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Research Article

Chemical and Microbial Characterizations of Bio-Yoghurt Made Using ABT Culture, Cow Milk and Coconut Milk

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Abstract

The aim of this study was to investigate the influence of utilization coconut milk and ABT (*L. acidophilus + B. bifidum + S. thermophiles*) culture on various yoghurt properties. Six treatments of yoghurt were manufactured from mixtures of cow and coconut milk using classic and ABT-5 starters. The results showed that Yoghurt made from cow and coconut milk mixtures possessed the lowest acidity, redox potential, ash, total nitrogen and water soluble nitrogen levels and the highest pH, total solids, fat, total volatile fatty acids and medium chain fatty acids especially lauric acid. Incorporation of coconut milk with cow milk in yoghurt production increased the counts of *Lactobacillus bulgaricus, Streptococcus thermophillus, Lactobacillus acidophilus* and *bifidobacteria*. Mixing 50% coconut milk with cow milk slightly increased color, appearance, body, texture and flavour scores of the produced yoghurt. Using of ABT culture in yoghurt preparation lowered titratable acidity, redox potential, water soluble nitrogen, total volatile fatty acids and medium chain fatty acids while increased pH values as compared with that made by classic culture. Results of total solids, fat, ash and total nitrogen were close to each other in both kinds of yoghurt. Numbers of *Streptococcus thermophillus* in ABT yoghurt were more double than those of classic starter for fresh samples and during storage. Using ABT culture in yoghurt manufacturing slightly improved flavour of yoghurt.

Keywords: *Coconut Milk; ABT Culture; Bifidobacteria; Lauric Acid; Yoghurt*

Introduction

Yoghurt, as a fermented diary product is regarded as a probiotic carrier, is nutritionally rich in available protein, calcium, milk fat, potassium, magnesium, vitamin B_2, B_6 and vitamin B_{12} [1]. It has nutritional benefits beyond those of milk, because people who are moderately lactose intolerant can enjoy yoghurt without ill effects, as most of the lactose in the milk precursor has been converted to lactic acid by the bacterial culture [2]. Yoghurt also has medical uses because of the probiotic characteristics, in helping out on a variety of gastro intestinal conditions and in preventing antibiotic associated diarrhea [3].

The role(s) of probiotics bacteria in dairy fermentations is to assist in: (i) the preservation of the milk by the generation of lactic acid and possibly antimicrobial compounds; (ii) the production of flavour compounds (e.g. acetaldehyde in yoghurt and cheese) and other metabolites (e.g. extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; (iii) to improve the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins; and (iv) the provision of special therapeutic or prophylactic properties as cancer [4-6] and control of serum cholesterol levels [7].

On the other hand, Coconut milk is the aqueous emulsion of coconut kernel, which is prepared by hand or machine pressing fresh grated coconut kernel. It is different from animal milk such as cow's milk. While cow's milk has equal amounts of oil and proteins, coconut milk has ten times more oil than proteins [8]. As a general, nutrient components of coconut milk include lipids, sugars, proteins and several other minor compounds. So that it is used in many culinary applications. Coconut milk is consumed directly or with cooked food. Therefore, the aim of this study was to investigate the possibility of combining nutritional and health benefits of both yoghurt and coconut milk in one bio-product.

Material and Methods

Materials

Fresh cow's milk was obtained from private farm in Damiette Governorate, Egypt. Coconut (*Cocos nucifera* L) was purchased from a local grocery in Damiette Governorate.

A commercial classic yoghurt starter containing *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) and ABT-5 culture which consists of *S. thermophiles*, *Lactobacillus acidophilus* + *B. bifidum* (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.

Methods

Preparation of Coconut milk

Coconut milk was prepared as described by Kolapo and Olubamiwa [9]. Coconut seed was cracked manually and the coconut meat removed with sharp knife. The brown part of the coconut meat was gently scraped off. It was cut into smaller pieces to enhance quicker blending. Two hundred grams of white coconut meat were blended with one liter of distilled water. The slurry obtained was further diluted with 1 liter of distilled water. It was then sieved with double layers of cheese cloth. The filtrate obtained is coconut milk.

Manufacture of yoghurt

Six treatments of yoghurt were made as fallow:

A: Yoghurt made from cow milk and classic starter

B: Yoghurt made from 75 % cow milk + 25 % coconut milk and classic starter

C: Yoghurt made from 50 % cow milk + 50 % coconut milk and classic starter

D: Yoghurt made from cow milk and ABT culture

E: Yoghurt made from 75% cow milk + 25% coconut milk and ABT culture

F: Yoghurt made from 50 % cow milk + 50 % coconut milk and ABT culture

Mixtures of cow and coconut milk were tempered to 85°C for 15 min then cooled to 40°C and inoculated with cultures (0.1 g/L of yoghurt mix). The inoculated milk was transferred to 100-ml plastic cups, incubated at 40°C. After fully coagulation, yoghurt treatments were stored at 4°C for 14 days and tested when fresh and after 7 and 14 days of cold storage.

Methods of Analysis

Chemical analysis

Total solids (TS), fat, total nitrogen (TN) and ash contents of samples were determined according to AOAC [10]. 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 [11]. Total volatile fatty acids (TVFA) were determined according to Kosikowiski [12].

Determination of fatty acids composition

The extraction of yoghurt 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 (CH_3ONa) was used [13]. The fatty acid composition of yoghurt was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC™- WAX, 30 m, ID 0.25 mm, Film:0,25 µm. Fatty acids were identified by comparison of the retention times to standard fatty acid methyl esters.

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 [14]. The count of bifidobacteria was determined according to Dinakar and Mistry [15].

Sensory properties judging

Samples of yoghurt 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.

Statistical Analysis

The obtained results were statistically analyzed using a software package [16] based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan [17] for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

Results and Discussion

Chemical composition of yoghurt as affected by milk and culture types

Data cleared in Table 1 illustrate the effect of using coconut milk and ABT-5 culture in yoghurt manufacturing on the titratable acidity (% lactic acid), pH and redox potential (Eh) during the refrigerated storage for 14 days. Using of coconut milk in yoghurt preparation clearly impacted on the above mentioned values as compared with that made from cow's milk. Yoghurt made from cow and coconut milk mixtures possessed the lowest acidity and redox potential (Eh) levels and the highest pH values. Similar findings are reported by Ladokun and Oni [18] who found that the pH results of milk fermented with cultures of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* at 0 hour of yoghurt production were goat milk (5.24), cow milk (5.85), soymilk (5.73) and coconut milk (5.98),

Properties	Treatments	Storage period (day)			Means
		Fresh	7	14	
Acidity %	A	0.85	1.07	1.23	1.05^a
	B	0.79	0.99	1.13	0.97^b
	C	0.71	0.86	0.97	0.85^d
	D	0.76	0.94	1.07	0.92^c
	E	0.71	0.87	0.98	0.85^d
	F	0.64	0.75	0.84	0.74^e
	Means	0.74^I	0.91^H	1.04^G	

pH values	A	4.61	4.50	4.41	4.51^{f}
	B	4.68	4.58	4.50	4.59^{e}
	C	4.79	4.70	4.63	4.71^{c}
	D	4.72	4.64	4.57	4.64^{d}
	E	4.83	4.72	4.66	4.74^{b}
	F	4.88	4.77	4.71	4.79^{a}
	Means	4.75^{G}	4.65^{H}	4.58^{I}	
E_h mV	A	161	169	176	169^{a}
	B	156	163	168	162^{b}
	C	150	156	160	155^{c}
	D	154	161	167	161^{b}
	E	148	153	157	153^{c}
	F	144	149	155	149^{d}
	Means	152^{I}	159^{H}	164^{G}	

Table 1: *Effect of mixing coconut milk with cow milk on acidity, pH and redox potential (E_h) values of yoghurt during storage period.*

^{abcde}*Letters indicate significant differences between yoghurt treatments*

^{GHI}*Letters indicate significant differences between storage times*

**mV: millivolts*

On the other side, using of ABT culture in yoghurt production lowered titratable acidity and E_h while increased pH values as compared with that made by classic culture. Furthermore, the acidity development rates through storage period were lower in ABT yoghurt than those of classic one. Acidity percentages of fresh samples A and D were 0.85 and 0.76% respectively. The acidity development rates for samples A, B and C were 44.70, 43.04 and 36.62% respectively. The respective values of samples D, E and F were 40.79, 38.03 and 31.25% respectively. These results agreed with Shihata and Shah [19] and disagreed with Kehagias., *et al* [20]. Shihata and Shah [19] reported that the ABT cultures are known to produce yoghurt with a fine, mild taste and low post acidification whereas Kehagias., *et al.* [20] stated that the addition of bifidobacteria to yoghurt starter increased acidity of yoghurt which attributed to the formation of both acetic and lactic acids by *B. bifidum*. In bio-yoghurt special attention should be given to avoid over acidification since this could affect the stability of bifidobacteria during storage.

Regardless of the milk or starter types used, titratable acidity and E_h values of various yoghurt treatments increased during storage due to the activity of the starter culture. These results agreed with Vijayalakshmi., *et al*. [21] 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.

Mixing of 25 and 50% coconut milk with cow milk increased TS and fat values in the resultant yoghurt (Table 2). Fat percentages of samples A, B and D at the end of storage period were 3.6, 5.5 and 6.7% respectively. On the contrary, ash levels of yoghurt contained coconut milk were slightly lower than those of control (cow milk yoghurt). Imele and Atemnkeng [22] and Sanful [23] also reported increased fat content, specific gravity and total solids with the addition of coconut milk to plain yoghurt. Ndife., *et al*. [24] stated that the fat content increased as the proportion of coconut-cake increased in the yoghurts.

As shown in Table 2, no pronounced differences in TS, fat and ash contents between yoghurt made with classic or ABT cultures at zero time or during storage period. Similar results were also reported by Ayad., *et al.* [25] who stated that TS, solids not fat (SNF), fat, Fat/Dry Matter (F/DM) and protein values in *bifidus* yoghurt-like products were not affected by Bifidobacteria incorporation with yoghurt-like products.

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
TS %	A	14.62	14.70	14.82	14.71^{e}
	B	15.24	15.30	15.36	15.30^{d}
	C	16.13	16.18	16.25	16.19^{a}
	D	14.49	14.53	14.61	14.54^{f}
	E	15.29	15.33	15.43	15.35^{c}
	F	16.10	16.15	16.20	16.15^{b}
	Means	15.31^{I}	15.37^{H}	15.45^{G}	
Fat %	A	3.6	3.6	3.7	3.6^{c}
	B	5.5	5.6	5.7	5.6^{b}
	C	6.7	6.8	6.8	6.8^{a}
	D	3.5	3.6	3.6	3.6^{c}
	E	5.5	5.5	5.6	5.5^{b}
	F	6.8	6.8	6.9	6.8^{a}
	Means	5.3^{G}	5.3^{G}	5.4^{G}	
Ash %	A	0.77	0.80	0.84	0.80^{a}
	B	0.73	0.75	0.78	0.75^{b}
	C	0.71	0.74	0.76	0.74^{b}
	D	0.76	0.79	0.82	0.79^{a}
	E	0.72	0.76	0.77	0.75^{b}
	F	0.70	0.74	0.75	0.73^{b}
	Means	0.73^{I}	0.76^{H}	0.79^{G}	

Table 2: *Effect of mixing coconut milk with cow milk on TS, fat and ash values of yoghurt during storage period.*

^{abcde} *Letters indicate significant differences between yoghurt treatments*

^{GHI} *Letters indicate significant differences between storage times*

Total solids, fat and ash contents of various yoghurt samples slightly increased due to the loss of moisture during storage. Similar observation was reported by Farag., *et al*. [26] and Ammarm., *et al.* [27].

Changes in TN, WSN and TVFA of yoghurt during cold storage

Results of Table 3 represent the contents of TN, WSN and TVFA of yoghurt as affected by using coconut milk and different cultures. Cow milk yoghurt had the highest content of TN comparing with that in yoghurt made from mixture of cow and coconut milk. Values of TN of samples A, B and C at the end of storage period were 0.630, 0.608 and 0.604% respectively. Consequently, the contents of WSN were higher in cow milk yoghurt.

Total volatile fatty acids (TVFA) are taken as a measure of the degree of fat hydrolysis during yoghurt storage (Table 3). 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, L. acidophilus* and *S. thermophilus. Lactobacillus* produces more TVFA than *S. thermophilus.* 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 [28]. Concentrations of TVFA of yoghurt contained coconut milk were higher than those of cow milk yoghurt. This may be attributed to the high fat content of coconut milk.

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
TN %	A	0.625	0.628	0.630	0.628[b]
	B	0.601	0.606	0.608	0.605[c]
	C	0.596	0.599	0.604	0.600[d]
	D	0.627	0.630	0.635	0.631[a]
	E	0.602	0.605	0.610	0.606[c]
	F	0.594	0.598	0.606	0.599[d]
	Means	0.608[I]	0.611[H]	0.616[G]	
WSN %	A	0.115	0.141	0.154	0.137[a]
	B	0.108	0.129	0.140	0.126[c]
	C	0.104	0.123	0.132	0.120[d]
	D	0.110	0.134	0.145	0.130[b]
	E	0.104	0.124	0.133	0.120[d]
	F	0.099	0.116	0.124	0.113[e]
	Means	0.107[I]	0.128[H]	0.138[G]	
TVFA %	A	9.2	10.8	11.8	10.6[d]
	B	10.2	12.0	13.1	11.8[b]
	C	10.6	12.3	13.6	12.2[a]
	D	8.5	9.9	10.7	9.7[e]
	E	9.3	10.7	11.6	10.5[d]
	F	9.8	11.2	12.3	11.1[c]
	Means	9.6[I]	11.2[H]	12.2[G]	

Table 3: *Effect of using coconut milk and ABT-5 culture on TN, WSN and TVFA of yoghurt.*

[abcde] Letters indicate significant differences between yoghurt treatments

[GHI] Letters indicate significant differences between storage times

Both types of yoghurt prepared by classic and ABT cultures had nearly the same TN contents. Nevertheless, the results of WSN test indicated that utilization of classic starter raised WSN values of the resulted yoghurt as compared with using ABT culture. This may be due to proteolytic activity (endopeptidase) of *L. delbrueckii* subsp. *bulgaricus* which hydrolyzed casein to polypeptides then; the latter was hydrolyzed to amino acids with exopeptidases produced by *S. thermophilus* [28]. Not only WSN but also TVFA values of yoghurt manufactured using classic starter were higher than those of yoghurt made using ABT culture. Also, the development rates of WSN and TVFA contents within storage were higher in classic yoghurt than that of ABT yoghurt. These findings are in agreement with the findings of Ismail [29].

Fatty acids content of yoghurt

Gas-liquid chromatography (GLC) provides the ultimate in identification of free fatty acids [12]. Fox., *et al*. [30] cleared that free fatty acids are released usually by the actions of lipases (from different sources) during lipolysis. They contribute directly to yoghurt and cheese flavours, particularly when they are properly balanced with products of proteolysis and other reactions. On the other hand, Collins., *et al*. [31] showed that ruminant milk fats contain a wide range of fatty acids and 437 distinct have been identified in bovine milk fats. This situation is reflected to dairy products. In our current study, FFA contents were measured in fresh yoghurt samples. Results are presented in Tables 4 and 5.

Fatty acids	C	Treatments					
		A	B	C	D	E	F
		Saturated fatty acids (SFA) %					
Caprylic	8:0	0.450	2.189	3.772	0.396	2.026	3.330
Capric	10:0	2.058	2.008	3.196	2.031	1.911	3.116
Lauric	12:0	3.276	16.45	25.53	3.149	15.82	24.94
Myristic	14:0	9.011	10.86	12.53	8.059	10.539	11.84
Pentadecanoic	15:0	1.845	0.297	0.265	1.617	0.189	0.219
Palmitic	16:0	29.73	23.09	19.60	29.33	22.82	18.34
Heptadecanoic	17:0	1.901	0.284	0.203	1.693	0.251	-
Stearic	18:0	16.99	13.82	10.76	15.81	12.92	10.10
Arachidic	20:0	0.189	0.504	0.642	0.164	-	-
Total		65.45	68.50	73.10	62.25	66.48	71.89
Unsaturated fatty acids (USFA) %							
Myristioleic acid	14:1	0.378	0.232	0.228	0.472	0.356	0.246
	15:1	0.185	0.150	0.131	-	-	-
Palmitioleic	16:1	2.195	1.781	1.043	2.473	1.817	1.235
Oleic	18:1	26.22	24.68	21.97	27.88	25.81	22.53
	18:2	1.061	0.992	0.546	1.615	1.353	0.711
Linoleic	18:2	2.891	2.736	2.542	3.473	3.213	2.899
α-Linolenic	18:3	0.764	0.293	0.285	0.866	0.437	0.315
Gamma linolenic	18:3	0.322	0.386	0.155	0.444	0.534	0.210
	20:2	0.194	0.101	-	0.210	-	
	22:2	0.343	0.149	-	0.371	-	
Total		34.55	31.50	26.90	37.75	33.52	28.11

Table 4: *Effect of using coconut milk and ABT-5 culture on fatty acids content (%) of fresh yoghurt.*

Treatments	SFA	USFA	MCFA	LCFA
A	65.45	34.55	5.784	94.216
B	68.50	31.50	20.647	79.353
C	73.10	26.90	32.498	67.502
D	62.25	37.75	5.576	94.424
E	66.48	33.52	19.757	80.243
F	71.81	28.11	31.386	68.614

Table 5: *Effect of using coconut milk on free fatty acid indices ratios of fresh yoghurt.*

SFA: saturated fatty acids; USFA: unsaturated fatty acids; MCFA: medium chain fatty acids (C8 to C12); LCFA: long chain fatty acids (> C12).

Saturated and unsaturated fatty acids

Incorporation 25 or 50% coconut milk with cow milk increased the concentration of saturated fatty acids (SFA) and decreased the level of unsaturated fatty acids (USFA) in the resulted yoghurt. The contents of SFA for samples A, B and C were 65.45, 68.50 and 73.10% respectively. Corresponding results of USFA were 34.55, 31.50 and 26.90% respectively. In general, SFA percentages were higher than USFA for all yoghurt treatments. However increasing of SFA levels in coconut and its products, but the health benefits of these products were proved by many authors. By giving coconut oil, Kurup and Rajamohan [32] found no statistically significant alteration in the serum levels of total cholesterol, HDL cholesterol or LDL cholesterol from the baseline values. They also noted a beneficial effect of adding the coconut kernel to the diet. The polyphenol fraction of virgin coconut oil was found to be capable of preventing LDL oxidation [33].

The study *in vitro* and *in vivo* lipid peroxidation and the levels of antioxidant enzymes in rats showed that virgin coconut oil (VCO) is beneficial as an antioxidant. VCO is superior in antioxidant action than copra oil (CO) and groundnut oil (GO). Polyphenol fraction from VCO was found to have more inhibitory effect on microsomal lipid peroxidation compared to that from the other two oils [34].

As cleared in Tables 4 and 5, utilization ABT culture in yoghurt production lowered SFA and increased USFA contents. These results mentioned to the effect of bacteria species on the fatty acids composition of yoghurt. These findings are in agreement with the findings of Caglayan., *et al*. [35] who found that the levels of USFA were slightly higher than SFA in probiotic Turkish yoghurt as compared with whole one. Lactic acid bacteria possess enzymes that are able to hydrolyze mono-, di- and triacylglycerols. The activity of the lipases depends strongly on the genera and bacteria species, as well as on the temperature and presence of calcium and magnesium ions [36].

Among the saturated fatty acids in A and D treatments, the most abundant was palmitic acid (C16:0) followed by stearic acid (C18:0). In samples B and E also palmitic acid was the highest but followed by lauric acid (C12:0). Lauric acid was the predominant followed by palmitic acid in samples C and F. Palmitic is one of the major SFA's; it raises serum cholesterol while stearic acid does not [37,38]. For the unsaturated fatty acids the prevailing acid was oleic acid (18:1 ω9) followed by linoleic (18:2 ω6) in various yoghurt treatments.

Medium chain fatty acids (C8 – C12)

The concentrations of medium chain fatty acids (MCFA) were considerably higher in yoghurt made from cow and coconut milk mixtures (treatment B, C, E and F) than those of yoghurt made from cow milk only (samples A and D). Values of MCFA were 5.784, 20.647, 32.498, 5.576, 19.757 and 31.386% for samples A, B, C, D, E and F respectively. This may be attributed to the very high content of MCFA especially lauric acid (C12:0) in coconut milk. Nutritionally, coconut oil is composed predominately of medium-chain fatty acids (MCFA) also known as medium chains triglycerides (MCT), unlike the long chain fatty acids (LCFA) of saturated and unsaturated oils found in meat, milk, egg and some vegetable oils [39]. MCFA are very different from LCFA, because they do not have negative effect on cholesterol and help to lower the risk of both arthrosclerosis and heart diseases [22,40].

On the other side, utilization of ABT culture in yoghurt production slightly lowered MCFA levels. Generally, lauric acid (C12:0) was the predominant in medium chain fatty acids in different yoghurt treatments.

Long chain fatty acids (> C12)

Contrary to medium chain fatty acids, long chain fatty acids (LCFA) were markedly higher in cow milk yoghurt than those of yoghurt made from cow and coconut milk mixtures. Also, using ABT culture in yoghurt making slightly raised LCFA levels as compared with classic starter. The prevailing acid of long chain fatty acids varied between yoghurt treatments. In samples A and D the abundant acid was palmitic (C16) followed by oleic acid (C18:1). In other treatments the opposite trend was noted where oleic acid were the predominant in LCFA followed by palmitic acid.

Microbial analysis of yoghurt

Outcomes presented in Table 6 show changes detected in *Lactobacillus bulgaricus, Streptococcus thermophillus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* of yoghurt at zero time and during storage period. The counts of various microbial groups for all yoghurt treatments 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 [41].

Incorporation of coconut milk with cow milk in yoghurt production clearly increased the count of *L. bulgaricus.* Moreover, the loss of viability during storage was low in yoghurt contained coconut milk. The highest numbers of *L. bulgaricus* were in yoghurt contained 50% coconut milk followed by yoghurt contained 25%. These results refer to the positive effect of coconut milk on *L. bulgaricus.*

It is quite apparent from the results of Table 6 that the counts of *Str. thermophillus* were higher in coconut milk yoghurt. Because of high acidity content, yoghurt made from cow milk (sample A) had the highest loss of survival rates for *Str. thermophillus* recorded 44.44%. Mixing 25 or 50% coconut milk with cow milk (samples B and C) reduced loss of viability of *Str. thermophillus* to 27.27 and 26.09% respectively. These results are confirmed with the results found in Table 3 which showed that cow milk yoghurt possessed the highest values of acidity development through storage.

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
Lactobacillus bulgaricus (cfu×x10^5/g)	A	11	9	5	8^b
	B	14	13	10	12^a
	C	16	15	12	14^a
	D	-	-	-	
	E	-	-	-	
	F	-	-	-	
	Means	14^A	12^A	9^B	
Streptococcus thermophillus (cfu×x10^5/g)	A	18	15	10	14^d
	B	22	20	16	19^c
	C	23	21	17	20^c
	D	41	36	32	36^b
	E	49	45	38	44^a
	F	50	45	40	45^a
	Means	34^G	30^H	26^I	
Lactobacillus acidophilus (cfu×x10^5/g)	A	-	-	-	
	B	-	-	-	
	C	-	-	-	
	D	15	13	9	12^b
	E	21	20	17	19^a
	F	22	20	18	20^a
	Means	19^G	18^{GH}	15^H	

Bifidobacterium bifidum (cfu×x10^5/g)	A	-	-	-	
	B	-	-	-	
	C	-	-	-	
	D	31	28	20	26^{b}
	E	35	33	29	32^{ab}
	F	38	35	31	35^{a}
	Means	35^{G}	32^{GH}	27^{H}	

Table 6: *Effect of using coconut milk and ABT-5 culture on starter bacteria counts of yoghurt.*

abcde Letters indicate significant differences between yoghurt treatments

GHI Letters indicate significant differences between storage times

Numbers of *Str. thermophillus* in ABT yoghurt were more double than those of classic starter for fresh samples and during storage. The activity of ABT culture produced lower acidity in yoghurt than that of classic starter, therefore the loss of survival values were lower in the former than that the later. These results are in accordance with those of Ismail [29].

The results of Table 6 showed that the viable counts of *L. acidophilus* significantly ($P < 0.05$) affected by mixing of coconut milk with cow milk. The greatest numbers of these bacteria were detected in yoghurt contained coconut milk. On the other side, numbers of *L. acidophilus* in yoghurt contained 25 or 50% coconut milk were close to each other. Values of the loss of survival during yoghurt storage were 40.00, 19.05 and 18.18% for samples D, E and F respectively.

The trend of results of bifidobacteria were similar to that of *L. acidophilus* where samples contained coconut milk possessed higher counts than those of cow milk yoghurt which may be caused by carbohydrates presence in coconut milk. This means that that coconut milk acted as prebiotic of bifidobacteria. These results are in agreement with those of Correˆa., *et al* [42].

Coconut milk not only increased bifidobacteria counts but also lowered loss of viability throughout cold storage of yoghurt. Loss of viability rates of samples D, E and F were 35.48, 17.14 and 18.42% respectively. On the other hand, loss of viability of bifidobacteria during storage was more pronounced than was that of lactic acid bacteria. Viability losing of probiotic bacteria in fermented milk was reported to be due to acid injury to the organisms [43]. However, reducing of bifidobacteria numbers during storage period, but the recommended level of 10^6 or 10^7 cfu.g^{-1} of bifidobacteria as a probiotic was exceeded for all treatments of bio-yoghurt and remained above 10^6 or 10^7 cfu g^{-1} until the end of storage period. Ouwehand and Salminen [44] stated that in order to exhibit positive health effects of probiotics, they have to deliver in certain numbers. As a guide, the International Dairy Federation (IDF) suggested a minimum of 107 cfu of probiotics/g product should be alive at the time of consumption. Similar results and recommendations were obtained by Moreno., *et al.* [45] and Jayamanne and Adams [46].

El Bakri and Zubeir [47] stated that the high lactobacilli count in the enriched yoghurts is suggestive of its viability with coconut enrichment. *Lactobacillus* and Bifidobacterium species are the most commonly used probiotics in dairy functional foods [48,49]. Moreover their ability to utilize coconut fiber as feed stock (prebiotics) is in dare need of further research in the development of symbiotic functional yoghurts [49,50].

Bifidobacterium sp., *Lactobacillus acidophilus*, and *L. casei* have been associated with health-promoting effects and are classified as probiotic organisms since they are thought to improve the microbial balance in the human gastrointestinal tract. Health benefits attributed to probiotics include antimicrobial, antimutagenic, anticarcinogenic and antihypertensive properties [51]. Many studies indicate that soymilk is a good substrate for probiotic bacteria [52] and good base for fermentation process [53].

Changes in sensory evaluation of yoghurt

Organoleptic properties evaluation is an important indicator of potential consumer preferences. The popularity of yogurt as a food component depends mainly on its sensory characteristics and addition of different flavours to yogurt has been found to increase options for consumers and helps in marketing yoghurt and retaining consumer interests [54]. Effect of starter type and blinding of coconut milk on sensory quality of yoghurt is given in Table 7.

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
Color&Appearance (15)	A	13	13	12	13[a]
	B	13	13	12	13[a]
	C	14	13	13	13[a]
	D	13	13	12	13[a]
	E	13	13	12	13[a]
	F	14	14	13	14[a]
	Means	13[G]	13[G]	12[G]	
Body&Texture (35)	A	33	33	31	32[a]
	B	33	33	31	32[a]
	C	33	33	32	33[a]
	D	31	30	27	29[b]
	E	31	31	29	30[ab]
	F	32	31	29	31[ab]
	Means	32[G]	32[G]	30[H]	
Flavor (50)	A	45	44	41	43[a]
	B	47	47	45	46[a]
	C	47	46	44	46[a]
	D	46	45	43	45[a]
	E	47	46	44	46[a]
	F	48	47	45	47[a]
	Means	47[G]	46[G]	44[G]	
Total (100)	A	91	90	84	88[ab]
	B	93	93	88	91[a]
	C	94	92	89	92[a]
	D	90	88	82	87[b]
	E	91	90	85	89[ab]
	F	94	92	87	91[a]
	Means	92[G]	91[G]	86[H]	

Table 7: *Effect of using coconut milk and ABT-5 culture on sensory evaluation of yoghurt.*

[abcde] Letters indicate significant differences between yoghurt treatments

[GHI] Letters indicate significant differences between storage times

Mixing 50% coconut milk with cow milk slightly increased color and appearance scores of the produced yoghurt. This may be white color of coconut milk which prefer for the majority of Egyptian consumers.

Color and appearance of yoghurt made using of classic starter were found to be comparable to those of yoghurt samples manufactured by ABT culture at zero time and during storage period. Scores of color for fresh samples A and F were 8.75 and 8.75 respectively. These results are in agreement with those obtained by Ammar., *et al* [55].

As color and appearance improved, also addition of coconut milk slightly improved body and texture scores of yoghurt especially at the end of storage period. Because body of ABT yoghurt was little weak, the texture and body scores slightly lowered than classic starter yoghurt.

The flavour evaluation tests of coconut milk yoghurt gained the highest scores as compared with that made from cow milk. Mixing of cow milk with 25 or 50 % coconut milk had approximately the same effect on yoghurt flavour.

On the other side, using ABT culture slightly improved flavour of yoghurt. Scores of flavour evaluation of fresh A and D treatments were 45 and 46 respectively. El-Sayed., *et al.* [56] reported a different trend where the addition of the adjunct cultures (*L. plantarum* or *B. bifidum*) to normal yoghurt starter had no adverse effect on the appearance, flavour and body & texture of yoghurt. This is however contrary to what was observed by Abd El-Salam., *et al*. [57] who cleared that the yoghurt sample made by addition of *Bifidobacterium* to yoghurt culture gained the highest scores for flavour, body& texture and appearance among all the treatments.

On a general note, fresh samples ranked the highest scores of color, appearance, body, texture, and flavour. 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 [56]. These trends are similar to other works in literature. Badawi., *et al.* [58] 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 [54] found that the storage time had a negative impact on the flavour scores of yoghurt which they attributed to changes in the aroma compounds.

Conclusion

Incorporation of 25 or 50% coconut milk with cow milk and using of ABT culture produced bio-yoghurt with highly nutritional value. This yoghurt contained high amounts of lauric acids. The recommended level of 107 cfu.g-1 of bifidobacteria as a probiotic was exceeded for bio-yoghurt. The results of sensory evaluation cleared that bio-yoghurt made from mixtures of coconut milk with cow milk using ABT-5 culture was acceptable in properties of color, appearance, body, texture and flavour.

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