



Effect of Fortification with Guava Pulp on Some Properties of Bio-Rayeb Milk Made from Goat's Milk

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Abstract

Guava fruit has several vital vitamins, minerals, and antioxidant that play a pivotal role in the prevention of cancers, aging, and infections. Also fermented dairy products have several nutritional and health benefits. Six treatments of bio-Raybe milk were made from goat's milk fortified with 5% sugar and 3, 6, 9, 12 and 15% guava pulp with using ABT-5 culture. Adding guava pulp to goat's milk improved starter activity, decreased coagulation time and syneresis and increased curd tension and water holding capacity.

Guava Raybe milk had slight low acidity, fat and saturated fatty acids and higher carbohydrate, total solids, dietary fiber, ash, total protein, water soluble nitrogen, total volatile fatty acids, unsaturated fatty acids, oleic, linoleic, α -linolenic acids and antioxidant activity values as compared with control. Guava Raybe milk also had the highest numbers of *Str. thermophilus*, *L. acidophilus* and *Bifidobacterium* and the lowest loss of viability ratios. The recommended level of 10^7 cfu.g⁻¹ of bifidobacteria as a probiotic was exceeded for various Raybe milk treatments and remained above 10^7 cfu g⁻¹ until the end of storage. Adding 6 or 9% guava pulp to goat's milk highly improved the sensory properties of Raybe milk.

Keywords: Goat's Milk; ABT; Guava Pulp; Raybe Milk

Introduction

Nutritional value of goat's milk is higher while allergen city is lower than that of cow's milk, especially in non-sensitized children [1], which led to an increased interest in goat's milk as a functional food, and it now forms a part of the current trend to healthy eating in developed countries [2,3]. Thus, using of milk with particular nutritional properties (e.g., goat's milk), alone or after fermentation with bacterial strains having probiotic properties and producing physiologically active metabolites, represents one of the technology options for manufacturing new dairy functional food [4]. Unfortunately, goat's milk isn't preferable for many of Egyptians because of its goats flavour.

Because of its highly nutritional and healthy benefits, fermented dairy products fortified with fruits received a lot of interest in recent decades. The use of different fruits and additives in fruit yogurt production has improved its nutritional and sensory properties [5]. Peaches, cherries, apricots, and blueberries are frequently used in yogurt production [6]. Guava is a rich source of vitamin C, pectin and good source of calcium and phosphorous. It is referred as 'apple of plains' due to its high nutritive value with mild flavour [7].

Traditional fermented dairy products vary considerably in composition, flavour and texture, depending on the properties of the fermenting organisms, type of milk, region of production and method of manufacture. These products have always proved very popular with consumers. Rayeb milk is one such popular indigenous dairy drink. It plays a major role in the diet of rural communities [8]. Rayeb milk is traditionally made from the raw milk of cows, ewes or goats, placed in earthenware pots and kept undisturbed without temperature control for 24h. It is produced through spontaneous fermentation of the milk, and is sometimes started via back slopping (inoculation of raw milk with a small quantity of the previous successful fermentation). On an industrial scale, it is produced from pasteurized cows' milk, with the addition of starter cultures and rennet. So, the purpose of this study was firstly, to produce bio-Rayeb milk using goat's milk and ABT-5 culture and secondly, to improve the sensory properties of fermented goat's milk by adding guava pulp.

Material and Methods

Materials

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

Agricultural Research Center, Egypt. ABT culture (ABT-5) with mixed strains of *S. thermophilus* (as sole fermenting organism) and LA+ *Bifidobacterium* BB-12 (as probiotic organisms) (Chr. Hansen's Lab A/S Copenhagen, Denmark) were used. The starter culture was in freeze-dried direct-to-vat set form. After procurement, the starter cultures were stored at -18°C in the absence of atmospheric air. Guava (*Psidium guajava* L.) pulp was obtained from Masr Italia for food Industries at a factory of fruit pulps in Damietta El-Jadida city, Damietta Governorate, Egypt. Guava pulp had pH 4.34, acidity (% citric acid) 0.25%, total soluble solids 8.7 (° Brix), total sugar 5.85%, ash 0.35%, and pectin 1.79%. Sugar was obtained from local market in Damietta Governorate, Egypt.

Methods

Preparation of Rayeb milk

Six treatments of Rayeb milk were made from goat's milk and guava pulp as follows:

- A: Rayeb milk made from goat's milk (control)
- B: Rayeb milk made from goat's milk + 5% sugar + 3% guava pulp
- C: Rayeb milk made from goat's milk + 5% sugar + 6% guava pulp
- D: Rayeb milk made from goat's milk + 5% sugar + 9% guava pulp
- E: Rayeb milk made from goat's milk + 5% sugar + 12% guava pulp
- F: Rayeb milk made from goat's milk + 5% sugar + 15% guava pulp

After heating to 90°C for 15 min, milk of various treatments were immediately cooled to 45°C then control milk was inoculated with cultures (0.1 g/L of milk mix), incubated at 40°C for fully coagulation and stored at 4°C overnight. In other treatments, 5% sugar was mixed with milk then the mentioned amounts of guava pulp were added and blended at 2000 rpm for 3 min and carefully filtered. All milk samples were 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 preserved at 4°C for 14 days. Rayeb milk samples were analyzed when fresh and after 7 and 14 days of refrigerated storage.

Methods of Analysis

Rheological Analyses

The curd tension was determined using the method of Chandra sekhar, *et al* [9]. The used apparatus consisted of knives of constant weight (5 gm) has H shaped with a needle in the middle ending with a hook, and a wire crossing a freely rotating wheel attached to the knife at one end a pan (5 gm) at the other. The knife was placed in a 100 ml beaker. The milk was heated to 40°C and inoculated with starter, and then it was added to the beaker and in-

cubated at 40°C until coagulation. The curd tension was measured after holding the curd in the refrigerator overnight. It is expressed as weight in grams required removing the knife from the prepared curd.

The susceptibility to syneresis (STS) was measured as given by Kpodo, *et al.* [10] by placing 100 ml of fermented milk sample on a filter paper placed on top of a funnel. After 6 hours of drainage, the volume of the whey collected in a beaker was measured and used as an index of syneresis. The following formula was used to calculate STS:

STS (%) = $V1/V2 \times 100$, where: V1 = Volume of whey collected after drainage; V2 = Volume of fermented milk sample.

For test of starter coagulation time during Rayeb milk making, milk was inculcated with starts and incubated at 40°C then coagulation was noticed at 30 min intervals. Water holding capacity (WHC) was measured according to Yousef, *et al.* [11]. Twenty g sample of native fermented milk (NY) was centrifuged for 10 min at 669 rpm and supernatant was removed and weighted (whey expelled (WE)). The WHC % was defined as:

$$\text{WHC (\%)} = 100 \times \text{NY-WE/NY}$$

Chemical Analysis

Total solids, fat, total nitrogen and ash contents of samples were determined according to AOAC [12]. Titratable acidity in terms of % lactic acid was measured by titrating 10g 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; In-gold (now Mettler Toledo), Urdorf, Switzerland] connected to a pH meter (model H 18418; Hanna Instruments, Padova, Italy). Total dietary fiber (TDF) was calculated as described by AOAC [13]. Total carbohydrates were calculated by difference as described by Ceirwyn [14]. Water soluble nitrogen (WSN) of Rayeb milk was estimated according to Ling [15]. Total volatile fatty acids (TVFA) were determined according to Kosikowski [16]. 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* [17].

Determination of Fatty Acids Composition:

The extraction of milk fat was done using the method of Rose-Gottlieb using diethyl ether and petroleum ether (Methodenbuch, Bd. VI VDLUFA-Verlag, Darmstadt, 1985). After that the solvents were evaporated on a vacuumrotary evaporator. For obtaining methyl esters of the fatty acids, sodium methylate (CH₃ONa) was used [18]. The fatty acid composition of Rayeb milk was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC™- WAX, 30m, ID 0.25 mm, Film:0,25 µm.

Microbial Analysis

Rayeb milk samples were analyzed for *Streptococcus thermophilus* and *Lactobacillus acidophilus* counts according to the methods described by Tharmaraj and Shah [19]. The count of bifidobacteria was determined according to Dinakar and Mistry [20].

Sensory Properties Judging

Samples of Rayeb milk were organoleptically scored by the staff of the Dairy Department, Faculty of Agricultural, Damietta University. The score points were 50 for flavour, 35 for body and texture and 15 for colour and appearance, which give a total score of 100 points.

Results and Discussion

Physiochemical Composition of Goat's Milk and Guava Pulp Mixtures

Titrateable acidity, pH, redox potential (E_h), total solids, fat and total protein values of goat's milk mixed with various guava concentrations were presented in Table 1. The chemical composition of goat's milk was within the normal range. Blending of high levels of guava pulp (9, 12 and 15%) with goat's milk slightly increased acidity and E_h while decreased pH values. With increasing of guava pulp amounts added to goat's milk, total solids (TS) content raised but fat and total protein concentrations slightly lowered.

Treatments	Acidity (%)	pH values	E _h (mV*)	TS (%)	Fat (%)	TP (%)
A	0.17	6.66	33.4	13.42	4.5	3.78
B	0.17	6.65	33.6	15.56	4.4	3.71
C	0.17	6.66	33.3	17.11	4.3	3.70
D	0.18	6.64	35.1	18.95	4.1	3.66
E	0.18	6.64	35.2	20.86	4.0	3.62
F	0.18	6.63	35.4	23.14	4.0	3.57

Table 1: Chemical Composition of Goat's Milk and Guava Pulp Mixtures.

*mV: millivolts

Starter Activity in Goat's Milk Fortified with Guava Pulp

Acidity, E_h and pH values of goat's milk contained guava pulp and inoculated with ABT-5 starter were tested at 30 min intervals during fermentation period. Measurements were stopped after 240 min in all of samples. Results were taken as indicator for starter activity.

With progressive of fermentation, titrateable acidity and E_h levels gradually increased while pH values decreased in all milk treatments (Figure 1, 2 and 3). These changes of course are attributed to the activity of starter bacteria which ferment milk lactose to lactic acid. Similar results were reported by Samet-Bali, *et al.* [21] who stated that as Leben (Rayeb milk) starters grow during fermentation, they produce acid which causes a decrease in pH and increase in acidity values.

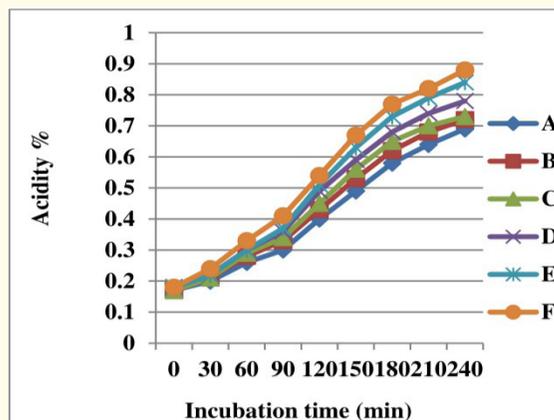


Figure 1: Changes in Acidity During Fermentation

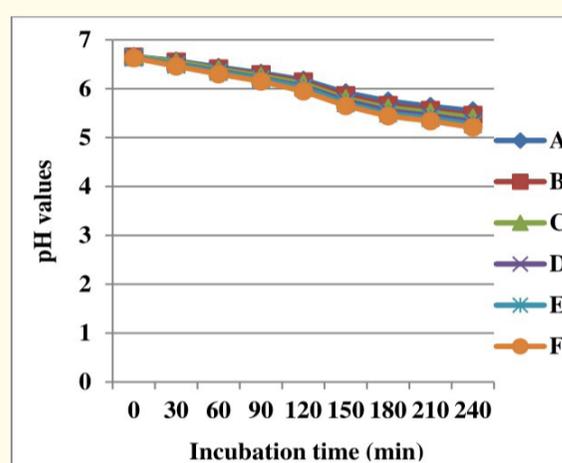


Figure 2: Changes in pH values during fermentation Of goat's milk and guava pulp mixtures of goat's milk and guava pulp mixtures.

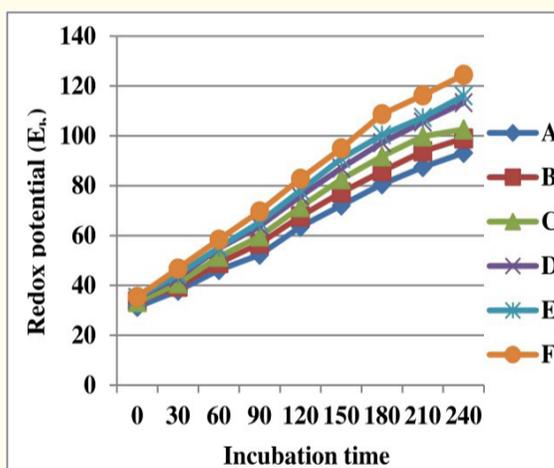


Figure 3: Changes in Redox Potential (E_h) during Fermentation of Goat's Milk and Guava Pulp Mixtures.

Acidity and E_h values and the development in both during 240 min of fermentation were higher in goat's milk supplemented with guava pulp than that of goat's milk only (control). Also, the reducing in pH was higher in the former than that of the latter. An increase in the concentration of guava pulp added positively affected the rate of acid production.

Coagulation time of goat's milk fortified with guava pulp

Because of stimulation effect of guava pulp on starter bacteria which caused faster acid production as cleared in Figure 1, 2 and 3, coagulation times of goat's milk fortified with guava pulp were shorter than that of control (Table 2). Coagulation times were reduced by 3.28, 6.56, 10.16, 14.10 and 16.39% for samples B, C, D, E and F respectively.

Rheological Properties of Fermented Goat's Milk Fortified with Guava Pulp

Curd tension

As previously mentioned in Table 1, mixing guava pulp with goat's milk increased total solids content. Therefore, curd tension values of goat's milk and guava pulp mixtures were higher as compared with control (Table 2). Curd tension values of different treatments slightly increased after 7 or 14 days of storage period, which may be due to moisture evaporation and total solids increasing through storage period. These results are in line with those of EL-Boraey, *et al.* [22].

Syneresis

Properties	Treatments	Storage period (day)		
		Fresh	7	15
Coagulation time (min)	A	305	-	-
	B	295	-	-
	C	285	-	-
	D	274	-	-
	E	262	-	-
	F	255	-	-
Curd tension (gm)	A	35.14	36.88	36.31
	B	36.80	37.96	37.50
	C	38.10	38.56	38.73
	D	40.20	40.06	40.11
	E	41.20	41.50	41.62
	F	44.60	44.02	44.41
Susceptibility to syneresis (%)	A	21.2	20.2	19.3
	B	20.1	18.4	17.5
	C	18.4	17.4	15.3
	D	15.1	13.6	12.4
	E	14.2	13.4	12.2
	F	12.2	11.1	10.9
Water holding capacity (%)	A	83.6	84.0	85.2
	B	84.9	85.3	87.1
	C	85.2	86.7	88.9
	D	88.4	88.9	89.6
	E	91.4	91.9	93.0
	F	92.3	93.8	94.9

Table 2: Effect of Adding Guava Pulp on Some Rheological Properties of Goat's Milk Coagulated with ABT Culture.

Table 2 shows the results of syneresis as measured by means of both drainage (susceptibility to syneresis) and centrifugal (water holding capacity) methods. Levels of susceptibility to syneresis (STS) were significantly ($P < 0.05$) low in fermented goat's milk

treatments contained guava pulp. The decrease in STS was inversely proportional with the increase in amounts of guava pulp added. Values of STS for fresh A, B, C, D, E and F samples were 21.2, 20.1, 18.4, 15.1, 14.2 and 12.2% respectively. In various fermented milk samples, STS values lowered during storage period.

On the contrary to STS, goat's milk contained guava pulp possessed the highest water holding capacity (WHC) levels. After 7 and 14 days of cold storage, values of WHC slightly increased in different treatments.

Chemical Composition of Rayeb Milk made from Goat's Milk Fortified with Guava Pulp

The changes in the titratable acidity (% lactic acid), pH, Eh, carbohydrate, total solids (TS) and fat contents of Rayeb milk as affected by incorporation of guava pulp are cleared in Table 3. The values of titratable acidity and Eh gradually increased within cold storage period of all treatments of Rayeb. The outcomes of the pH values followed an opposite trend to that observed for titratable acidity measurements, i.e., as the acidity increased, the pH decreased. This may be due to fermentation of lactose, which produces lactic and acetic acid during fermentation and storage period. These outcomes are consistent with those of Hamad, *et al.* [23].

Properties	Treatments	Storage period (days)		
		Fresh	7	14
Acidity %	A	0.74	0.99	1.17
	B	0.75	0.98	1.16
	C	0.72	0.94	1.11
	D	0.70	0.92	1.10
	E	0.68	0.90	1.07
	F	0.66	0.89	1.04
pH values	A	4.79	4.58	4.45
	B	4.77	4.59	4.47
	C	4.81	4.63	4.52
	D	4.84	4.67	4.54
	E	4.88	4.70	4.57
	F	4.91	4.73	4.59
E _h mV	A	151.3	178.1	192.2
	B	152.6	176.0	191.7
	C	150.8	173.7	189.7
	D	147.8	171.5	186.1
	E	145.2	170.0	185.4
	F	141.4	168.3	183.7

Carbohydrate %	A	4.61	4.44	4.22
	B	8.00	7.79	7.75
	C	9.78	9.54	9.42
	D	10.99	10.62	10.57
	E	13.84	13.77	13.44
	F	14.98	14.76	14.66
TS %	A	14.69	14.64	14.70
	B	18.04	18.16	18.10
	C	19.92	19.93	19.96
	D	21.08	21.03	21.07
	E	24.04	24.07	24.04
	F	25.38	25.40	25.37
Fat %	A	4.7	4.6	4.7
	B	4.5	4.6	4.4
	C	4.4	4.4	4.4
	D	4.2	4.3	4.2
	E	4.1	4.0	4.1
	F	4.1	4.1	4.0

Table 3: Effect of Adding Guava Pulp to Goat's Milk on Some Physicochemical Properties of Rayeb Milk.

Unexpectedly, the acidity and Eh levels of control Rayeb (treatment A) were slightly higher than that of Rayeb made from goat's milk blended with guava pulp (treatments B, C, D, E and F). Moreover, the developments in titratable acidity and Eh or drop in pH during storage were also higher in control Rayeb than that detected in goat's milk contained guava pulp. According to Celik and Bakirci [24], adding fruit juice decreases viscosity and the rate of acid enhancing, but increases whey separation of yoghurt. On the contrary, Güven and Karaca [25] reported that acidity increased parallel to the increase in fruit content. In their study, an increase in the acidity in ice creams was associated with probiotic cultures and pulp addition.

As a result of adding 5% sugar and also high carbohydrate content of guava pulp, treatments B, C, D, E and F characterized with high carbohydrate levels comparing with control (sample A). The same trend was observed for TS contents which increased by addition sugar and guava pulp. Total solids contents of samples A, B and C at the end of cold storage were 14.70, 18.10 and 19.96% respectively. Conversely, fat concentrations of guava pulp Rayeb were slightly lower than control. Anyway, the fat content of guava pulp Rayeb milk was not far from that of commercial Rayeb milk available in the markets. The obtained results were in accordance with that found by Kavas and Kavas [26] who noticed that the in-

crease in dry matter of probiotic frozen yoghurt contained 20% guava pulp (GPFY) was higher than in plain probiotic frozen yoghurt (PFY). The increase in acidity measured in GPFY on the 1st and the 120th days of the storage was higher than those measured in PFY. Fat values in GPFY were lower than those in PFY. Protein values decreased in GPFY and PFY samples during storage. The highest decrease was measured in GPFY.

During storage period carbohydrate content of different samples gradually lowered which due to fermentation process. In contrast, total solids and fat contents were almost constant through storage.

It can easily be noted from Table 4 that there is a substantial impact of the supplementation with guava pulp on dietary fiber, ash, total nitrogen, total protein, WSN and TVFA contents of Rayeb milk. Because of using goat's milk only in its preparing, sample A (control) was free from dietary fiber. On the other side utilization guava pulp in Rayeb milk production slightly increased the dietary fiber content. In the same trend, significant increases in ash, total nitrogen, total protein, WSN and TVFA contents were obtained with incorporation of guava pulp in Rayeb milk. Buriti., *et al.* [27] showed that pulp of guava was the sole source of dietary fiber in beverages made from mixture of fermented whey of goat's milk and guava. Some other studies also reported that protein values of fermented milk increased as the fruit pulp ratio increased [28]. Bisla., *et al.* [29], in their study on ice cream fortified with soy milk, watermelon seed milk and guava pulp (50g), associated high protein (11.12%) and fat (7.26%) values with soy milk and watermelon seed milk.

Properties	Treatments	Storage period (days)		
		Fresh	7	14
Dietary Fiber %	A	0	0	0
	B	0.051	0.050	0.053
	C	0.105	0.107	0.106
	D	0.162	0.165	0.166
	E	0.204	0.201	0.203
	F	0.251	0.250	0.254
Ash %	A	0.95	0.93	0.96
	B	0.97	0.96	0.97
	C	0.98	0.99	0.97
	D	1.01	1.00	1.02
	E	1.04	1.03	1.05
	F	1.07	1.06	1.08

TN %	A	0.579	0.577	0.572
	B	0.591	0.593	0.590
	C	0.616	0.619	0.620
	D	0.630	0.631	0.629
	E	0.655	0.653	0.656
	F	0.678	0.680	0.681
TP %	A	3.69	3.68	3.65
	B	3.77	3.78	3.76
	C	3.93	3.95	3.95
	D	4.02	4.02	4.01
	E	4.18	4.17	4.18
	F	4.32	4.34	4.34
WSN %	A	0.133	0.182	0.199
	B	0.146	0.198	0.220
	C	0.157	0.211	0.237
	D	0.164	0.231	0.246
	E	0.175	0.249	0.263
	F	0.185	0.261	0.278
TVFA*	A	7.2	10.5	12.3
	B	7.3	10.9	12.8
	C	7.8	11.1	13.4
	D	7.9	11.3	13.7
	E	8.1	11.7	14.0
	F	8.4	12.0	14.5

Table 4: Effect of Adding Guava Pulp to Goat's Milk on Some Chemical Properties of Rayeb Milk.

General spiking, dietary fiber, ash, total nitrogen, total protein contents of different Rayeb milk samples nearly didn't change during storage period. On the contrary, WSN contents gradually increased in various Rayeb milk treatments as storage period advanced which may be due to degradation of protein by activity of starter bacteria. The increasing levels of WSN during storage were higher in guava Rayeb than control.

Values of WSN development through storage for samples A, B, C, D, E and F were 49.62, 50.68, 50.95, 50.00, 50.28 and 50.27% respectively. These results might be interpreted to mean that guava pulp components have stimulation effect on proteolysis bacteria. As a result of starter bacteria activity and secretion of lipase, TVFA concentrations gradually increased in all Rayeb milk samples during storage period. Also, the increasing rates were higher in guava Raybe milk.

Free Fatty Acids Content (FFA) of Rayeb Milk

Free fatty acids (FFA) are generated by both lipolytic processes (C₄ - C₂₀) and bacterial fermentation (C₂ -C₄). Quantification of the

levels of short-chain FFAs would be important since their concentration can cause flavor changes and defects in milk based foods [30]. The FFA profile in fresh Rayeb milk was illustrated in Tables 5 and 6.

Saturated and Unsaturated Fatty Acids

The saturated fatty acids (SFA) values of various Rayeb milk samples were inversely proportional with the amount of unsaturated fatty acids (USFA). The concentration of SFA was higher than USFA in all treatments. Values of SFA and USFA for sample A were 72.62 and 26.99 (as percent of total fat) respectively.

It could be viewed from Tables 5 and 6 that fortification of Rayeb milk with guava pulp caused a markedly decrease in SFA and increase in USFA contents. Levels of SFA were 72.62 and 66.58% for samples A and B respectively. Based on these results, combination of guava pulp, ABT culture and goat's milk in one fermented dairy product like Rayeb milk greatly lowered SFA content whereas highly increased USFA concentration. Decreasing of SFA and increasing USFA values in bio-Rayeb milk obtained in our study raise the healthy benefit of this product because it is well known that unsaturated fatty acids are more important in human nutrition.

Fatty acids	C	Treatments					
		A	B	C	D	E	F
		Saturated fatty acids (SFA) %					
Caprylic	8:0	2.39	2.33	2.29	2.11	2.18	2.04
Capric	10:0	9.61	8.35	8.10	8.07	8.10	7.99
Undecanoic	11:0	0.30	0.25	0.24	0.18	0.18	0.21
Lauric	12:0	3.79	3.30	3.25	3.20	3.24	3.38
Myristic	14:0	10.3	9.10	8.75	8.38	8.49	8.27
Pentadecanoic	15:0	1.64	1.73	1.74	1.75	1.76	1.48
Palmitic	16:0	34.20	30.98	30.80	29.90	29.23	28.94
Heptadecanoic	17:0	1.36	2.20	2.00	2.64	2.52	2.64
Stearic	18:0	8.82	8.10	8.13	8.07	7.97	7.94
Arachidic	20:0	0.21	0.24	0.23	0.24	0.19	0.26
Total		72.62	66.58	65.53	64.54	63.86	63.15
		Unsaturated fatty acids (USFA) %					
5-Tetradecenoic (phytosteric)	14:1 ω5	0.17	0.17	0.16	0.16	0.17	0.18
	16:1 ω5	0.50	-	-	-	-	-

	16:1 ω7	1.13	1.20	1.30	1.44	1.21	1.37
Palmitioleic	16:1 ω9	0.45	0.51	0.51	0.67	0.61	0.66
Hexagonic	16:3 ω4	0.33	0.35	0.35	0.46	0.38	0.43
Octadecosaenoic	18:1 ω5	0.13	0.19	0.20	0.56	0.55	0.55
Vaccenic	18:1 ω7	0.37	0.67	0.67	0.15	0.16	0.16
Oleic	18:1 ω9	21.30	25.76	26.30	25.54	26.21	27.24
	18:2 ω4	-	0.18	0.18	0.30	0.37	0.25
	18:2 ω5		-	-	0.31	0.33	0.21
Linoleic	18:2 ω6	2.30	3.10	3.11	3.69	3.75	3.70
	18:2 ω7	-	0.21	0.21	0.60	0.60	0.50
α-Linolenic	18:3 ω3	0.14	0.22	0.23	0.25	0.26	0.29
Gamma linolenic	18:3 ω6		-	-	0.23	0.24	0.12
Octadecatetraenoic	18:4 ω3	-	0.41	0.44	0.42	0.62	0.66
Arachidonic	20:4 ω6	0.17	0.20	0.20	0.24	0.23	0.26
Total		26.99	33.17	33.86	35.02	35.69	36.58
Non identified fatty acid		0.39	0.25	0.61	0.44	0.45	0.27

Table 5: Effect of adding guava pulp to goat's milk on free fatty acids (%) of fresh Rayeb milk.

Treatments	SFA	USFA	MUFA	PUFA	SCFA	MCFA	LCFA
A	72.62	26.99	24.05	2.94	16.09	48.72	34.80
B	66.58	33.17	28.50	4.67	14.23	44.04	41.48
C	65.53	33.86	29.14	4.72	13.88	43.61	41.90
D	64.54	35.02	28.52	6.50	13.56	42.70	43.30
E	63.86	35.69	28.91	6.78	13.70	41.85	44.00
F	63.15	36.58	30.16	6.42	13.62	41.33	44.78

Table 6: Effect of adding guava pulp to goat's milk on free fatty acid indices ratios of fresh Rayeb milk.

SFA: saturated fatty acids; USFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids (C:1); PUSFA: polyunsaturated fatty

acids (C:2+ C:3+ C:4); SCFA: short chain fatty acids (C₈ to C₁₂); MCFA: medium chain fatty acids (C₁₃ to C₁₆); LCFA: long chain fatty acids (> C₁₆).

Generally, the most predominant SFA found in various Rayeb milk samples was palmitic acid (C₁₆) followed by capric acid (C₁₀). The highest acid ratio of USFA was oleic acid (18:1 ω9) followed by linoleic acid (18:2 ω6).

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

Values of MUSFA and PUSFA markedly affected with using guava pulp in manufacturing of Rayeb milk. Guava Rayeb milk had higher contents of MUSFA than that of control one. Unexpectedly, PUSFA values of guava Rayeb milk nearly doubled two or three times from those detected in control Rayeb (sample A).

In different Rayeb milk samples, MUSFA values were considerably higher than those of PUSFA. Oleic acid was found to have the greatest concentration of MUSFA in various Rayeb samples. The dominant fatty acid of PUSFA was linoleic acid.

As it is well known, omega fatty acids are a group of essential fatty acids very important for human health.

Rayeb made from goat's milk supplemented with guava pulp characterized by high contents of α-linolenic acid (omega-3), linoleic acid (omega-6) and oleic acid (omega-9) as compared with Rayeb made from just goat's milk. The values of these fatty acids gradually increased in Rayeb milk by increasing of guava pulp amounts mixed with goat's milk. Fatty acids are long chain or short chain organic acids with a terminal carboxylic acid group. Certain fatty acids like palmitic and oleic can be synthesized in the body, while fatty acids like the polyunsaturated fatty acids (PUFA) need to be given through the diet. The human body does not have any mechanism for synthesizing polyunsaturated fatty acids [31], the essential fatty acids that include linoleic and linolenic acids. These fatty acids must therefore have to be supplied from external sources through the diet.

Short Chain Fatty Acids (C8-C12)

A difference in the concentrations of short-chain fatty acids was noticed between the experimental and the control Rayeb at the beginning of storage period. Utilization of guava pulp in Rayeb milk preparation reduced the levels of SCFA. The values of SCFA in treatments A, B, C, D, E and F were 16.09, 14.23, 13.88, 13.56,

13.70 and 13.62% respectively. Because Rayeb milk was made from goat’s milk, the fatty acid capric acid (C10:0) was the predominant SCFA followed by lauric (C:12) in all samples.

Medium Chain Fatty Acids (C13-C16)

Medium chain fatty acids (MCFA) of Rayeb milk had the same trend of SCFA where using of guava pulp in manufacturing led to decreasing of the content from these fatty acids. Values of MCFA for samples A, B, C, D, E and F were 48.72, 44.04, 43.61, 42.70, 41.85 and 41.33% respectively. In different Rayeb treatments, the concentration of palmitic acid (C16) was the highest of medium chain fatty acids and was followed by myristic acid (C14).

Long Chain Fatty Acids (> C16)

The levels of long chain fatty acids (LCFA) were higher in guava Rayeb milk as compared with those of control. Among all the long chain fatty acids measured, the value of oleic acid was the highest in various Rayeb samples.

Antioxidants Activity of Rayeb Milk

Ratios of antioxidant activity of fresh Rayeb milk made from goat’s milk fortified with or without guava pulp were tabulated in Table 7. Guava Rayeb milk contained higher antioxidant activity percentages than those found in control Rayeb. The antioxidant effect in Rayeb milk increased with rising guava pulp levels added to milk. Values of antioxidants activity of fresh samples A, B, C, D, E and F were 42.16, 47.23, 54.14, 60.89, 70.56 and 75.11% respectively. Jimenez-Escrig, *et al.* [32] stated that peel and pulp of Psidium guajava fruit presented high levels of dietary fiber, indigestible fraction, and phenolic compounds.

Treatments	Antioxidant activity (DPPH inhibition %)
A	42.16
B	47.23
C	54.14
D	60.89
E	70.56
F	75.11

Table 7: Effect of Adding Guava Pulp to Goat’s Milk on Antioxidant Activity of Fresh Rayeb Milk.

There was a statistically significant correlation between estimated extractable phenol content and radical scavenging activity/

ferric reducing power. These bioactive compounds contributed significantly to the high antioxidant capacity of guava fruit as measured by these methods. In the same trend, Musa, *et al.* [33] reported that pink guava shows valuable nutraceutical properties in terms of high antioxidant activity as well as vitamin C and lycopene. The flavonoid content is reported with kaempferol as the main flavonoid compound. Moreover, since these fruits show the highest antioxidant content in the peel, they seem to be particularly suitable for unpeeled whole fresh fruit consumption and thus promote health related benefits.

Changes in Microbial Counts of Rayeb Milk during Storage

Data cleared in Table 8 represent the counts of *Str.thermophilus*, *L. acidophilus* and *Bifidobacterium* of Rayeb milk supplemented with guava pulp.

Bacteria	Treatments	Storage period (day)		
		Fresh	7	15
<i>Streptococcus thermophilus</i> (cfu×x10 ⁷ /g)	A	86	78	65
	B	99	87	76
	C	134	123	112
	D	153	139	128
	E	169	157	146
	F	188	177	169
<i>Lactobacillus acidophilus</i> (cfu×x10 ⁵ /g)	A	60	51	45
	B	77	68	62
	C	90	81	77
	D	117	110	102
	E	128	123	119
	F	134	127	121
<i>Bifidobacterium</i> (cfu×x10 ⁵ /g)	A	21	14	10
	B	30	25	23
	C	40	35	29
	D	51	45	41
	E	59	56	50
	F	63	58	54

Table 8: Effect of Adding Guava Pulp to Goat’s Milk on Starter Bacteria Counts of Rayeb Milk.

The counts of *Str.thermophilus* increased in Rayeb milk samples fortified with guava pulp as compared with control. The greatest counts of *Str.thermophilus* were observed in Rayeb milk contained 15% guava pulp (sample F). To the contrary, losses of viability levels of *Str. thermophilus* during storage were lower in guava Rayeb milk than that of control. Values of loss of viability for

samples A, B, C, D, E and F were 24.42, 23.23, 16.42, 16.34, 13.61 and 10.11% respectively. The increase in the cell counts and the survival of culture in the fruit beverage makes it suitable as a probiotic fruit beverage [34].

Utilization of guava pulp in Rayeb milk manufacture increased the numbers of *L. acidophilus* in fresh product and during storage period. In addition to this, guava Rayeb milk possessed the lowest levels of survival loss during storage. Values of loss of survival through storage were 25.00, 19.48, 14.44, 12.82, 7.03 and 9.70% for samples A, B, C, D, E and F respectively. Bramari, et al. [35] showed that the probiotic Guava fruit beverage contained lactic acid bacteria could serve as a healthy beverage for consumers with dairy allergy, beneficial to gut health, prevention of diarrhea and best nutrient source for under nourished vulnerable population.

It has been reported that the addition of fruit juices or pulps might be deleterious to the survivability of some species and strains of probiotic microorganisms in food products, particularly due to acidity and the presence of antimicrobial compounds [36,37]. In the present study, however, it was verified that *Bifidobacterium* maintained good viability in the presence of guava pulp. Rayeb milk contained guava had the highest numbers and the lowest loss of viability levels of *Bifidobacterium*. Loss of viability rates during 14 days of storage for samples A, B, C, D, E and F were 52.38, 30.43, 27.50, 19.61, 15.2 and 14.28% respectively. This may be attributed to two reasons, first is high acid production and accumulation in control Rayeb milk and second provide a lot of nutrients in guava Rayeb milk. The variances in survival were interpreted by the metabolic activity of *Bifidobacterium* in different fermented products, which might be affected by the composition and availability of nitrogen and carbon sources in growth media [38]. Buriti, et al. [27] reported that fermented whey-based goat beverages prepared using *Str. thermophiles* TA-40 as starter culture, with added guava pulp, showed to be good vehicles for *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus rhamnosus* Lr-32, maintaining their viability above 7 log CFU/ml during 21 days.

In different Rayeb milk samples, there were pronounced reduction in viable counts of *Str. thermophilus*, *L. acidophilus* and *Bifidobacterium* during storage which due to the accumulation of acids or reduction of availability of nutrient required for the growth [39]. Giyarto, et al. [40] reported that during storage 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 [41]. Akalin, et al. [42] 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. [43] 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.

However, lowering in counts during the entire storage period, but the viability of bifidobacteria in guava Rayeb milk was always above the minimum recommended level of 107cfu.g-1, suggested for beneficial health effects in the gut. The number of probiotic bacteria in the food should be at least 107cfu/ml or per g at the time of consumption in order to exert beneficial effects in the host [44].

From the results of Table 8, plus outcomes of FFA, and antioxidants activity in Tables 5, 6 and 7, it can be said that using of probiotics in fermentation of goat's milk supplemented with guava pulp give very health fermented dairy product.

Changes in Sensory Evaluation of Rayeb Milk during Storage

Fermentation of goat's milk may serve as one possibility that can improve sensory properties and consumption. Table 9 clears the organoleptic properties of Rayeb milk manufactured from goat's milk and fortified with various amounts of guava pulp. Taking into account adding both of sugar and guava pulp to goat's milk, it was noticed that the sensory evaluation scores were generally improved.

Properties	Treatments	Storage period (day)		
		Fresh	7	15
Color and Appearance (15)	A	14	14	13
	B	14	14	13
	C	14	14	13
	D	13	13	12
	E	13	13	12
	F	13	13	11

Body and Texture (35)	A	33	33	31
	B	33	33	32
	C	34	34	33
	D	34	34	32
	E	33	32	30
	F	31	29	26
Flavor (50)	A	42	42	41
	B	46	46	45
	C	48	48	47
	D	48	48	48
	E	47	47	46
	F	46	45	43
Total (100)	A	89	89	85
	B	93	93	90
	C	96	96	93
	D	95	95	92
	E	93	92	88
	F	90	87	80

Table 9: Effect of Adding Guava Pulp to Goat's Milk on Sensory Evaluation of Raybe Milk.

Scores of color and appearance attributes tested on the first day and during storage period were slightly lower for Rayeb milk contained 9, 12, 15% guava pulp as compared with control. This may be due to the simple change of white color of Rayeb milk. As it is known, Egyptian consumers prefer fermented dairy products that have bright white color.

The addition of guava pulp enhanced the body and texture of Rayeb milk, since trials C and D exhibited higher scores than other samples for this attribute. The scores of body and texture for trials A and B were low in comparison with treatments C and D probably due to a low concentration of total solids. Nonetheless, samples E and F which had higher TS levels also gained low scores of body and texture. These trials were perceived as "sticky", while Rayeb C and D were perceived as "creamy".

In addition to fermentation, fortification of Rayeb milk with guava pulp improved the flavour evaluation scores. When compared with plain (control) Rayeb treatment, guava Rayeb samples were preferred by the panelists that tasted the samples who attributed that to the sweet taste of guava comparing with goaty taste non preferable for the Egyptians. The addition of fruits and fiber ingredients appears as alternative to improve the sensory acceptability of whey-based goat beverages [45], besides contributing with nutrients which are not contained in milk, particularly dietary fiber [37].

Treatment F clearly varied from other trials concerning the scores of flavour. It gained the lowest scores however high amounts of guava pulp added. Sample F presented higher carbohydrate content in relation to the other trials (Table 3), and therefore, this parameter influenced negatively its flavour. Walkunde., *et al.* [7] stated that the average score for flavour, body and texture, acidity, colour and appearance of yoghurt contained 5, 10 and 15% guava pulp differed significantly ($P < 0.05$) from control sample. Sample 5% pulp and 6% sugar had good blend of natural flavour of guava and yoghurt, desired body, smooth texture with attractive colour and appearance and hence, rated best among all treatments including control sample.

The sensory parameters evaluated (color, appearance, body, texture and flavour) negatively changed throughout the storage period in all Rayeb samples. Our results are in agreement with Ahmed [46] who cleared that significant ($p < 0.001$) decreases in the total organoleptic scores of bio-Rayeb milk were noticed when storage period progressed.

Conclusion

Supplementation of Rayeb milk with guava pulp increased the total solids, dietary fiber, unsaturated fatty acids, oleic (omega 9), linoleic (omega 6), α -linolenic (omega 3) acids contents which increased its nutritional value. Blending 6 or 9% guava pulp with goat's milk highly improved the sensory evaluation scores of Rayeb milk. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were greatly activated by mixing guava pulp with Rayeb milk which main that guava pulp could be utilized as sweeter and prebiotic in bio-Rayeb milk production.

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Bibliography

1. Albenzio M and Santillo A. "Biochemical characteristics of ewe and goat milk: Effect on the quality of dairy products". *Small Ruminant Research* 101 (2011): 33-40.
2. Olalla M., *et al.* "Nitrogen fractions of Andalusian goat milk compared to similar types of commercial milk". *Food Chemistry* 113.3 (2009): 835-838.
3. Abbas HM., *et al.* "Physicochemical Characteristics of Goat's Milk". *Life Science Journal* 11 (2014): 307-317.
4. Minervini F., *et al.* "Fermented goats milk produced with se-

- lected multiple starters as a potentially functional food". *Food Microbiology* 26.6 (2009): 559-564.
5. Çakmakç S., et al. "Probiotic properties, sensory qualities, and storage stability of probiotic banana yogurts". *Turkish Journal Veterinary and Animal Sciences* 36.3 (2012): 231-237.
 6. Arslan S and Özels. "Some properties of stirred yoghurt made with processed grape seed powder, carrot juice or a mixture of grape seed powder and carrot juice". *Milchwissenschaft* 67.3 (2012): 281-285.
 7. Walkunde TR., et al. "Sensory quality of yoghurt from cow milk by utilizing guava fruit". *Asian Journal of Animal Science* 3.2 (2008): 99-102.
 8. Benkerroum N and Tamine AY. "Technology transfer of some Moroccan traditional dairy products (Iben, jbenand smen) to small industrial scale". *Food Microbiology* 21.4 (2004): 399-413.
 9. Chandrasekhara MR., et al. "The use of mammalian milk and processed milk foods in the feeding of infants". *Indian Journal of Child Health* 6 (1957): 701-710.
 10. Kpodo KFM., et al. "Effect of Ingredient Variation on Microbial Acidification, Susceptibility to Syneresis, Water Holding Capacity and Viscosity of Soy-Peanut-Cow Milk Yoghurt". *Journal of Nutritional Health and Food Engineering* 1.2 (2014): 1-6.
 11. Yousef M., et al. "Effect of different concentration of fruit additives on some physicochemical properties of yoghurt during storage". *Annals of Biological Research* 4.4 (2013): 244-249.
 12. AOAC. "Association of Official Analytical Chemists. Official Methods of Analysis". 17th ed, Washington, DC, USA, 2000.
 13. AOAC. "Association of Official Analytical Chemists. Official Method of Analysis". 18th ed, Washington, DC, USA, 2005.
 14. Ceirwyn SJ. "Analytical Chemistry of Foods". Part I in Book.p.1 English. Published London 1995.
 15. Ling ER. "A Text - Book of Dairy Chemistry". Vol. 2, Practical, 3rd ed., Champan and Hall, London, England, 1963.
 16. Kosikowski FV. "Cheese and Fermented Milk Foods". 2nd ed. Cornell Univ. Ithaca, New York (1978).
 17. Olivera P, et al. "Chemical composition and antioxidant activity of essential oils of twelve spice plants". *Croatica Chemica Acta Journal* 79.4 (2006): 545-552.
 18. Jahreis GJ., et al. "Conjugated linoleic acid in milk fat: High variation depending on production system". *Nutrition Research* 17.9 (1997): 1479-1484.
 19. Tharmaraj N and Shah NP. "Selective Enumeration of Lactobacillus delbrueckii sp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and Propionibacteria". *Journal of Dairy Science* 86.7 (2003): 2288-2296.
 20. Dinakar P and Mistry VV. "Growth and viability of Bifidobacterium bifidum in Cheddar cheese". *Journal of Dairy Science* 77.10 (1994): 2854-2864.
 21. Samet-Bali O., et al. "Development of fermented milk "Leben" made from spontaneous fermented cow's milk". *African Journal of Biotechnology* 11.8 (2012): 1829-1837.
 22. EL-Boraey NA., et al. "Chemical composition, sensory evaluation, rheological properties and starter activity of admixtures of buffalo's, cow's and soymilk". *American Journal of Food Science and Nutrition Research* 2.4 (2015): 119-127.
 23. Hamad EH., et al. "Quality aspects of the Sudanese fermented milk (Robe) supplemented with gum Arabic powder". *Discourse Journal of Agriculture and Food Sciences* 1.1 (2013): 8-7.
 24. Celik SF and Bakirci I. "Some properties of yoghurt produced by adding mulberry pekmez (concentrated juice)". *International Journal of Dairy Technology* 56.1 (2003): 50-57.
 25. Güven M and Karaca OB. "The effects of the varying sugar content and fruit concentration on the physical properties of vanilla and fruit ice-cream type frozen yoghurts". *International Journal of Dairy Technology* 55.1 (2002): 27-31.
 26. Kavas N and Kavas G. "Probiotic frozen yoghurt production using camel milk (Camelus dromedarius) with improved functions by strawberry guava (Psidium littorale var. cattleianum) Fortification". *British Journal of Applied Science and Technology* 14.5 (2016): 1-12.
 27. Buriti FCA., et al. "Effects of tropical fruit pulps and partially hydrolysed galactomannan from Caesalpinia pulcherrima seeds on the dietary fibre content, probiotic viability, texture and sensory features of goat dairy beverages". *LWT - Food Science and Technology* 59.1 (2014): 196-203.
 28. Aliyev C. "Effect of physicochemical, sensory and microbiological properties of ice cream use of Blueberry and kefir". Master Thesis, Nineteen May University of Science and Technology Institute, Food Engineering Department 70 (2006).
 29. Bisla G., et al. "Development of ice creams from soybean milk and watermelon seeds milk and evaluation of their acceptability and nourishing potential". *Advances in Applied Science Research* 3.1 (2012): 371-376.
 30. Güler Z and Park YW. "Characteristics of physico-chemical properties, volatile compounds and free fatty acid profiles of

- commercial set-type Turkish yoghurts, Chemical properties of Turkish yoghurts". *Journal of Animal Sciences* 1.1 (2011): 1-9.
31. How JSL and Young CT. "Comparison of Fatty Acid Content of Imported Peanuts". *Journal of the American Oil Chemists' Society* 60.5 (1983) 945- 947.
 32. Jimenez-Escrig A., et al. "Guava Fruit (PsidiumguajavaL.) as a New Source of Antioxidant Dietary Fiber". *Journal of Agriculture and Food Chemistry* 49.11 (2001): 5489-5493.
 33. Musaa KH., et al. "Flavonoid profile and antioxidant activity of pink guava". *ScienceAsia* 41 (2015): 149-154.
 34. Yoon KY., et al. "Production of probiotic cabbage juice by lactic acid bacteria". *BioresTechnology* 97.12 (2005): 1427-1430.
 35. Bramari SG., et al. "Formulation of Guava Fruit Beverage with Isolated Probiotic Strains". *Research Journal Science and IT Management* 3(2014): 59-70.
 36. Buriti FCA., et al. "Activity of passion fruit (Passifloraedulis) and guava (Psidiumguajava) pulps on Lactobacillus acidophilus in refrigerated mousses". *Brazilian Journal of Microbiology* 38.2 (2007): 315-317.
 37. do Espírito Santo AP., et al. "Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts". *International Journal of Food Microbiology* 154.3 (2012): 135-144.
 38. Chou CC and Hou JW. "Growth of bifidobacteria in soymilk and their survival in the fermented drink during storage". *International Journal of Food Microbiology* 56 (2000): 113-121.
 39. Kabeir BM., et al. "Roasted peanut milk partially substituted with millet thin porridge as a carrier for BifidobacteriumlongumBaB536". *International Journal of Current Microbiology and Applied Science* 4.8 (2015): 299-308.
 40. Giyarto TF., et al. "Fermentation of peanut milk by Lactobacillus acidophilus SNP-2 for production of non-dairy probiotic drink". The 3rd International Conference of Indonesian Society for Lactic Acid Bacteria (3rd IC-ISLAB), 21-22 January, Yogyakarta, Indonesia, (2011).
 41. Shah NP and Jelen P. "Survival of lactic acid bacteria and their lactases under acidic conditions". *Journal of Food Science* 55.2 (1990): 506-509.
 42. Akalin AS., et al. "Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage". *International Journal of Food Science and Technology* 39.6 (2004): 613-621.
 43. Vinderola CG., et al. "Survival of probiotic microflora in Argentinian yoghurts during refrigeration storage". *Food Research International* 33.2 (2000): 97-102.
 44. Ouwehand AC and Salminen SJ. "The health effects of cultured milk products with viable and non-viable bacteria". *International Dairy Journal* 8 (1998): 749-758.
 45. Tranjan B., et al. "Development of goat cheese whey-flavoured beverages". *International Journal of Dairy Technology* 62.3 (2009): 438-443.
 46. AhmedNawal MR. "Study on ABT starter bacteria activity in milk from vegetarian source". M. Sc. Thesis, Botany Department, Faculty of Science, Damietta University, Egypt (2016).

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