

Chemical Composition, Microbial Properties and Sensory Evaluation of Bio-Yoghurt Made From Admixture of Cow and Coconut Milk And Honey

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ABSTRACT

The aim of this study was the possibility of improvement of the nutritional and health values of bio-yoghurt made from cow and coconut milk by adding 5% honey where eight treatments of yoghurt were made from cow milk and mixtures of cow and coconut milk and with or without adding 5% honey, Yoghurt samples were stored in refrigerator at 5°C for 14 days. Samples were analyzed when fresh and after 7 and 14 days of storage period. Acidity, E_h, total solids and ash values of yoghurt treatments contained 5% honey were significantly (p<0.05) higher than that of control and increased WSN and TVFA contents, while, caused a markedly decrease in SFA and increase in USFA contents and was not so much pronounced in color and appearance but improved body, texture and flavour of yoghurt. Fat contents of treatments with or without addition honey were close to each other, while, the mixing 25 or 50% coconut milk with cow milk decreased acidity, E_h, ash and total nitrogen values and increased pH, total solids, fat levels and medium chain fatty acids especially lauric acid in yoghurt. On the other sides, acidity and E_h values of classic starter yoghurt samples were relatively higher while pH data were lower than those made using ABT culture. The color and appearance scores of yoghurt made using classic or ABT cultures were close to each other whereas body and texture properties were slightly higher in the former than the latter. The opposite trend was found for flavour scores.

Keywords: Yogurt, Coconut milk, Fatty acids content, Honey.

I. INTRODUCTION

In recent years, there has been increasing interest in the use of natural and healthy food additives and incorporating health promoting substances into the diet due to its healthy and natural image (Chen *et al.*, 2000). Honey is a natural, sweet, syrupy fluid collected by bees from nectar of flowers. The pleasant aroma and taste of this viscous liquid ranging in color from pale yellow to dark amber varies according to geographical and seasonal conditions. The use of natural honey as food and medicine by mankind has been in existence from time immemorial. Natural honey is accepted by all generations, traditions and civilizations, both ancient and modern. Also, it is recommended in all religious books. The religion of Islam recommended the use of honey as food and medicine, and even named an entire

chapter in the Holy Qur'an called Surah al-Nahl meaning chapter of the Honey Bee. In the book of hadith, Prophet Muhammad strongly advocated the use of honey for curative and healing purposes (Al-Waili, 2004). The health benefits of honey have long been realized by humans to treat a variety of ailments. Besides its sugar composition, honey consists of a number of bioactive compounds such as phenolic compounds, flavonoids, carotenoid-like derivatives, organic acids, Maillard reaction products, catalase, ascorbic acid, and other compounds which function as antioxidants (Bogdanov *et al.*, 2008). Several therapeutic and medicinal effects such as antibacterial, antimutagenic, antiproliferative, hepatoprotective, hypoglycemic, and antioxidant effects have been ascribed to honey through last years (Erejuwa *et al.*, 2010 and Ghashm *et al.*, 2010). Poorani *et al.* (2012) stated that honey which is naturally available good

product with high nutritive and medicinal value can be used preparing a bifidiogenic milk product by assessing the content of bifidus growth factor and further incorporation will give a valuable product. Therefore, the aim of this study was the possibility of improvement of the nutritional and health values of bio-yoghurt made from cow and coconut milk by adding 5% honey. The aim of this study was the possibility of improvement of the nutritional and health values of bio-yoghurt made from cow and coconut milk by adding 5% honey.

II. METHODS AND MATERIAL

Materials:

Raw cow milk was bought from private farm in Damiette Governorate, Egypt. Coconut (*Cocos nucifera* L) and honey were purchased from a local grocery in Damiette Governorate. A commercial classic yoghurt starter containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) and probiotic yogurt culture 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 (2012)**. 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 Supplemented with Honey:

Six treatments of yoghurt were made as follow:

- ■ Yoghurt made from cow milk using classic yoghurt starter+5% honey (Treatment A).
- ■ Yoghurt made from mixture of cow milk (75%) and coconut milk (25%) using classic yoghurt starter + 5% honey (Treatment B).

- Yoghurt made from mixture of cow milk (50%) and coconut milk (50%) using classic yoghurt starter + 5% honey (Treatment C).
- Yoghurt made from cow milk using ABT culture + 5% honey (Treatment D).
- Yoghurt made from mixture of cow milk (75%) and coconut milk (25%) using ABT culture + 5% honey (Treatment E).
- Yoghurt made from mixture of cow milk (50%) and coconut milk (50%) using ABT culture + 5% honey (Treatment F).

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

Methods of Analysis

Chemical Analysis:

Total solids, fat, total nitrogen and ash contents of samples were determined according to (**AOAC, 2000**). 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) was determined in yoghurt according to **Ling (1963)**. Total volatile fatty (TVFA) acids were determined as described by **Kosikowski (1978)**. The free fatty acids of fresh yoghurt were determined using gas liquid chromatography. 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 (CH_3ONa) was used (**Jahreis et al., 1997**). The fatty acid composition of Raybe milk was determined by gas chromatography "Pay-Unicam

304” with flame ionization detector and column ECTM-WAX, 30 m, ID 0.25 mm, Film:0,25 µm.

Microbial Analysis:

Media Preparation:

Different agar media (*Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus* counts) were used and diluents for serial dilutions were prepared as follows:

A. **Tharmaraj and Shah (2003)** who reported that *Lactobacillus acidophilus* could be enumerated using MRS agar at 43°C for 72h under anaerobic incubation. The mixture of antibiotics (5 ml) was added to 100 ml of MRS agar medium, it consists of:

Dextrose 20.0 g	Tween 80 1 ml
Bacteriological peptone 10.0 g	Ammonium citrate 2.0 g
Beef extract 8.0 g	Magnesium sulphate 0.29
Sodium citrate 5.0 g	Manganese sulphate 0.05 g
Yeast extract 4.0 g	Agar 15 g
Di potassium phosphate 2.0 g	Distillation water 1000.0 ml (pH 6±0.2 at 25°C)

The medium was sterilized in autoclave at 121°C for 15 minutes. Ten ml of membrane-filtered sterile solutions of 10% D-sorbitol were added to 90 ml of the sterilized mentioned medium just before pouring the agar medium. Inoculated plates were incubated anaerobically at 37°C for 48 h. The colony morphology were rough, dull, small (0.1-0.5 mm) brownish. Cysteine-HCl was added at the rate of 0.05% to decrease the redox potential of the medium. Plates were incubated at 37°C for 48 to 72 h under anaerobic condition.

- B. The counting of *Lactobacillus delbrueckii* subsp. *bulgaricus* was determined using MRS-Commercial medium (**Charteris, et al. 1997**). MRSpH 5.4 agar (MRS)-Commercial MRS medium (OXOID, Basingstoke, UK) was rehydrated in distilled water according to manufacturer’s instructions, and hydrochloric acid (HCl) was used to adjust the pH of the medium to 5.4. The agar medium was sterilized at 121°C for 15 min.
- C. The count of *bifidobacterim bifidium* was determined according to **Dinakar and Mistry**

(**1994**). It consists of a mixture of antibiotics, including:

2 g of neomycin sulfate.
0.3 g of nalidixic acid.
4 g of paromomycin sulfate.
60 g of lithium chloride (NPNL, Sigma Chemical Co.) prepared in 1 Liter of distilled water, filter-sterilized, and stored at 4°C until use.

D. The counting of *Streptococcus thermophilus* was determined using M17-lactose agar medium (**Saccaro et al. 2011**), which has the following composition:

Tryptone 5.0 g.
Soya peptone 5.0 g.
Meat digest 5.0 g.
Magnesium sulphate 0.25 g.
Di-sodium-glycerophosphate 19.0 g.
Agar 15 g
Distillation water 1000.0 ml (pH 6.9±0.2 at 25°C).

M17_{pH6.9} agar-Commercial M17 agar (Oxoid, Basingstoke, UK) was prepared according to manufacturer’s instructions. The rehydrated medium was sterilized in an autoclave at 121°C for 15 min. 5.3 ml of membrane-filtered sterile solutions of 10% lactose were added per 100 ml of the sterilized mentioned medium just before pouring the agar medium. Inoculated plates in duplicates were incubated aerobically at 37°C for 72h. The colony morphology were 0.1-0.5 mm, round yellowish.

Enumeration of micro-organisms

The counts of the yogurt starter cultures and probiotic microorganisms were enumerated as follows:

Re-activated pure culture test: The activated cultures (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidium*) were enumerated on M17 and MRS, respectively, to evaluate the appropriate selective media for each strain (**Talwalkar et al., 2003 and Kailasapathy et al., 2008**). The experiment was replicated twice.

Yogurt and fermented milks test: The cell counts of the yogurt starter cultures and probiotics bacteria prepared with mixed cultures were enumerated after 1, 7 and 14 days storage at <10°C. Samples (1 mL) were added to 9 ml of sterile peptone diluents (0.1g/L);

appropriate dilutions were made. Enumeration was carried out using pour plate technique. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were incubated aerobically at 37°C for 72h. The probiotic cultures (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) were enumerated after anaerobic incubation at 37°C for 48 to 72h. Anaerobic conditions were created using AnaeroGen (Oxoid, Basingstoke, UK). Plates containing 20 to 200 colonies were enumerated, and the counts were expressed as log₁₀cfu/g of the product. The selectivity of the growth conditions was confirmed by microscopic examination.

Sensory Evaluation:

Samples of 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.

Statistical Analysis:

The obtained results were statistically analyzed using a software package (SAS, 1991) based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan (1955) for the comparison between means. The data presented, in the tables, are the mean (\pm standard deviation) of 3 experiments.

III. RESULT AND DISCUSSION

Chemical composition of yoghurt fortified with honey:

For improvement of sensory evaluation of yoghurt especially flavour, 5% honey was added to cow and coconut milk and their mixtures. The added amount of honey was determined based on the findings of literatures. Different yoghurt samples were stored at 4°C for 14 days and analyzed in fresh and after 7 and 14 days.

The changes in the titratable acidity (% lactic acid), pH, and E_h during storage of yoghurt are presented in **Table 1**. The values of titratable acidity and E_h gradually increased during refrigerated storage of various treatments of yoghurt. The results 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., (2013)**. On the other hand, the acidity percentages and E_h values of yoghurt treatments contained 5% honey were significantly ($p < 0.05$) higher than that of control at zero time and during storage period. Moreover, the rises in titratable acidity and E_h or drop in pH during storage were higher in honey yoghurt than that of control. This may be due to the honey content of fructooligosaccharides (**Akalin et al., 2007**). Acidity values of fresh samples A and B were 0.85 and 0.94% respectively.

Mixing 25 or 50% coconut milk with cow milk decreased acidity and E_h values and increased pH levels of yoghurt. Values of E_h of treatments B, C and D at the seventh day of storage were 182, 172 and 161 respectively. This is in close agreement with the report of **Ladokun and Oni (2014)**.

Apart from the type of milk used in manufacturing, acidity and E_h values of classic starter yoghurt samples were relatively higher while pH data were lower than those made using ABT culture. Also, the rise in titratable acidity and E_h in classic starter yoghurt was more than that observed in the ABT one. This finding was in agreement with those of **Hussein (2010)**. Opposite outcomes were found by **El-Sayed et al., (2013)** who reported that the pH decreased at similar rates within yoghurt treatments made using different combinations of normal yoghurt starter and probiotic *B. bifidum* and *L. plantarum*. There were no significant differences in the pH of the control and all treatments. They concluded that supplementation with different starter cultures had no significant influence on pH of yoghurt during either fermentation process or post-fermentation changes through storage.

Morris (2000) reported that E_h of a growth medium has an inverse relationship with pH. Therefore, this increase in yogurt E_h from day 1 to 15 could be attributed to the decrease in pH over the same storage period and/or increase in oxygen tension due to air permeability through the plastic containers during storage. It is observed from Table 2 that there is a substantial effect of adding honey on TS and ash contents of yoghurt. Significant ($p < 0.05$) increases in TS and ash contents of yoghurt were obtained with fortification of milk by 5% honey. Similar results were reported by **Ammar et al.,**

(2015). Fat contents of treatments with or without addition honey were close to each other. Total solids and fat values were significantly ($p < 0.05$) higher while ash contents were slightly lower in yoghurt treatments contained coconut milk. On the other side, yoghurt prepared using classic starter possessed TS, fat and ash

concentrations similar to that prepared by ABT starter. During storage, TS, fat and ash contents of various yoghurt treatments slightly increased and could be ascribed to moisture loss.

Table 1. Effect of mixing 5% honey with cow or coconut milk on acidity, pH and redox potential (E_h) values of yoghurt during storage period

Properties	Treatments	Storage period (day)			Means
		Fresh	7	14	
Acidity %	A	0.85	1.07	1.23	1.05 ^c
	B	0.94	1.19	1.37	1.17 ^a
	C	0.90	1.13	1.30	1.11 ^b
	D	0.84	1.05	1.21	1.03 ^c
	E	0.76	0.94	1.07	0.92 ^e
	F	0.86	1.05	1.21	1.04 ^c
	G	0.81	0.99	1.13	0.98 ^d
	H	0.77	0.93	1.05	0.92 ^e
	Means	0.84 ^C	1.04 ^B	1.20 ^A	
pH values	A	4.61	4.50	4.41	4.51 ^c
	B	4.50	4.36	4.25	4.37 ^e
	C	4.56	4.44	4.34	4.45 ^d
	D	4.63	4.51	4.43	4.52 ^c
	E	4.72	4.64	4.57	4.64 ^b
	F	4.59	4.49	4.39	4.49 ^c
	G	4.74	4.65	4.54	4.64 ^b
	H	4.80	4.70	4.63	4.71 ^a
	Means	4.64 ^A	4.54 ^B	4.45 ^C	
E_h mV	A	161	169	176	169 ^{bc}
	B	170	182	191	181 ^a
	C	163	172	181	172 ^b
	D	154	161	168	161 ^d
	E	154	161	167	161 ^d
	F	162	170	178	170 ^b
	G	158	166	173	166 ^c
	H	153	158	164	158 ^d
	Means	159 ^C	167 ^B	175 ^A	

^{abcde} Letters indicate significant differences between yoghurt treatments

^{ABCD} Letters indicate significant differences between storage times

*mV: millivolts

A: Yoghurt made from cow milk and classic starter

B: Yoghurt made from cow milk + 5% honey and classic starter

C: Yoghurt made from 75 % cow milk + 25 % coconut milk + 5% honey and classic starter

D: Yoghurt made from 50 % cow milk + 50 % coconut milk + 5% honey and classic starter

E: Yoghurt made from cow milk and ABT culture

F: Yoghurt made from cow milk + 5% honey and ABT culture

G: Yoghurt made from 75% cow's milk + 25% coconut milk + 5% honey and ABT culture

H: Yoghurt made from 50 % cow milk + 50 % coconut milk + 5% honey and ABT culture

Table 2. Effect of mixing 5% honey with cow or coconut milk on TS, fat and ash values of yoghurt during storage period

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
TS %	A	14.62	14.70	14.82	14.71 ^g
	B	18.59	18.68	18.75	18.67 ^f
	C	19.61	19.77	19.89	19.76 ^d
	D	20.70	20.82	20.91	20.81 ^a
	E	14.49	14.53	14.61	14.54 ^h
	F	18.64	18.70	18.81	18.72 ^e
	G	19.70	19.79	19.91	19.80 ^c
	H	20.64	20.71	20.84	20.73 ^b
	Means	18.37 ^C	18.46 ^B	18.57 ^A	
Fat %	A	3.6	3.6	3.7	3.6 ^c
	B	3.5	3.6	3.6	3.6 ^c
	C	5.4	5.4	5.5	5.4 ^b
	D	6.5	6.5	6.6	6.5 ^a
	E	3.5	3.6	3.6	3.6 ^c
	F	3.6	3.6	3.6	3.6 ^c
	G	5.4	5.5	5.5	5.5 ^b
	H	6.6	6.6	6.7	6.6 ^a
	Means	4.8 ^A	4.8 ^A	4.9 ^A	
Ash %	A	0.77	0.80	0.84	0.80 ^{bc}
	B	0.84	0.87	0.92	0.88 ^a
	C	0.81	0.83	0.87	0.84 ^{ab}
	D	0.78	0.81	0.84	0.81 ^{bc}
	E	0.76	0.79	0.82	0.79 ^c
	F	0.82	0.85	0.90	0.86 ^a
	G	0.80	0.84	0.88	0.84 ^{ab}
	H	0.76	0.80	0.85	0.80 ^{bc}
	Means	0.79 ^C	0.82 ^B	0.87 ^A	

^{abcde} Letters indicate significant differences between yoghurt treatments

^{ABCD} Letters indicate significant differences between storage times

A: Yoghurt made from cow milk and classic starter

B: Yoghurt made from cow milk + 5% honey and classic starter

C: Yoghurt made from 75 % cow milk + 25 % coconut milk + 5% honey and classic starter

D: Yoghurt made from 50 % cow milk + 50 % coconut milk + 5% honey and classic starter

E: Yoghurt made from cow milk and ABT culture

F: Yoghurt made from cow milk + 5% honey and ABT culture

G: Yoghurt made from 75% cow's milk + 25% coconut milk + 5% honey and ABT culture

H: Yoghurt made from 50 % cow milk + 50 % coconut milk + 5% honey and ABT culture

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

Results shown in Table 3 illustrate the effect of supplementation of yoghurt with 5% honey and utilization coconut milk on total nitrogen (TN), water soluble nitrogen (WSN) and total volatile fatty acids (TVFA) contents during the refrigerated storage.

Mixing of 5% honey with cow or coconut milk slightly lowered TN values in yoghurt produced. Values of TN of fresh A and B samples were 0.625, and 0.615% respectively. As storage period advanced, TN values of all samples slightly increased. On the other hand, concentrations of TN were higher in cow milk yoghurt as compared with that made from cow and coconut milk mixtures. Total nitrogen contents of yoghurt treatments were not clearly affected by type of starter. Levels of TN of fresh samples A and E were 0.625 and 0.627% respectively.

Fortification of milk with 5% honey increased WSN contents in yoghurt which may refer to the stimulation effect of fructooligosaccharides in honey on bifidobacteria (Akalin *et al.*, 2004). Because of high TN content of cow milk as compared with coconut milk, yoghurt made from cow milk individually characterized by high concentrations of WSN comparing with that made from cow and coconut milk mixtures. Not only were those, but also cow milk yoghurt possessed the greatest rates of WSN development during storage period. Values of WSN development of samples B, C and D were 35.77, 33.90 and 32.74% respectively. Contents of WSN were higher in yoghurt made using classic culture as compared with that made by ABT. This may be due to the high proteolytic activity of *L. delbrueckii* subsp. *bulgaricus* (Ammar *et al.*, 2014). During refrigerated storage, WSN values obviously increased and the increasing rates were higher in yoghurt contained honey or made using classic culture as compared with other treatments. Increasing of WSN values may be due to the protein breakdown in the Labneh by milk enzymes and other microbial activities (El-Zeini *et al.*, 2007).

As known, lactic acid bacteria added as the starter culture or present as non-starter lactic acid bacteria are able to transform lactic acid, citrate, lactate, proteins and fat into volatile compounds (Ortigosa *et al.*, 1999). Total volatile fatty acids (TVFA) are taken as a measure of the degree of fat hydrolysis during storage (Table 3). As storage time increased, TVFA contents significantly ($p < 0.001$) increased in different yoghurt treatments.

Table 3. Effect of mixing 5% honey with cow or coconut milk on TN, WSN and TVFA of yoghurt

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
TN %	A	0.625	0.628	0.630	0.628 ^a
	B	0.615	0.620	0.623	0.619 ^b
	C	0.603	0.609	0.614	0.609 ^c
	D	0.595	0.601	0.606	0.601 ^d
	E	0.627	0.630	0.635	0.631 ^a
	F	0.613	0.621	0.622	0.619 ^b
	G	0.605	0.610	0.615	0.610 ^c
	H	0.593	0.600	0.607	0.600 ^d
	Means	0.610 ^C	0.615 ^B	0.619 ^A	
WSN %	A	0.115	0.141	0.154	0.137 ^{bc}
	B	0.123	0.152	0.167	0.147 ^b
	C	0.118	0.145	0.158	0.140 ^{bc}
	D	0.113	0.137	0.150	0.133 ^{cd}
	E	0.110	0.134	0.145	0.130 ^{cd}
	F	0.104	0.129	0.139	0.124 ^{de}
	G	0.099	0.122	0.131	0.117 ^e
	H	0.095	0.118	0.127	0.398 ^a
	Means	0.110 ^C	0.135 ^B	0.146 ^A	
TVFA %	A	9.2	10.8	11.8	10.6 ^f
	B	10.4	12.2	13.5	12.0 ^{cd}
	C	11.0	12.9	13.9	12.6 ^b
	D	11.6	13.3	14.5	13.1 ^a
	E	8.5	9.9	10.7	9.7 ^g

	F	9.6	11.3	12.3	11.1 ^e
	G	10.3	12.1	13.2	11.9 ^d
	H	10.8	12.7	13.6	12.4 ^{bc}
	Means	10.2 ^C	11.9 ^B	12.9 ^A	

^{abcde} Letters indicate significant differences between yoghurt treatments

^{ABCD} Letters indicate significant differences between storage times

A: Yoghurt made from cow milk and classic starter

B: Yoghurt made from cow milk + 5% honey and classic starter

C: Yoghurt made from 75 % cow milk + 25 % coconut milk + 5% honey and classic starter

D: Yoghurt made from 50 % cow milk + 50 % coconut milk + 5% honey and classic starter

E: Yoghurt made from cow milk and ABT culture

F: Yoghurt made from cow milk + 5% honey and ABT culture

G: Yoghurt made from 75% cow's milk + 25% coconut milk + 5% honey and ABT culture

H: Yoghurt made from 50 % cow milk + 50 % coconut milk + 5% honey and ABT culture

It is quite apparent from the results reported in **Table 3** that yoghurt contained 5% honey possessed the highest levels of TVFA values and also rates of TVFA development. Total volatile fatty acids rose during storage period by 28.26 and 29.81% for samples A and B respectively. In supplementary, **Chick et al., (2001)** mentioned that the organic acids production was enhanced when bifidobacteria were grown in the presence of honey, where various oligosaccharides found in honey may be responsible for enhancing organic acids production by bifidobacteria. Honey also contains a variety of organic acids such as acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic and succinic acids (0.17 to 1.17%), **NHB (1996)**.

Using cow and coconut milk mixtures in yoghurt preparation increased the concentrations of TVFA. This may be attributed to the high fat content of coconut milk. On the contrary, using of ABT culture in manufacturing of yoghurt lowered TVFA content as comparing with utilization classic starter.

Free fatty acids content (FFA) of yoghurt:

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 (**Güler and Park 2011**). The FFA profile in fresh yoghurt was illustrated in **Tables 4 and 5**.

Saturated and unsaturated fatty acids:

The levels of saturated fatty acids (SFA) of various yoghurt samples were inversely proportional with the concentrations of unsaturated fatty acids (USFA). The value of SFA was higher than USFA in all yoghurt treatments. Fortification of yoghurt with 5% honey caused a markedly decrease in SFA and increase in USFA contents. Ratios of SFA were 65.45 and 63.37% (as percent of total fat) for samples A and B respectively. Respective values for USFA were 34.55 and 36.63% respectively.

It could be viewed from **Tables 4 and 5** that addition 25 or 50% coconut milk to cow milk markedly increased the amount of SFA and inversely decreased the amounts of USFA of yoghurt. Increasing of SFA in coconut milk doesn't lower its healthy benefits. **Dayrit (2003)** showed that virgin coconut oil (VCO) is digested easily without the need for bile and goes directly to the liver for conversion into energy. On the other hand, VCO stimulates metabolism, boosts energy and prevents deposition of fats thereby preventing obesity. Also, **Five (2004)** stated that VCO possesses anti-inflammatory, antimicrobial and antioxidant properties which work together to protect arteries from atherosclerosis and the human heart from cardiovascular disease. VCO improves the nutritional values of food by increasing absorption of vitamins, minerals and amino acids.

Utilization of ABT starter caused a pronounced decrease in SFA and increase in USFA contents of yoghurt. Generally, the most predominant SFA found in different yoghurt samples (except samples D and H) was palmitic acid (C₁₆). In samples D and H, lauric acid (C₁₂) was the most abundant. The highest acid ratio of USFA was oleic acid (18:1 ω₉) for various yoghurt samples.

Medium chain fatty acids (C8 – C12):

Control and honey yoghurt (samples A, B, E and F) had similar medium chain fatty acids (MCFA) contents while differences in the levels of MCFA were noticed between the coconut milk and the control yoghurt. Using of coconut milk in yoghurt manufacturing considerably increased the concentrations of MCFA. The levels of MCFA in treatments B, C and D were 5.791, 21.433 and 30.501% respectively. This may be due to the very high content of MCFA especially lauric acid (C_{12:0}) in coconut milk. **Bawalan and Chapman (2006)** cleared that coconut oil is unique amid fats and oils, as it contains the highest percentage of medium chain fatty acids with a carbon-chain length of 8 to 12 carbon atoms. VCO behaves and metabolizes differently in the human body to other saturated and unsaturated fats or oils. MCFA in coconut oil is about 64% with lauric fatty acid (C₁₂) as the highest ranging from 47 to 53% depending on the coconut variety. The medium chain (C₈-C₁₂) fats in coconut oil are similar in structure to the fats in mother's milk that gives babies immunity to disease. There are also similar beneficial effects in adults (**Kabara, 2000**).

Yoghurt made using ABT culture had slight lower MCFA contents than that made by classic culture. **Beshkova et al., (1998)** found that the formation of volatile free fatty acids (C₂-C₁₀) was more active in the mixed yoghurt cultures than in the pure ones owing to the stimulating effect of protocol-operation between the two thermophillic species on the metabolic activities, which are responsible for the formation of free fatty acids. In fact, volatile acids is not only produced from lipolysis by lipases but also from several biochemical pathways including the fermentation of lactose or citrate and the degradation (oxidative deamination or decarboxylation) of amino acids (alanine and serine) which are the most important precursor of most volatile fatty acids (**Kneifel et al., 1992; Beshkova et al., 1998**).

In various yoghurt treatments, the fatty acid lauric (C:12) was the predominant MCFA followed by capric acid (C_{10:0}).

Long chain fatty acids (> C12):

The contents of long chain fatty acids (LCFA) were similar in yoghurt made with or without adding honey. Mixing of 25 or 50% coconut milk with cow milk decreased the content of yoghurt from these acids. Furthermore, LCFA levels of yoghurt slightly increased when ABT culture was used in production. Among all the long chain fatty acids measured, the value of palmitic acid was the highest in yoghurt samples A, B, E and F whereas oleic acid was the highest in treatments C, D, G and H.

Table 4. Effect of mixing 5% honey with cow or coconut milk on fatty acids content (%) of fresh yoghurt

Fatty acids	C	Treatments							
		A	B	C	D	E	F	G	H
		Saturated fatty acids (SFA) %							
Caprylic	8:0	0.450	0.550	2.451	3.011	0.396	0.551	2.222	3.010
Capric	10:0	2.058	2.031	3.132	3.980	2.031	2.062	3.071	3.781
Lauric	12:0	3.276	3.210	15.85	23.51	3.149	3.042	14.94	22.61
Myristic	14:0	9.011	9.161	10.29	12.76	8.059	7.458	9.47	11.57
Pentadecanoic	15:0	1.845	1.620	1.131	0.621	1.617	1.442	1.001	0.700
Palmitic	16:0	29.73	29.25	22.82	18.04	29.33	28.58	21.85	17.89
Heptadecanoic	17:0	1.901	1.452	0.362	-	1.693	1.283	0.500	-

Stearic	18:0	16.99	15.90	11.41	10.65	15.81	15.43	11.17	9.71
Arachidic	20:0	0.189	0.201	-	0.460	0.164	0.244	-	0.132
Total		65.45	63.37	67.45	73.03	62.25	60.09	64.22	69.04
Unsaturated fatty acids (USFA) %									
Myristioleic acid	14:1	0.378	1.130	0.641	0.251	1.222	1.290	0.712	0.423
	15:1	0.185	0.554	0.166	0.141	-	0.625	0.270	0.579
Palmitioleic	16:1	2.195	2.308	1.811	1.092	2.473	2.589	1.952	1.535
Oleic	18:1	26.22	27.44	25.00	22.03	27.88	28.07	26.68	23.91
	18:2	1.061	1.124	0.986	0.500	1.211	1.716	1.251	0.701
Linoleic	18:2	2.891	2.971	2.836	2.570	3.073	3.694	3.419	2.835
α-Linolenic	18:3	0.764	0.848	0.398	0.315	0.866	0.961	0.512	0.522
Gamma linolenic	18:3	0.322	0.157	0.400	0.071	0.444	0.439	0.307	0.155
	20:2	0.194	0.098	0.123	-	0.210	0.224	0.200	0.300
	22:2	0.343	-	0.487	-	0.371	0.302	0.477	
Total		34.55	36.63	32.55	26.97	37.75	39.91	35.78	30.96

Table 5. Effect of mixing 5% honey with cow or coconut milk on free fatty acid indices ratios of fresh yoghurt

Treatments	SFA	USFA	MCFA	LCFA
A	65.45	34.55	5.784	94.216
B	63.37	36.63	5.791	94.209
C	67.45	32.55	21.433	78.567
D	73.03	26.97	30.501	69.499
E	62.25	37.75	5.576	94.424
F	60.09	39.91	5.655	94.345
G	62.22	35.78	20.233	79.767
H	69.04	30.96	29.401	70.599

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

Microbial Analysis of Yoghurt:

Yoghurt treatments manufactured from cow and coconut milk supplemented with 5% honey and using classic or ABT cultures were analyzed microbiologically for *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Results were cleared in Table 6. In different yoghurt samples, the counts of mentioned bacteria decreased during storage period. This reduction may be attributed to the high acidity produced by microbial fermentation (**Dave and Shah, 1997**).

The counts of *Lactobacillus bulgaricus* were higher in yoghurt fortified with honey than control. To the contrary, losses of viability levels of *Lactobacillus bulgaricus* during storage were lower in honey yoghurt than those of other treatments. Values of loss of viability for samples A and B were 54.54 and 21.05% respectively. **Nagpal and Kaur (2011)** reported that honey added at the level of 5% improved the viability of lactobacilli pure cultures after 5 weeks storage and that improvement might be strain dependent.

Outcomes presented in Table 6 confirmed that yoghurt made from cow and coconut milk mixtures gained the greatest count of *Lactobacillus bulgaricus* dislike cow milk samples which recorded the lowest count.

Utilization of honey or coconut milk in yoghurt production significantly ($p < 0.05$) increased the numbers of *Streptococcus thermophilus* in fresh product and within storage period. In addition to this, honey yoghurt possessed

the lowest levels of survival loss during storage. Yoghurt made using ABT culture had higher *Str. thermophilus* counts than those made by classic starter, meaning that the presence of *L. acidophilus* and *B. bifidum* clearly encouraged *Str. thermophilus* growth. This effect may be attributed to the low activity of acidity production of *L. acidophilus* and *B. bifidum* as compared with *L. bulgaricus* found in classic starter. Therefore, loss of survival values of *Str. thermophilus* were lower in ABT yoghurt than those of classic starter one.

The effect of fortification of yoghurt with 5% honey on *L. acidophilus* numbers was similar to that of using coconut milk. Numbers of these probiotic bacteria highly increased in honey yoghurt especially treatments made from cow and coconut mixtures which also had the minimum of survival loss. Values of loss of survival through storage were 40.00, 26.31, 21.74 and 20.00% for samples E, F, G and H respectively.

Mixing of 5% honey with cow milk or mixture of cow and coconut milk increased counts while decreased loss of viability of *B. bifidum* in yoghurt. **Ustunol and Gandhi (2001)** found that the honey promotes of *Bifidobacterium bifidum* growth.

It is clear from the results of Table 6 that bifidobacteria counts were higher in yoghurt treatments contained coconut milk than those of cow milk which may be due to the activation effect of coconut milk components on bifidobacteria. This means that our treatments had no worthless effect on these healthy bacteria. Furtherance of these results, the loss of viability rates of bifidobacteria throughout cold storage of yoghurt also were lower in coconut milk samples than other treatments.

However, lowering of bifidobacteria counts during storage, but the recommended level of 10^7 cfu.g⁻¹ of bifidobacteria as a probiotic was exceeded for different yoghurt treatments and remained above 10^7 cfu g⁻¹ until the end of storage stage especially in honey and coconut milk samples.

Table 6. Effect of mixing 5% honey with cow or coconut milk on starter bacteria counts of yoghurt during storage period

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
<i>Lactobacillus bulgaricus</i> (cfu×x10 ⁵ /g)	A	11	9	5	8 ^c
	B	19	18	15	17 ^b
	C	22	20	17	20 ^{ab}
	D	25	22	19	22 ^a
	E	-	-	-	
	F	-	-	-	
	G	-	-	-	
	H	-	-	-	
	Means	19 ^A	17 ^{AB}	14 ^B	
<i>Streptococcus thermophilus</i> (cfu×x10 ⁵ /g)	A	18	15	10	14 ^f
	B	23	21	18	21 ^e
	C	24	22	18	21 ^{de}
	D	27	26	22	25 ^d
	E	41	36	32	36 ^c
	F	47	44	39	43 ^b
	G	52	49	46	49 ^a
	H	55	51	48	51 ^a
	Means	36 ^A	33 ^B	29 ^C	
	A	-	-	-	

<i>Lactobacillus acidophilus</i> (cfu×x10 ⁵ /g)	B	-	-	-	
	C	-	-	-	
	D	-	-	-	
	E	15	13	9	12 ^c
	F	19	17	14	17 ^{bc}
	G	23	22	18	21 ^{ab}
	H	25	23	20	23 ^a
	Means	21 ^A	19 ^{AB}	15 ^B	
<i>Bifidobacterium bifidum</i> (cfu×x10 ⁵ /g)	A	-	-	-	
	B	-	-	-	
	C	-	-	-	
	D	-	-	-	
	E	31	28	20	26 ^c
	F	40	37	34	37 ^b
	G	44	42	38	41 ^{ab}
	H	47	46	42	45 ^a
Means	41 ^A	38 ^A	34 ^B		

^{abcde} Letters indicate significant differences between yoghurt treatments

^{ABCD} Letters indicate significant differences between storage times

Changes in sensory evaluation of yoghurt:

Sensory analysis (quantitative and / or descriptive) is often used to assess the flavor, appearance, texture and other attributes of food products as a function of processing parameters (Kwok *et al.*, 2000). The results given in Table 7 described the influence of addition honey and using coconut milk and ABT culture on the sensory evaluation of yoghurt.

The effect of supplementation yoghurt with 5% honey was not so much pronounced in color and appearance. On the other hand, scores of color and appearance attributes tested in fresh samples and during storage period were slightly higher for yoghurt made from cow and coconut milk mixtures than those of yoghurt prepared from cow milk only. It is clear that the color and appearance scores of yoghurt made using classic or ABT cultures were close to each other. These results are in agreement with those obtained by Ammar *et al.*, (2015).

Addition honey increased body and texture scores in the produced yoghurt which may be due to the increasing of TS content. Also, texture and body scores were higher in yoghurt made from cow and coconut milk mixtures than that made from cow milk. The texture and body scores of ABT yoghurt slightly lowered than classic starter one.

Fortification of yoghurt with honey improved the flavour evaluation scores. When compared with plain (control) yoghurt samples, honey yoghurt samples were preferred by the panelists that tasted the samples who attributed that to the lovely sweet taste of honey. In similar report to our present work, Amiri *et al.*, (2010) found that the incorporation of honey led to development of sweetened synbiotic acidophilus milk. Addition of honey (7%) to acidophilus milk made by *Lactobacillus acidophilus* + *Bifidobacterium bifidum* + *Lactobacillus casei* increased the sensory score for colour, flavour, texture and overall acceptability of the product developed. They also mentioned that incorporation of *B. bifidum* increased the flavour of synbiotic acidophilus milk when compared to *L. acidophilus* as control, whereas *L. casei* culture showed thinner consistency in the product. Addition of prebiotic affected only the sensory scores, whereas the probiotics addition resulted in a marginal variation of pH and titratable acidity.

Table 7. Effect of mixing 5% honey with cow or coconut milk on sensory evaluation of yoghurt during storage period

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	14	13	14 ^a
	D	14	14	14	14 ^a
	E	13	13	12	13 ^a
	F	13	12	12	12 ^a
	G	14	13	13	13 ^a
	H	14	13	13	13 ^a
	Means	14 ^A	13 ^A	13 ^A	
Body & Texture (35)	A	33	33	31	32 ^a
	B	34	34	33	34 ^a
	C	34	34	33	34 ^a
	D	34	34	34	34 ^a
	E	31	30	27	29 ^b
	F	33	33	32	33 ^a
	G	33	33	32	33 ^a
	H	33	33	32	33 ^a
	Means	33 ^A	33 ^A	32 ^B	
Flavor (50)	A	45	44	41	43 ^c
	B	47	47	45	46 ^{ab}
	C	47	47	45	46 ^{ab}
	D	47	47	46	47 ^{ab}
	E	46	45	43	45 ^{bc}
	F	48	47	45	47 ^{ab}
	G	48	47	45	47 ^{ab}
	H	49	48	47	48 ^a
	Means	47 ^A	47 ^A	45 ^B	
Total (100)	A	91	90	84	88 ^c
	B	94	94	90	93 ^{ab}
	C	95	95	91	94 ^{ab}
	D	95	95	94	95 ^a
	E	90	88	82	87 ^c
	F	94	92	89	92 ^b
	G	95	93	90	93 ^{ab}
	H	96	94	92	94 ^{ab}
	Means	94 ^A	93 ^A	89 ^B	

^{abcde} Letters indicate significant differences between yoghurt treatments

^{ABCD} Letters indicate significant differences between storage times

Yoghurt manufactured from cow and coconut milk mixtures recorded the highest levels of flavour which may be due to the good coconut flavor. Because ABT culture produces mild acidity as compared with classic culture (Kurmann *et al.*, 1992), using it in yoghurt manufacture slightly improved the flavour. These findings agreed with that reported by Abd El-Salam *et al.*, (2011).

Fresh yoghurt treatments obtained the highest scores of sensory evaluation. During storage period, the sensory evaluation degrees of various samples decreased. Our results are in agreement with **Osman and Ismail (2004)** who cleared that significant ($p < 0.001$) decreases in the total organoleptic scores of bio-yoghurt were noticed when storage period progressed.

IV. CONCLUSION

Mixing of 5% honey with cow and coconut milk mixtures and using of ABT culture produced bio-yoghurt with highly nutritional value. This yoghurt characterized by acceptable in properties of color, appearance, texture and body and flavour.

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