



Soy milk yoghurt- A functional non-dairy alternative to cow milk yoghurt

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Abstract

In this study a new functional yoghurt was manufactured using soymilk. Three yoghurt samples were processed using traditional starter culture, and *Bifidobacterium bifidum*: (A) 100% cow milk yoghurt as a basis of comparison, (B) 100% soy milk yoghurt, (C) (50% cow milk+50% soy milk) yoghurt, samples were stored at 4°C for 15 days, and were analysed on day 1, day 7, and day 15. Results showed that all samples kept a vital force of 10^6 colony /g till day 15 of cooled storage period, Soy milk yoghurt had the highest content of protein (3.75%), and highest levels of unsaturated fatty acids, also had acceptable taste, smell, and firm texture, and it could be considered a functional probiotic vegan alternative to cow milk yoghurt, since adding *Bifidobacterium* prolonged the shelf-life, improved the undesirable flavor of soy milk, and this non-dairy new yoghurt is nutritious, and healthy.

Keywords: dairy alternatives, functional food, oligosaccharides, soy yogurt, vital motivation

Introduction

Widespread use of dairy products has various nutritional benefits, and therapeutic effects on human's health, yoghurt is one of the most important fermented dairy products consumed widely in the world, it has a firm coagulum, acidic taste, and other flavor compounds. It is generally produced by fermentation of animal milk using LAB (Lactic Acid Bacteria) as probiotics, and when the viable cells count of LAB is about ($10^6 - 10^8$) cfu/g it will be a kind of probiotics functional food for several age categories (Gasmalla *et al.*, 2013) [6]. It has been observed that consuming cow milk yoghurt causes allergic reaction and health problems; lactose intolerance, cardiovascular diseases. Moreover, There are insufficient quantities of animal protein to meet the human daily requirement of proteins in developing countries, besides of the groups who prefer a vegetarian diet, therefore nutritional industries sector tends to concentrate on finding vegetarian nutritional alternatives to meet the demand of vegetarians, and to prevent some diseases (Yadav *et al.*, 2017) [24].

Producing dairy alternative products as functional foods started in Hong Kong in 1940, it was limited firstly in preparing emulsions of oily seeds, and soaked legumes, such as soy bean milk in China, and extractions of coconuts, and almond in Korea, Bulgaria, and Turkey. However soy milk the most popular non-dairy extraction among dairy alternatives is considered as a highly nutritious healthy food, since it has a considerable amount of high quality protein, and a good level of poly unsaturated fatty acids (Sethi *et al.*, 2016) [20]. On contrast marketing of soymilk is limited due to decrease of its acceptance between consumers because of the beany flavor which is related to undesirable flavor components such as methanol, hexanal, pentanal, and ethanol (Blagden & Gilliland, 2005) [4]. Another factor

related to flatulence which is resulted from oligosaccharides exist in soymilk such as Raffinose, and Stachyose. Several studies assured that fermentation of soy milk by lactic acid bacteria, helps to remove the undesirable flavor, and flatulence problems of it, beside soy milk considered a typical substrate for the growth of lactic acid bacteria together with *Bifidobacterium* by utilizing oligosaccharides in this milk, and giving the special probiotic flavor of the fermented product (yoghurt) through a symbiosis catalyzing relation between the two genus (*Lactobacillus*, *Bifidobacterium*). Shilpa *et al* (2011) [21] stated that species of *Bifidobacterium* compose a considerable part of colon microflora, and tend to grow in anaerobic conditions in the large intestine consuming parts of food "prebiotics" which aren't digested by human enzymes (Gibson *et al.*, 2010) [7]. While species of *Lactobacillus* are aero-tolerant, or anaerobic, it can inhabit the small intestine, therefore probiotics products that contain *Lactobacilli*, and *Bifidobacterium* with a viable cells count of 10^6 cfu/g, can affect all the intestinal channel, serving a nutritional, and therapeutic effects for individual.

This study suggested to manufacture a therapeutic, vegetarian yoghurt, supplemented by *Bifidobacterium*, using soy milk, for a more functional nutritious product since dairy alternatives supplemented with functional microorganisms can solve the nutritional problems related to dairy products.

Materials and Methods

Materials

Fresh cow milk was brought from the Faculty of Agriculture farm, Damascus university, soybeans were brought from Crop Department of the General Commission For Scientific Agriculture Research, and a commercial traditional starter

culture of lactic acid bacteria YC-X11 (*Streptococcus thermophiles*, *Lactobacillus bulgaricus*), and a starter culture of (*Bifidobacterium bifidum*), were purchased.

Methods

Soybean Milk Preparation

Soybeans were soaked in water for 3 hours to remove the hulls, then soaked water was discarded, hulls were manually removed, after that the dehulled beans were resoaked in 0.05% NaHCO₃ water for 20 minutes, then boiled for 5 minutes at 100 °C, (NaHCO₃ used to blanch soy beans), boiling water was discarded, and the beans were rinsed with pure water. Then water was added to beans by a ratio of 6:1 respectively, and it was mixed at high speed for 10 minutes, the slurry was filtered through double layers of cheese cloths, and the pure extraction was heated at 85 °C for 30 minutes (Park *et al.*, 2012) [19].

Enrichment of Starter Culture

Two closed bottles containing 100 ml of non-fat milk supplemented with 0.2% yeast extract, and 0.1% sucrose were used to activate the starter cultures, they were heated at 90 °C for 10 min, then cooled to 42 °C, one was inoculated with 1 g of starter culture (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*), and the other was inoculated with 1g of *Bifidobacterium bifidum*, then both bottles were incubated at 45 °C until the pH reached point 4.6, then stored at 4 °C to be used later in processing yoghurt samples (Horrall *et al.*, 1950) [11].

Manufacturing of yoghurt samples

Following three samples of yoghurt were prepared:

A: Yoghurt made from cow milk 100%.

B: Yoghurt made from soy milk 100%.

C: Yoghurt made from (50 % cow milk + 50 % soymilk).

Milk of each sample was heated to 85 °C, for 30 min, cooled rapidly to 43 °C, inoculated with enriched cultures (3% of raw milk), incubated at 43 °C until the pH reached point 4.6, and stored at 4 °C for 15 days. The microbiological, chemical, and sensory analysis were carried out in triplicate at day1, day7, and day15 of cooled storage.

Methods of Analysis

Microbiological Analysis

Each yoghurt sample was tested for the vital count of each bacterial species (*Streptococcus thermophiles*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum*) using petri dishes. Pepton water was used to prepare sequent dilutions starting from 10⁻¹ to 10⁻⁹, 1 ml of each dilution placed in the petri dish, then 15 ml of suitable media for each bacterial species were added to the petri dish; acidified MRS Agar medium (pH=5.2) (Ashraf & Shah., 2014) [3] was used for the enumeration of *Lactobacillus bulgaricus*, incubated anaerobically at 44 °C for 72 h, M17 Agar medium used for *Streptococcus thermophiles* incubated at 37 °C, for 72 h, and BSM Agar used for the enumeration of *Bifidobacterium bifidum*, incubated anaerobically at 37 °C, for 5 days. The colonies of each species were counted in each sample and get the total vital bacterial count (total vital force).

Chemical Analysis

The content of total protein, total fat, and acidity were conducted for yoghurt samples according to standard methods (AOAC, 2002). Fatty acids content was determined using Gas Chromatography, with flame ionization detector (FID), and the carrier gas was Helium, and Nitrogen (ISO, 1990).

Sensory Evaluation

The sensory properties of the yoghurt samples were evaluated according to Likert scale for the following indicators: (color, smell, taste, texture, and general acceptable) at the following degrees: 1: Strongly agree, 2: Agree, 3: Neither, 4: Disagree, 5: Strongly disagree (Joshi *et al.*, 2015) [14].

Statistical Analysis

Data were analysed by Genstat 12, L.S.D 0.05 using variation analysis test (ANOVA).

Results and Discussion

Microbiological Analysis of Yoghurt Samples

Table 1: log (cfu/g) of bacterial growth in yoghurt samples during storage period

Samples	Storage period(day)		
	1	7	15
A	9.43 ^a ±0.02	7.57 ^c ±0.05	7.22 ^d ±0.04
B	6.86 ^e ±0.02	6.72 ^f ±0.06	6.57 ^g ±0.02
C	8.41 ^b ±0.1	7.23 ^d ±0.06	6.54 ^g ±0.01

Different small letters in the same row refer to a significant difference at (p<0.05)

Different small letters in the same column refer to a significant difference at (p<0.05)

Values explained means±SD for all samples

As mentioned in table (1) the counts of all three bacterial species used in the manufactured yoghurt samples showed that all yoghurt samples kept a vital force of bacterial count at a level of 6 log (cfu/g) at least till the end of storage period (15) days. As compared to cow yoghurt (A), sample (C) showed a good type of probiotics product with a bacterial count of (8.41±0.1) log (cfu/g), sample (B) also gained a significant vital force of total bacterial count at a degree of probiotics (6.86±0.02) log (cfu/g). This is proved that *Bifidobacterium*, and Lactic Acid Bacteria (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*) showed a good cohabitation in fermented vegan substrate like soymilk by stimulating the activity and growth of each other in a term of probiotic product which served an adequate, alive number of microorganisms when consumed, this was agreed with the definition of probiotics products (Hill *et al.*, 2014) [9]. Since *Bifidobacterium* secretes α -galactosidase, and utilizes prebiotics (oligosaccharides) exist in soymilk, it reduces flatulence caused by oligosaccharides and serves a type of symbiotic food with a healthy effect on the human intestinal system, preserves the balance in the gut microflora. That led to the possibility of producing functional yoghurt by fermentation of soymilk with a ratio of 50%, or 100%, in the mixture, using strains of the two genus *Lactobacillus* and *Bifidobacterium* (Horáčková *et al.*, 2015; Akusu and Wordu, 2017) [10, 1].

Chemical analysis of yoghurt samples

Table (2) showed that mean values of protein, fat, and acidity in all manufactured yoghurt samples had increased through storage period as a result of partial loss of moisture in samples, Similar findings were mentioned with (Hassan and Amjad, 2010) [8].

Table 2: Mean±SD values of some chemical properties of yoghurt samples

Samples	Storage period (day)	Protein%	Fat%	Acidity%
A	1	3.14 ^g ±0.01	3.58^f ±0.01	0.75 ^c ±0.01
	7	3.16 ^f ±0.01	3.60^f ±0.01	1.09 ^b ±0.01
	15	3.20 ^e ±0.01	3.63^f ±0.01	1.16 ^a ±0.01
B	1	3.72 ^b ±0.01	2.89^g ±0.01	0.51 ^g ±0.02
	7	3.73 ^b ±0.01	2.91^g ±0.01	0.57 ^f ±0.01
	15	3.75 ^a ±0.01	2.94 ^f ±0.01	0.63 ^c ±0.01
C	1	3.44 ^d ±0.02	3.23^f ±0.01	0.65 ^{de} ±0.02
	7	3.45 ^d ±0.02	3.25^e ±0.01	0.67 ^d ±0.02
	15	3.47 ^c ±0.02	3.28^d ±0.01	0.73 ^c ±0.01

Different small letters in the same column refer to a significant difference at (p<0.05)

As expected the highest value of protein, was in (soy milk yoghurt 100%) sample B (3.75% ±0.01), compared to cow milk yoghurt sample A which had the lowest value of protein (3.20% ±0.01) with a significant difference between protein mean values at (p<0.05)of the two yoghurt samples B,A, which leads to consider soy milk yoghurt 100% as an essential nutritional source of high quality protein which is required for the individual in daily diet, in the cases of shortage in animal protein amounts (Opara *et al.*,2013) [17]. It was noted that the highest value of acidity through storage period was in sample (A) (1.16%±0.01), while the lowest one was in sample (B) (0.63%±0.01) with a significant difference among all manufactured yoghurt samples, however in spite of low value of acidity in sample (B), it is still considered to be in the acceptable range of lactic acid

products. Since *Bifidobacterium bifidum* multiplied in soymilk, fermented oligosaccharides, and produced desirable acidity, although lactose in cow milk yoghurt is considered the typical substrate for yoghurt starter culture to produce lactic acid. Similar results were found with (Ming, 2012). For the fat content, sample (B) had the lowest value of fat (2.94 ±0.01), while cow milk yoghurt sample (A) had the highest values (3.63% ±0.01), followed by sample (C) (3.28% ±0.01) with a significant difference between fat values at (p<0.05) through storage period. The fat content in cow milk yoghurt was high due to the high content of fat in cow milk and this is in agreement with the results stated by (Ismail *et al.*, 2016) [12].

Fatty Acids Composition in Yoghurt Samples

Table 3: Differences in fatty acids values between manufactured yoghurt samples

Saturated Fatty Acids	Mean values of saturated fatty acid in yoghurt samples%		
	A	B	C
Butyric C4:0	4.23 ^a ± 0.1	n.d	3.74 ^b ±0.1
Caproic C6:0	2.17 ^a ±0.1	n.d	1.33 ^b ±0.1
CaprylicC8:0	2.30 ^a ±0.1	n.d	1.43 ^b ±0.1
Capric C10:0	3.63 ^a ±0.1	n.d	2.13 ^b ±0.1
Lauric C12:0	4.70 ^a ±0.1	n.d	2.83 ^b ±0.1
MyristicC14:0	10.60 ^a ±0.1	4.20 ^c ±0.1	9.83 ^b ±0.1
PalmiticC16:0	42.40 ^a ±0.1	22.67 ^c ±0.1	28.77 ^b ±0.1
Stearic C18:0	4.72 ^a ±0.3	3.76 ^c ±0.1	4.30 ^b ±0.1
Unsaturated Fatty Acids	Mean values of unsaturated fatty acid in yoghurt samples%		
	A	B	C
Myristioleic	0.52 ^b ±0.4	1.73 ^a ±0.1	0.85 ^b ±0.1
Palmitioleic	2.76 ^a ±0.1	0.24 ^c ±0.2	1.07 ^b ±0.01
Oleic ω9	19.67 ^c ±0.5	26.80 ^a ±0.1	22.67 ^b ±0.5
Linoleic ω6	2.13 ^c ±0.1	32.03 ^a ±0.1	14.10 ^b ±0.1
α-Linolenic ω3	n.d	3.74 ^a ±0.1	1.23 ^b ±0.1

Different small letters in the same row refer to a significant difference at (p<0.05)

Different small letters in the same column refer to a significant difference at (p<0.05)

n.d: Not detected.Each sample were analyzed for each type of fatty acid using one-way ANOVA followed by post hoc L.S.D to see significant differences (p<0.05)

Table (3) showed that sample (B) soy milk yoghurt had the highest values of all unsaturated fatty acids except Palmetioleic acid, and the lowest values of all saturated fatty acids among the manufactured yoghurt samples followed by sample (C) the mixed yoghurt. Also soy milk yoghurt contained the highest level of the essential fatty acids; olic acid (26.80%±0.1), linoleic acid (32.03%±0.1), and α-linolinic acid (3.74%±0.1), followed by sample (C) the mixed yoghurt. Consequently for healthy therapeutic issues adding soy milk with a ratio of 50%, or 100% during manufacturing yoghurt had a good effect in lowering the ratio of saturated fatty acids, and raised the level of

unsaturated ones (Mazumder & Begum, 2016) [15], and provided the final product with essential fatty acids in a significant ratio, which might offer soy milk yoghurt as a good therapeutic diet; helps in limiting the high concentration of low density cholesterol (LDL), and prevents high blood pressure by serving high levels of unsaturated fatty acids, similler results was found with (Stradling *et al.*, 2013) [23].

Sensory Evaluation of Yoghurt Samples

Sensory evaluation of yoghurt samples, was carried out on the days (1, 7, 15) of storage period as shown in table (4).

Table 4: Sensory evaluation of yoghurt samples during storage period

Storage Period (days)	Treatments	Quality attribute				
		Color	Smell	Taste	Texture & Body	Overall acceptability
1	A	4.90 ^a ±0.3	4.20 ^a ±0.7	4.70 ^a ±0.4	3.60 ^b ±0.8	4.40 ^a ±0.6
	B	2.20 ^c ±0.4	3.60 ^b ±0.4	2.80 ^c ±0.4	5.00 ^a ±0	2.97 ^c ±0.2
	C	3.80 ^b ±0.4	4.10 ^{ab} ±0.3	4.10 ^b ±0.3	4.90 ^a ±0.3	4.00 ^b ±0.3
7	A	4.80 ^a ±0.4	3.70 ^a ±0.6	4.20 ^a ±0.4	3.60 ^b ±0.8	4.08 ^a ±0.3
	B	1.90 ^c ±0.8	3.00 ^b ±0.4	2.70 ^b ±0.6	5.00 ^a ±0	3.15 ^b ±0.2
	C	3.00 ^b ±0.8	3.50 ^{ab} ±0.5	4.10 ^a ±0.5	4.90 ^a ±0.3	3.87 ^a ±0.3
15	A	4.60 ^a ±0.5	3.70 ^a ±0.6	4.10 ^a ±0.3	3.60 ^b ±0.8	4.03 ^a ±0.3
	B	1.90 ^c ±0.8	3.00 ^b ±0.5	2.70 ^b ±0.6	5.00 ^a ±0	3.10 ^b ±0.2
	C	3.00 ^b ±0.8	3.50 ^{ab} ±0.5	4.00 ^a ±0.4	4.90 ^a ±0.3	3.85 ^a ±0.3

Different small letters in the same column refers to a significant difference at ($p < 0.05$)

Evaluation of taste, and smell in sample (A) got the highest scores (4.70 ± 0.4 , 4.20 ± 0.7) respectively, followed by sample (C) which had the score of (4.10 ± 0.3) for taste and smell through storage period. and it was not unpredicted that soymilk yoghurt had the lowest scores for taste (2.80 ± 0.4) inspite of the positive effect of lactic acid bacteria (Prbiotics) and *Bifidobacterium* on the resultant fermented soymilk as they improved and reduced the undesirable beany flavor caused by n-hexanal, and pentanal this result was similar to that reported by (Sebastian *et al.*, 2018) [19]. For color indicator sample (A) also had the highest scores (4.90 ± 0.3) compared to other yoghurt samples, sample (A) had a white color till the end of storage period, while sample (B) had a yellowish creamy color and gained the lowest scores for color, and smell evaluation. Obviously, color of soymilk yoghurt was less favorable because of lecithin; a brown yellowish phospholipid, similar results recorded by (Choudhary *et al.*, 2013) [5]. Contrarily, for texture evaluation sample (B) had the highest evaluation for texture (5.00 ± 0) followed by sample (C) (4.90 ± 0.3), it was noted that when the proportion of soymilk in the manufactured yoghurt increases, the more firm the texture of the yoghurt was achieved and the separation of yoghurt serum decreased because globulin; the major soy milk protein whose molecules are small, can entrap water inside the matrix firmly more than large casein molecules of cow milk protein which have large spaces in between and caused more separating of yoghurt serum, this result conforms with what stated by (Stephen *et al.*, 2017) [22].

Conclusion

Soy milk yoghurt is a good non-dairy alternative to cow milk yoghurt. It has functional and healthy effects since *Bifidobacterium bifidum*, added a clear effect in prolonging shelf- life of yoghurt by the ability to survive alive till the end of storage period, and save the vital force of the end product at the level of probiotic functional products count (10^6 cfu/g). Besides it gave acceptable results in sensory properties; taste, smell, and removed the beany flavor of soy milk in the final product. Soy yoghurt had the highest ratio of protein, unsaturated fatty acids which are needed to fence human's health, also it has more firm texture, but less desirable taste that can be modified with natural flavors, and agent colors to get a functional, tasty therapeutic product.

Conflicts of interest

Authors state no conflicts of interest.

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