International Journal of Microbiology and Application
2017; 4(1): 1-9
http://www.openscienceonline.com/journal/ijma
ISSN: 2381-442X (Print); ISSN: 2381-4438 (Online)

Utilization ABT Culture, Coconut Milk, and Honey in Bio-Yoghurt Manufacture

Magdy Mohamed Ismail[1, *], Mohamed Farid Hamad[2], Sherif Lotfy El-Kadi[3], Mohamed Shalaby Zidan[4]

[1]Dairy Technology Department, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

[2]Department of Dairying, Faculty of Agriculture, Damietta University, Damietta, Egypt

[3]Agriculture Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt

[4]General Organization for Export and Import Control, Ministry of Trade and Industry, Damietta, Egypt

Email address

abo-omar98@hotmail.com (M. M. Ismail)

[*]Corresponding author

To cite this article

Magdy Mohamed Ismail, Mohamed Farid Hamad, Sherif Lotfy El-Kadi, Mohamed Shalaby Zidan. Utilization ABT Culture, Coconut Milk, and Honey in Bio-Yoghurt Manufacture. *International Journal of Microbiology and Application*. Vol. 4, No. 1, 2017, pp. 1-9.

Received: March 22, 2017; **Accepted**: May 1, 2017; **Published**: August 2, 2017

Abstract

In this study, impact of using probiotic culture, coconut milk, and honey on chemical composition, microbial and sensory properties of bio-yoghurt was evaluated. Eight treatments of yoghurt were made from cow and coconut milk or from their mixtures with or without adding 5% honey and using classic or ABT-5 starters. The obtained results showed that acidity, total solids, ash, water soluble nitrogen (WSN) and total volatile fatty acids (TVFA) values of yoghurt treatments contained 5% honey were higher than that of control. Fortification of yoghurt with 5% honey caused a markedly decrease in saturated fatty acids (SFA) and increase in unsaturated fatty acids (USFA) contents. The counts of *Lactobacillus bulgaricus*, *S. thermophiles*, *L. acidophilus* and bifidobacteria were higher in yoghurt fortified with honey than control. Supplementation yoghurt with 5% honey improved body, texture and flavour of yoghurt. Mixing 25 or 50% coconut milk with cow milk decreased values of acidity, ash, total nitrogen and WSN while increased pH, total solids, fat and TVFA levels of yoghurt. Blending coconut milk with cow milk highly increased medium chain fatty acids especially lauric acid in yoghurt. Yoghurt made from cow and coconut milk mixtures recorded the greatest count of *Lactobacillus bulgaricus*, *S. thermophiles*, *L. acidophilus* and bifidobacteria. Scores of sensory attributes were higher for yoghurt made from cow and coconut milk mixtures than those of yoghurt prepared from cow milk only. Incorporation 5% honey with cow and coconut milk mixtures and using of ABT culture produced bio-yoghurt with highly nutritional value.

Keywords

Bio-Yoghurt, Cow Milk, Coconut Milk, Honey, Storage Period

1. Introduction

Yoghurt is one of the most popular fermented milk products worldwide because it has many health benefits such as improving lactose intolerance, reducing risk of certain cancers, anti-cholesterolaemic effects, prevention of genital and urinary tract infections and other health attributes associated with probiotic bacteria [1, 2]. Probiotic is defined as a live microbe that protects its host and prevents disease. Therefore, a probiotic is a living microorganism that when administered in sufficient numbers is beneficial to the host and exerts health benefits beyond inherent basic nutrition.

On the other hand, populations around the world use coconuts as their source of meat, juice, milk, and oil. Coconut is highly nutritious and is full of fiber, vitamins, and minerals. It also provides many health benefits beyond its nutritional content. Coconut has also been one of the sources of economy to nearly one third of the world's population. Among these cultures, the coconut has a long and respected history. Coconut milk is an oil-in-water emulsion formed from the aqueous extract of coconut solid endosperm. Coconut milk has been used as a vital ingredient in a variety of Asian foods and desserts especially in China, India and

Southeast Asia. In general, coconut milk is milky white juice prepared by pressing grated coconut flesh with or without added water. The composition of fresh coconut milk typically contains aqueous 55 ± 3%, fat 37 ± 2% and protein 8 ± 2% [3]. According to its composition, coconut milk is an oil-in-water emulsion which is stabilized by natural emulsifiers such as globulins and albumins proteins and phospholipids [4]. Fat is considered as a key component because it effects on the appearance and sensorial attributes of coconut milk products and the food that applied them as an ingredient.

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 [5]. Several therapeutic and medicinal effects such as antibacterial, anti-mutagenic, anti-proliferative, hepatoprotective, hypoglycemic, and antioxidant effects have been ascribed to honey through last years [6, 7]. Poorani et al. [8] 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.

2. Materials and Methods

2.1. Materials

Fresh cow's milk was obtained 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 thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) and ABT-5 culture which consists of *S. thermophiles*, *Lactobacillus acidophilus* + *Bifidobacterium* (Chr. Hansen's Lab A/S Copenhagen, Denmark) were used. Starter cultures were in freeze-dried direct-to-vat set form and stored at –18°C until used.

2.2. Methods

2.2.1. Preparation of 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.

2.2.2. Manufacture of Yoghurt Supplemented with Honey

Eight treatments of yoghurt were made as fallow:

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

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.

2.2.3. Methods of Analysis

(1) Chemical Analysis

Total solids, fat, total nitrogen 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].

(2) Determination of fatty acids composition

The extraction of milk fat was done using the method of Rose-Gottlieb using diethyl ether and petroleum ether (Method enbuch, Bd. VI VDLUFA-Verlag, Darmstadt, 1985). After that the solvents were evaporated on a vacuum rotary evaporator. For obtaining methyl esters of the fatty acids, sodium methylate (CH_3ONa) was used [13]. The fatty acid composition of yoghurt was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC^{TM}- WAX, 30 m, ID 0.25 mm, Film:0,25 µm.

(3) 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].

(4) 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.

(5) 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.

3. Results and Discussion

3.1. Chemical Composition of Cow and Coconut Milk and Their Mixtures

Acidity and E_h levels of cow's milk were slightly higher than those of coconut milk (Table 1). Therefore, blinding of different amounts of cow's milk with coconut milk increased acidity and E_h values of the resultant milk. Values of pH of various treatments possessed the opposite trend of acidity and E_h.

There is a higher amount of total solids (almost one and a half) and fat (almost three times) in the coconut milk than in the cow's milk. On the contrary, total nitrogen and ash contents of the former were lower than the latter. Mixing of coconut milk with cow's milk increased TS and fat values and decreased total nitrogen and ash contents of the resulted mixtures. These results are in agreement with the work of Ladokun and Oni [18] who cleared that coconut milk contains higher total solids and fat and lower crude protein and ash than cow and goat milk. The ash content which was highest in goat milk and lowest in coconut milk could be due to the salt lick activities done by the herbivores [19].

Generally, the chemical composition values of coconut milk were within ranges described by Arumughan et al. [20] while were lower than recommended by Law et al. [21]. Arumughan et al. [20] showed that total solids, fat and ash contents of coconut milk produced in Singapore were 15.60, 11.00 and 0.70% respectively while Law et al. [21] cleared that total solids, fat and ash values of raw coconut milk were 33.89, 24.75 and 0.81% respectively. The variation in coconut to water ratio used for coconut milk extraction affects the coconut milk composition.

Table 1. *Chemical composition of cow and coconut milk and their mixtures.*

Treatments	Acidity (%)	pH values	E_h (mV*)	TS (%)	Fat (%)	TN (%)	Ash (%)
A	0.18^a	6.60^a	47.2^a	13.22^b	3.4^d	0.613^a	0.71^a
B	0.16^{ab}	6.70^a	37.1^c	18.26^a	9.0^a	0.424^{ab}	0.60^a
C	0.18^a	6.62^a	47.3^a	15.41^b	5.2^c	0.589^a	0.68^a
D	0.17^a	6.65^a	42.5^b	16.30^{ab}	6.6^b	0.526^a	0.65^a
E	0.16^{ab}	6.69^a	37.4^c	17.66^a	7.4^b	0.479^{ab}	0.64^a

*mV: millivolts
A: Cow milk
B: Coconut milk
C: 75% Cow milk + 25% Coconut milk
D: 50% Cow milk + 50% Coconut milk
E: 25% Cow milk + 75% Coconut milk

3.2. 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.

The changes in the titratable acidity (% lactic acid), pH, and E_h during storage of yoghurt are presented in Table 2. 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. [22].

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 is may be due to the honey content of fructooligosacchrides [23].

Mixing 25 or 50% coconut milk with cow milk decreased acidity and E_h values and increased pH levels of yoghurt. This is in close agreement with the report of Ladokun and Oni [18].

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 [24]. Opposite outcomes were found by El-Sayed et al. [25] who reported that the pH decreased at similar rates within yoghurt treatments made using different combinations of normal yoghurt starter and probiotic *Bifidobacterium* 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.

It is observed from Table 3 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. [26]. 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 2. *Effect of mixing 5% honey with cow or coconut milk on acidity, pH and redox potential (Eh) values of yoghurt.*

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
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 3. *Effect of mixing 5% honey with cow or coconut milk on TS, fat and ash values of yoghurt.*

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
Total Solids %	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

3.3. Changes in TN, WSN and TVFA of Yoghurt during Cold Storage

Mixing of 5% honey with cow or coconut milk slightly lowered TN values in yoghurt produced (Table 4). 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 [27]. 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. 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* [28]. 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 yoghurt by milk enzymes and other microbial activities [29].

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 [30]. Total volatile fatty acids (TVFA) are taken as a measure of the degree of fat hydrolysis during storage (Table 4). As storage time increased, TVFA contents significantly ($P< 0.001$) increased in different yoghurt treatments.

It is quite apparent from the results reported in Table 4 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. [31] 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%), [33].

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.

Table 4. *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	
Total protein %	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}	
Water soluble nitrogen %	A	0.115	0.141	0.154	0.137^{bc}
	B	0.123	0.152	0.167	0.147^{a}
	C	0.118	0.145	0.158	0.140^{b}
	D	0.113	0.137	0.150	0.133^{c}
	E	0.110	0.134	0.145	0.130^{cd}
	F	0.104	0.129	0.139	0.124^{d}
	G	0.099	0.122	0.131	0.117^{de}
	H	0.095	0.118	0.127	0.113^{e}
	Means	0.110^{C}	0.135^{B}	0.146^{A}	
Total volatile fatty acids*	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
* expressed as ml 0.1 NaOH 100 g^{-1} yoghurt

3.4. 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 [33]. The FFA profile in fresh yoghurt was illustrated in Tables 5 and 6.

3.4.1. 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 form Tables 5 and 6 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 health benefits. Dayrit [34] 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, Fife [35] 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 (C_{16}). In samples D and H, lauric acid (C_{12}) was the most abundant. The highest acid ratio of USFA was oleic acid (18:1 ω9) for various yoghurt samples.

3.4.2. 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 (C12:0) in coconut milk. Bawalan and Chapman [36] 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 chin (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 [37].

Yoghurt made using ABT culture had slight lower MCFA contents than that made by classic culture. Beshkova et al. [38] 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 [39, 38].

In various yoghurt treatments, the fatty acid lauric (C:12) was the predominant MCFA followed by capric acid (C10:0).

3.4.3. 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 5. *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 6. *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).

3.5. Microbial Analysis of Yoghurt

In different yoghurt samples, the counts of *Lactobacillus bulgaricus*, *Streptococcus thermophiles*, *Lactobacillus acidophilus* and *Bifidobacterium* decreased during storage period (Table 7). This reduction may be attributed to the high acidity produced by microbial fermentation [40].

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 [41] 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 7 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 thermophiles* 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 *S. thermophiles* counts than those made by classic starter, meaning that the presence of *L. acidophilus* and *Bifidobacterium* clearly encouraged *S. thermophiles* growth. This effect may be attributed to the low activity of acidity production of *L. acidophilus* and *Bifidobacterium* as compared with *L. bulgaricus* found in classic starter. Therefore, loss of survival values of *S. thermophiles* 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 *Bifidobacterium* in yoghurt. Ustunol and Gandhi [42] found that the honey promotes of *Bifidobacterium* growth.

It is clear from the results of Table 7 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.

3.6. 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 [43]. The results given in Table 8 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. [26].

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.

Table 7. *Effect of mixing 5% honey with cow or coconut milk on starter bacteria counts of yoghurt.*

Properties	Treatments	Storage period (day)			Means
		Fresh	7	15	
Lactobacillus bulgaricus (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	
Streptococcus thermophiles (cfu×x10^5/g)	A	18	15	10	14^f
	B	23	21	18	21^e
	C	24	22	18	21de
	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	
Lactobacillus acidophilus (cfu×x10^5/g)	A	-	-	-	
	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	
Bifidobacterium bifidum (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

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. [44] 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

Bifidobacterium 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.

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 [45], using it in yoghurt manufacture slightly improved the flavour. These findings agreed with that reported by Abd El-Salam et al. [46].

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 [47] who cleared that significant ($p< 0.001$) decreases in the total organoleptic scores of bio-yoghurt were noticed when storage period progressed.

4. Conclusion

It can be concluded that novel acceptable yoghurt could be successfully made from mixtures of 25 or 50% coconut milk with cow milk. For increasing the nutritional and health values of this product, ABT culture which contains probiotic can also be used. Utilization coconut milk in yoghurt production increased the amounts of lauric acids. The recommended level of 10^7 cfu.g^{-1} of bifidobacteria as a probiotic was exceeded for bio-yoghurt. Furthermore, mixing 5% honey with cow and coconut milk mixtures highly improved the sensory properties of bio-yoghurt. This yoghurt characterized by acceptable in properties of color, appearance, texture and body and flavour.

Table 8. *Effect of mixing 5% honey with cow or coconut milk on sensory evaluation of yoghurt.*

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

References

[1] Mckinley MC. (2005) The nutrition and health benefits of yoghurt. *Inter. J. Dairy Technol.,* 58:1–12.

[2] Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. (2006) Bacteriocins and lactic acid bacteria. A mini review. *African J. Biotechnol.,* 5: 678-683.

[3] Seow CC, Gwee CN. (1997). Review, coconut milk: chemistry and technology. *J. Food Sci. Tech.,* 32: 189-201.

[4] Onsaard E, Vittayanont M, Sringam S, McClements DJ. (2005) Properties and stability of oil-in-water emulsion stabilized by coconut skim milk protein. *J. Agri. Food Chem.,* 53 (14), 5747–5753.

[5] Bogdanov S, Jurendic T, Sieber R, Gallmann P. (2008) Honey for Nutrition and Health: A Review. *J The American College of Nut.,* 27 (6): 677–689.

[6] Erejuwa OO, Gurtu S, Sulaiman SA, Wahab MSA, Sirajudeen KNS, Salleh MSM. (2010) Hypoglycemic and antioxidant effects of honey supplementation in streptozotocininduced diabetic rats. *Int. J. for Vitamin and Nutrition Res.,* 80(1): 74–82.

[7] Ghashm AA, Othman NH, Khattak MN, Ismail NM, Saini R. (2010) Antiproliferative effect of Tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines. BMC *Complementary and Alternative Medicine,* 10 (49).

[8] Poorani A, Subramonian BS, Elango A, Pughazhenthi TR, Dhanalakshmi B. (2012) Synbiotic bifido milk powder in weaned piglets: Effect on growth, hematology and intestinal histology. *Egyptian J. Dairy Sci.,* 40:91-97.

[9] Kolapo AL, Olubamiwa AO. (2012) Effect of different concentrations of coconut milk on the chemical and sensory properties of soy-coconut milk based yoghurt. *Food and Public Health,* 2(4): 85-91.

[10] AOAC (2000) Association of Official Analytical Chemists. Official Methods of Analysis. 17th ed, Washington, DC, USA.

[11] Ling ER. (1963) A Text - Book of Dairy Chemistry. Vol. 2, Practical, 3rd ed., Champan and Hall, London, England.

[12] Kosikowski FV. (1978) Cheese and Fermented Milk Foods. 2nd ed. Cornell Univ. Ithaca, New York.

[13] Jahreis GJ, Fritsche MSC, Steinhart H. (1997) Conjugated linoleic acid in milk fat: High variation depending on production system. *Nutrition Res.,* 17 (9): 1479-1484.

[14] Tharmaraj N, Shah NP. (2003) Selective Enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*, Bifidobacteria, *Lactobacillus casei, Lactobacillus rhamnosus*, and Propionibacteria. *J. Dairy Sci.,* 86:2288–2296.

[15] Dinakar P, Mistry VV (1994). Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. *J. Dairy Sci*., 77:2854-2864.

[16] SAS. (1991) SAS User's guide: statistics. SAS Inst, Inc, Cary, NC.

[17] Duncan DB. (1955) Multiple Range and Multiple F-test. *Biometrics,* 11:1–42.

[18] Ladokun O, Oni Sarah (2014) Fermented milk products from different milk types. *Food and Nutrition Sci.,* 5:1228-1233.

[19] Aworh S, Akinniyi A. (1989) Proximate Analysis and Nutritional Component of Locally Produced Cheese. *Nigerian J Food Sci and Techn.,* 9: 220-227.

[20] Arumughan C, Balachandran C, Sundaresan A. (1993) Development of process for coconut cream on commercial scale. *J Food Sci and Techno.,* 30:408-412.

[21] Law HY, Ong CI, Ab Aziz N Taip, Farah S, Muda, N. (2009) Preliminary work on coconut milk fouling deposits study. *Int J Engineering & Technology,* 9: 8-13.

[22] Hamad HE, Sulieman AE, Salih ZA. (2013) Quality aspects of the Sudanese fermented milk (*Robe*) supplemented with gum Arabic powder. *Discourse J Agri and Food Sci.,* 1(1): 8-7.

[23] Akalin AS, Gonc S, Unal G, Fenderya S. (2007) Effects of fructoooligo-saccharide and whey protein concentrate on the viability of starter culture in reduced-fat probiotic yoghurt during storage. *J. Food Sci.,* 72:222-227.

[24] Hussein GAM. (2010) Preparation of yoghurt and probiotic yoghurt- like fermented products for bottle-fed infants. *Egyptian J. Dairy Sci.,* 38:171-182.

[25] El-Sayed SM, Hagrass AE, Asker AA, Malhat FM, El-Sayed MM, Abd El-Salam MH. (2013) Effect of using some vitamin B producing microorganisms as adjunct cultures in the manufacture of yoghurt. *Egyptian J. Dairy Sci.,* 41:127-136.

[26] Ammar El-Tahra MA, Ismail MM, Khalil AE, Eid MZ. (2015) Impact of fortification with honey on some properties of bio-yoghurt. *J Micro, Biotechn and Food Sci.,* 4 (6): 503-508.

[27] Akalin AS, Gönç S, Dinkçi N. (2004) Liquid chromatographic determination of thiamin in dairy products. *Int J Food Sci and Nut* 55: 345–349.

[28] Ammar El-Tahra MA, Ismail MM, Khalil AE, Eid MZ. (2014) Effect of supplementation with olive oil on some properties of bio-yoghurt. *The Asia J Applied Microb.,* 1(4): 66-77.

[29] El-Zeini HM, El-Aasser MA, Anis SMK, Romeih EAH. (2007) Influence of some processing treatments on chemical composition, rheological properties and micro-structure of cast UF-white soft cheese. *Egyptian J. Dairy Sci.,* 35: 57-72.

[30] Ortigosa M, Barcenas P, Arizcun C, Perez-Elortondo F, Albisu M, Torre P. (1999) Influence of the starter culture on the microbiological and sensory characteristics of ewe's cheese. *Food Microbiology,* 16, 237–247.

[31] Chick H, Shin HS, Ustunol Z (2001). Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey. *J. Food Sci.,* 66:478-4886.

[32] National Honey Board (NHB). (1996) Non-peroxide antibacterial activity in some New Zealand honey. *J. Apicul Res.,* 27:62-69.

[33] Güler Z, Park YW. (2011) Characteristics of physico-chemical properties, volatile compounds and free fatty acid profiles of commercial set-type Turkish yoghurts. Chemical properties of Turkish yoghurts. *J Animal Sci.,* 1: 1-9.

[34] Dayrit CS. (2003) Coconut for better health. Lecture delivered during the Symposium on coconut. Philippine Coconut Authority Auditorium. Quezon City, Philippines.

[35] Five B. (2004) The coconut oil miracle. Piccadilly Books, Ltd., Colorado Springs. CO 80936. U.S.A.

[36] Bawalan DD, Chapman KR. (2006) Virgin coconut oil production manual for micro and village-scale processing. FAO Regional Office for Asia and Pacific, Bangkok, Thailand.

[37] Kabara JJ. (2000) Health oils from the tree of life (Nutritional and Health Aspects of Coconut Oil). Paper delivered at the Asian and Pacific Coconut Community (APCC) COCOTECH Meeting held in Chennai, India.

[38] Beshkova D, Simova E, Frengova G, Simov Z. (1998) Production of flavour compounds by yogurt starter cultures. *J. Ind. Micro. Biotechn.,* 20: 180-186.

[39] Kneifel W, Ulbert F, Erhard F, Jaros D. (1992) Aroma profiles and sensory properties of yoghurt and yoghurt related products. I. Screening of commercially available starter cultures. *Milchwissenchaft,* 47: 362-365.

[40] Dave RI, Shah NP. (1997) Viability of probiotic bacteria in yoghurts made from commercial starter culture. *Int. Dairy J.,* 7:31-38.

[41] Nagpal R, Kaur A. (2011) Synbiotic Effect of Various Prebiotics on *In Vitro* Activities of Probiotic Lactobacilli. *Eco. Food Nutr.,* 50(1): 63-68.

[42] Ustunol Z, Gandhi H. (2001) Growth and viability of commercial *Bifidobacterium ssp*. in honey-sweetened skim milk. *J. of Food Protection,* 64:1775-1780.

[43] Kwok K, Basker D, Niranjan K. (2000) Kinetics of sensory quality changes in soymilk during thermal processing, by parametric and non-parametric data analyses. *J Sci. Food and Agric.,* 80(5): 595.

[44] Amiri ZR, Khandelwal P, Aruna BR. (2010) Development of acidophilus milk via selected probiotics & prebiotics using artificial neural network. *Advances in Bioscience and Biotechnology,* 1: 224-231.

[45] Kurmann JA, Rasic JL, Kroger M. (1992) Encyclopedia of Fermented Fresh Milk Products: An International Inventory of Fermented Milk, Cream, Buttermilk, Whey and Related Products. 1st Edn., E. van Nostrand Reinhold, New York.

[46] Abd El-Salam BA, Zayan AF, Mailam MA. (2011) Effect of fortification with honey and Bifidobacterium strain on the characteristics of yoghurt. *Egyptian J. Dairy Sci.,* 39:65-74.

[47] Osman MM, Ismail MM. (2004) Effect of fortification with zinc, iron and ascorbic acid on the chemical, microbiological and organoleptic properties of buffalo's milk bio-yoghurt. *J. Agric., Sci., Mansoura Univ.,* 29 (1): 237-251.