samples, the highest content of total free amino acids was that of glutamic acid, which is responsible for protection from cardiovascular diseases, followed by aspartic acid. On the contrary, methionine and cystine acids had the lowest content of total amino acids.

3.3.2. Essential Amino Acids (EAA)

Generally, slight lowering was observed in the amounts of the essential amino acids as a result of substitution of 25% buffalo or cow milk with soy milk in yoghurt production. Yoghurt made from buffalo milk possessed the highest amounts of threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine and lysine. The contents of these essential acids in cow milk yoghurt were somewhat higher than those found in soy milk one. Consequently, samples of soy milk contained the lowest ratios of essential amino acids to total amino acids (E/T) among the treatments. This could be explained on the basis of difference in fat content between treatments. Bao, *et al.* [22] showed that there were clearly positive relationship between the amount of fat in the milk base and the total FAA contents after fermentation with *L. casei* GBHM-21.

Table-2. Effect of using soymilk on free fatty acids content (%) of fresh yoghurt

Fatty acids	**C**	**Treatments**				
		A	**B**	**C**	**D**	**E**
		Saturated fatty acids (SFA) %				
Caprylic	8:0	0.41	0.77	0.61	0.66	0.49
Capric	10:0	2.14	3.09	2.98	2.55	2.40
Undecanoic	11:0	0.16	0.31	0.18	0.18	0.17
Lauric	12:0	2.54	3.32	3.50	2.99	3.04
Tridecanoic	13:0	0.41	0.61	0.34	0.38	0.48
Myristic	14:0	4.36	10.90	10.01	8.14	8.20
Pentadecanoic	15:0	1.33	3.19	3.02	2.94	2.85
Palmitic	16:0	18.66	26.60	26.23	24.26	24.46
Heptadecanoic	17:0	0.67	2.54	2.49	2.30	2.28
Stearic	18:0	6.50	11.80	11.71	10.15	10.04
Arachidic	20:0	0.31	0.20	0.21	0.23	0.27
Behenic acid	22:0	0.27	-	-	0.10	0.13
Total		37.76	63.33	61.28	54.88	54.81
		Unsaturated fatty acids (USFA) %				
	12:1 ω5	0.53	0.49	0.49	0.43	0.49
5-Tetradecenoic (phytosteric)	14:1 ω5	-	0.42	0.40	0.42	0.67
	14:1 ω7	0.34	0.25	0.40	0.40	0.31
Myristioleic acid	14:1 ω9	0.53	0.30	0.33	0.45	0.31
	16:1 ω5	-	-	0.12	0.16	-
Palmitioleic	16:1 ω7	0.43	2.20	2.23	2.29	2.19
	16:2 ω4	-	0.38	0.30	0.34	0.25
Hexagonic	16:3 ω4	-	0.33	0.40	0.45	0.36
	18:1 ω4	-	0.30	0.25	0.35	0.25
Octadecosaenoic	18:1 ω5	-	0.41	0.41	0.52	0.41
Vaccienic	18:1 ω7	1.10	1.07	1.13	1.10	1.08
Oleic	18:1 ω9	20.54	25.92	26.10	24.54	24.62
	18:2 ω4	-	0.60	0.75	0.73	0.76
	18:2 ω5	-	0.35	0.42	0.52	0.50
Linoleic	18:2 ω6	33.16	1.91	2.29	8.54	9.91
	18:2 ω7	-	-	0.33	0.23	-
α-Linolenic	18:3 ω3	4.23	0.72	0.87	1.90	1.86
	18:3 ω4	-	-	0.15	0.19	0.15
Gamma linolenic	18:3 ω6	-	0.48	0.31	0.36	0.18
Octadecatetraenoic	18:4 ω3	-	-	0.21	0.17	0.18

Gadoleic acid	20:1 ω9	0.14	-	-	-	-
Eicosaenoic	20:1 ω11	-	-	0.14	0.29	0.19
Eicosatrienoic	20:3 ω6	-	-	-	-	-
Total		61.00	36.22	38.03	44.38	44.67
Non identified fatty acid		1.24	0.54	0.69	0.74	0.52

With the increase in fat concentration, the concentration of some FAAs, such as Glu, Leu, Trp, Phe, and Lys, were significantly increased. Additionally, some FAAs, such as Ala and Cys, were not significantly affected by the fat concentration. 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.

Table-3. Effect of using soymilk on free fatty acid indices ratios of fresh yoghurt

Treatments	SFA	USFA	MUSFA	PUSFA	SCFA	MCFA	LCFA
A	37.76	61.00	23.61	37.39	5.78	26.06	66.92
B	63.33	36.22	31.36	4.86	7.98	45.48	46.00
C	61.28	38.03	32.00	6.03	7.76	43.78	47.77
D	54.88	44.38	30.95	13.43	6.81	40.23	52.22
E	54.81	44.67	30.52	14.15	6.59	40.08	52.81

SFA: saturated fatty acids; USFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids (C:1); PUSFA: polyunsaturated fatty acids (C:2+ C:3); SCFA: short chain fatty acids (C8 to C12); MCFA: medium chain fatty acids (C13 to C16); LCFA: long chain fatty acids (> C16).

Notably, soymilk yoghurt were most limited in sulfur amino acids (methionine and cysteine), whereas buffalo milk yoghurt was richer in these amino acids. Chaiwanon, *et al.* [23] reported that soymilk and cow milk have similar protein content 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. Also, Blmstrand, *et al.* [24] cleared that the concentration of sulfur-containing amino acids is low in soy protein isolates.

In various yoghurt treatments, the major essential amino acid was leucine followed by lysine. Methionine content was the lowest.

3.3.3. Nonessential Amino acids (Non-EAA)

On the reverse of essential amino acid, the contents of nonessential amino acid were higher in soy milk yoghurt treatments than those of buffalo or cow milk samples. Mixing of 25% soy milk with buffalo and cow milk increased the levels of nonessential amino acids by 4.35 and 3.14% for treatments D and E respectively. The soy milk yoghurt, 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. Our study is in accordance with the results obtained by Ma *et al.* (2014) who illustrated that soy and milk proteins contained 3.32 and 3.10 alanine, 5.70 and 3.40 arginine, 4.31 and 5.00 serine and 3.34 and 5.00 (g/100g protein) tyrosine respectively.

3.3.4. Branched-chain Amino Acids (BCAA)

In the present study, data of Tables 4 and 5 indicate that yoghurt made from buffalo or cow milk had higher amounts of total BCAA than that made from soy milk. Thus, it would appear that blinding of 25% soy milk with buffalo and cow milk slightly decreased the values of total BCAA in yoghurt treatments. The declining rates were more pronounced in leucine content. Both human clinical studies and animal research have demonstrated that soy protein products are comparable in digestibility to other high-quality protein sources, such as meat, milk, fish, and egg [25].

Table-4. Effect of using soymilk on free amino acids content (g/100mL) of fresh yoghurt

Amino acids	Treatments				
	A	B	C	D	E
Aspartic (ASP)	0.38	0.28	0.25	0.31	0.26
Threonine (THR)	0.16	0.19	0.17	0.18	0.14
Serine (SER)	0.20	0.26	0.26	0.23	0.22
Glutamic acid (GLU)	0.61	0.51	0.49	0.58	0.57
Proline (PRO)	0.23	0.36	0.33	0.34	0.31
Glycine (GLY)	0.14	0.08	0.08	0.10	0.08
Alanine (ALA)	0.23	0.15	0.13	0.17	0.15
Valine (VAL)	0.23	0.29	0.27	0.26	0.23
Methionine	0.07	0.12	0.10	0.10	0.09
Isoleucine (ILE)	0.19	0.23	0.20	0.21	0.17
Leucine (LEU)	0.30	0.40	0.34	0.34	0.29

Tyrosine (TYR)	0.14	0.17	0.15	0.17	0.14
Phenylalanine (PHE)	0.21	0.30	0.26	0.27	0.22
Histidine (HIS)	0.12	0.18	0.14	0.17	0.13
Lysine (LYS)	0.26	0.40	0.37	0.36	0.33
Arginine (ARG)	0.20	0.16	0.12	0.17	0.16
Cystine (CYS)	0.06	0.10	0.10	0.09	0.08

Table-5. Effect of using soymilk on free amino acid indices ratios of fresh yoghurt

Treatments	Total amino acids (g/100mL)	Total EAA (g/100mL)	Total Non-EAA (g/100mL)	Total BCAA (g/100mL)	E/T (%)	Total BCAA/Total (%)
A	3.73	1.54	2.19	0.72	41.29	19.30
B	4.18	2.11	2.07	0.92	50.49	22.01
C	3.76	1.85	1.91	0.81	49.20	21.54
D	4.05	1.89	2.16	0.81	46.67	20.00
E	3.56	1.59	1.97	0.68	44.66	19.10

Total EAA: total essential amino acids; Total Non-EAA: total nonessential amino acids; Total BCAA: total branched-chain amino acids; E/T: Ratio of essential amino acids to total amino acids.

3.4. Microbial Analysis of Yoghurt

Data presented in Table 6 show changes occurred in *Streptococcus thermophiles*, and *Lactobacillus bulgaricus* of yoghurt at zero time and during preservation period. The numbers of different microbial groups for all yoghurt samples pronounced decreased within storage. This decrease could be evidently attributed to the increase in titratable acidity which controlled the rate of bacterial growth or acted as bactericidal agent [26].

It is quite apparent from the results of Table 7 that the count of *S. thermophiles* was almost similar between fresh samples of soy, buffalo and cow milk yoghurt. However, the loss of its survival during storage was the lowest for soy milk yoghurt being 33.33% (sample A). Because of high acidity content, yoghurt made from buffalo milk (sample B) had the highest loss of survival rates for *S. thermophiles* recorded 61.54% while cow milk yoghurt (sample C) just recorded 45.45%. Mixing 25% soy milk with buffalo or cow milk (samples D and E) reduced loss of viability of *S. thermophiles* to 26.31 and 20.00% respectively. These results are confirmed with the results found in Table 1 which showed that buffalo milk yoghurt possessed the highest values of acidity development through storage.

Utilization of soy milk only or mixed with buffalo or cow milk in yoghurt manufacture clearly decreased the count of *L. bulgaricus*. Moreover, the loss of viability during storage was also high in soy milk yoghurt. The highest counts of *L. bulgaricus* were in cow milk yoghurt followed by buffalo milk one. These results refer to the negative effect of soy milk on *L. bulgaricus*. In supplementary, Mital, *et al.* [27] showed that certain organisms such as *S. thermophiles*, *L. acidophilus*, *L. cellobiosis* and *L. plantarum* which utilize sucrose, exhibited significant growth and produced substantial amounts of acid in soymilk. Others such as *L. delbrueckii* ssp. *bulgaricus* grew poorly in soymilk because of their inability to ferment sucrose and other carbohydrates in soymilk. Sumarna [28] reported that *S. thermofilus*, 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.

3.5. Changes in Sensory Evaluation of Yoghurt

Impact of culture type and mixing of soy milk on sensory quality of yoghurt is given in Table 7. As it is expected, white color of buffalo milk which preferred for all Egyptian consumers granted yoghurt the highest scores of color and appearance. Soy milk yoghurt obtained the lowest scores so mixing of 25% soy milk with buffalo or cow milk slightly decreased color and appearance grades of the produced yoghurt. This is in close agreement with the report of Osman and Abdel Razig [12] 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%).

Table-6. Effect of using soymilk on starter bacteria counts of yoghurt during storage period

Treatments	Storage Period (days)	*Streptococcus thermophiles* (cfu×x10^5/g)	*Lactobacillus bulgaricus* (cfu×x10^5/g)
A	Fresh 7 15	12 11 8	8 7 4
B	Fresh 7 15	13 10 5	15 12 8
C	Fresh 7 15	11 9 6	17 15 10
D	Fresh 7 15	14 11 7	13 10 7
E	Fresh 7 15	13 11 8	14 11 8

Because the soy-yoghurt produced was yellowish in color and has a beany flavor, it was not surprising that the smell, taste and mouth feel evaluation tests of soy milk yoghurt gained the lowest scores as compared with that made from buffalo or cow milk. Mixing of buffalo and cow milk with soymilk markedly improved the above mentioned testes. For instance, incorporation of buffalo milk with soy milk (75+25%) increased smell and taste evaluation scores of fresh sample D by 12.12 and 16.87% respectively.

However, soymilk yoghurt failed to obtain high scores of color, appearance, smell, taste and mouth feel but the texture and body scores of it were higher than those of cow milk yoghurt. Buffalo milk yoghurt recorded the greatest texture and body scores because of high total solids content of milk. Finally, overall acceptability grades were low for soy milk yoghurt but addition of buffalo or cow milk to soy milk increased these grades.

On a general note, fresh treatments ranked the highest scores of color, appearance, smell, taste, mouth feel texture, body and overall acceptability. Unfortunately, with storage progressive the sensory evaluation degrees of various samples lowered. This may be attributed to the developed acidity and/or whey separation, which may impair the pleasant acid flavour of yoghurt [29]. These trends are similar to other works in literature. Badawi, *et al.* [30] mentioned that scores for sensory properties of yoghurt were almost unchanged during the first 6 days of storage and then decreased. In their study, Routray and Mishra [31] found that the storage time had a negative impact on the flavour scores of yoghurt which they attributed to changes in the aroma compounds.

Table-7. Effect of using soymilk on sensory evaluation of yoghurt during storage period

Treatments	Storage Period (days)	Quality attribute						
		Color	Appearance	Smell	Taste	Mouth feel	Texture & Body	Overall Acceptability
A	Fresh 7 15	8.75 8.75 8.60	8.75 8.75 8.70	8.25 8.10 7.85	8.00 7.70 7.45	8.50 8.35 8.15	9.75 9.75 9.70	8.00 7.85 7.65
B	Fresh 7 15	10.00 10.00 9.80	10.00 10.00 9.85	10.00 9.75 9.40	10.00 9.60 9.25	10.00 9.55 9.10	10.00 10.00 9.95	10.00 9.60 9.10
C	Fresh 7 15	9.10 9.10 9.10	9.20 9.20 9.15	9.75 9.55 9.25	9.25 9.00 8.65	9.15 8.85 8.50	9.50 9.50 9.45	9.15 8.85 8.50
D	Fresh 7 15	9.45 9.45 9.40	9.40 9.40 9.40	9.25 9.15 9.00	9.35 9.00 8.60	9.10 8.75 8.40	9.50 9.50 9.45	9.45 9.10 8.70
E	Fresh 7 15	9.00 900 9.00	9.05 9.05 9.00	9.50 9.35 9.15	8.75 8.60 8.30	9.00 8.85 8.55	9.75 9.75 9.70	9.10 8.85 8.60

4. Conclusion

Incorporation of 25% soymilk with buffalo or cow milk produced yoghurt with highly nutritional value. This yoghurt contained high amounts of unsaturated fatty acids and essential amino acids. The results of sensory evaluation cleared that yoghurt made from mixtures of soy milk with buffalo or cow milk was acceptable in properties of color, appearance, smell, taste, mouth feel texture and body.

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