J. Food and Dairy Sci., Mansoura Univ., Vol. 5 (9): 653-666, 2014

USING OF HONEY AND OLIVE OIL IN PRODUCTION OF SYNBIOTIC YOGHURT

El-Tahra M. A. Ammar*; M. M. Ismail; El-Shazly, A. K.* and M. Z. Eid****

*** Dairy Department, Faculty of Agriculture, Mansoura University, El-Mansoura, Egypt.**

**** Dairy Technology Department, Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.**

ABSTRACT

The effect of fortification with both honey and olive oil on yoghurt quality was investigated. Control yoghurt was made using classic yoghurt culture and whole milk. Fou other yoghurt treatments were made by ABT culture and whole milk fortified with 0+0, 2+1, 4+2, and 6+4% of honey and virgin olive oil respectively. The sixth treatment was prepared using ABT culture and skim milk contained 6% honey + 4% virgin olive oil. Changes in rheological, chemical, microbial and organoleptic properties of yoghurt were monitored during refrigerated storage (4°C) for 15 days. Mixing of yoghurt milk with honey and olive oil to milk showed a slight decrease in the rate of acidity development. Fortification of milk with honey and olive oil had no clear effect on coagulation time, but increased curd tension and decreased curd syneresis values. Addition of honey and olive oil increased acidity, TS, fat, ash and TVFA contents of the examined yoghurt while there were no differences in TN and WSN values. Yoghurt made with honey and olive oil had lower TVBC, moulds and yeasts numbers as compared with control. Adding of honey and olive oil stimulated the growth and viability of bifidobacteria which helped in maintenance their counts above the recommended levels (10^6cfu.g^{-1}) for a probiotic effect. Finally, supplementation of yoghurt with honey and olive oil greatly improved the sensory evaluation scores.

Keywords: ABT, yoghurt, bifidobacteria, honey, olive oil.

INTRODUCTION

Probiotics are technically defined as live microbial food ingredients that have a beneficial effect on human health. Some of the important beneficial effects are antimicrobial activity, immune system modulation, antimutagenic activity, colonization resistance activity, maintenance of micro-ecology of bowel, stimulation of *Bifidobacteria,* deactivation of carcinogens etc. Commercially available probiotic strains belong to genera *Lactobacilli*, *Bifidobacterium, Streptococcus, Bacillus, Bacteriodes, Pediococcus, Leuconostoc, Propionibacteruim* (Meile *et al.,* 2008, and Douglas and Sanders, 2008) *Saccharomyces cerevisia* and *Aspergillusoryzae* (Verma and Singh 1995). Yoghurt and probiotic fermented milk are beneficial to human health because of the type of bacteria and the large number of viable cells they should contain. Although quantitative standards vary from 10^6 to10^7 cfu/g viable cells as minimum requirements, it is generally recommended that yogurt or fermented milk should contain at least one million viable cells per gram at the time of consumption. To maintain these numbers, it is important to test probiotic bacteria for growth and viability during cold storage (Damin *et al.,* 2006).

The other key component of functional dairy or other products is prebiotic ingredient. Prebiotics are nondigestible food ingredients that beneficially

affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in colon that can improve the host health (Niness 1999). When prebiotics are used in combination with probiotics or live bacteria, the resultant has synergistic effects, referred to as “synbiotic”. This is because in addition to the action of probiotics that promote the growth of existing strains of beneficial bacteria in the colon, prebiotics such as inulin and oligofructose also act to improve the survival, implantation and growth of newly added probiotics strains. Jan Mei *et al.,* (2010), and Nagpal and Kaur (2011) concluded that honey contained oligosaccharides which would function well as prebiotics for probiotics. On the other hand, olive oil is considered as the pillar of the Mediterranean diet, since it improves the major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism and antithrombotic profile. Endothelial function, inflammation and oxidative stress are also positively modulated. Some of these effects are attributed beside the monounsaturated fatty acids (MUFA) to the minor components of virgin olive oil (Al Jamal and Ibrahim, 2011). Attempt is, therefore, made in this study to supplement yoghurt with combination of honey and olive oil for production of synbiotic yoghurt and examine the effect of this supplementation on bifidobacteria activity.

MATERIALS and METHODS

Commercial classic yoghurt starter containing *Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbruckii* subsp. *bulgaricus* (1:1) and ABT culture (ABT-5) with mixed strains of *S. thermophilus* (as sole fermenting organism) and LA + *B. bifidum* (as probiotic organisms) (Chr. Hansen’s Lab A/S Copenhagen, Denmark) were used. Starter culture was in freeze-dried direct-to-vat set form. After procurement, the starter cultures were stored at –18°C in the absence of atmospheric air. Honey was obtained from local market in Damiette Governorate, Egypt. Extra virgin olive oil was obtained from El-Wadi Company for Food Industries (Wadi Food), Alexandria, Egypt.

Yoghurt samples were prepared from fresh buffalo's and cow's milk mixture 1:1 (acidity 0.17%, pH 6.61, fat 5.1, TS 14.56 and total protein 3.87%) in Dairy Laboratory of El-Serw Animal Production Research Station, Animal Production Research Institute, Agriculture Research Center. Six yoghurt treatments were made using classic yoghurt or ABT cultures. The first yoghurt sample was manufactured using yoghurt starter and whole milk as control, whereas the treatments from two to five were made by ABT culture and whole milk fortified with 0+0, 2+1, 4+2, and 6+4% of honey and virgin olive oil, respectively. The last yogurt sample was prepared by ABT culture and skim milk with 6% honey + 4% olive oil. The whole or skim milk was tempered to 60°C and fortified with 1, 2 and 4% (wt/wt) virgin olive oil. The mix was blended at 2000 rpm for 3 min, reheated to 85°C for 15 min, cooled to 40°C, fortified with 2, 4 and 6% (wt/wt) honey, inoculated with commercial yoghurt culture (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 15 days. Yoghurt samples were analyzed in fresh and after 7 and 15 days of refrigerated storage. Three replicates of each treatment were conducted.

Total solids, fat, TN and ash contents of samples were determined according to (AOAC, 2000). Titratable acidity in terms of % lactic acid was measured by titrating 10 g of sample mixed with 10 ml of boiling water against 0.1 N NaOH using phenolphthalein indicator to an end point of faint pink color (Parmar 2003). pH of the sample was measured using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Water soluble nitrogen (WSN) of yoghurt was estimated according to Ling (1963). Total volatile fatty acids (TVFA) were determined according to Kosikowiski (1978).

The curd tension was determined using the method of Chandrasekhara *et al.,* (1957) whereas the curd syneresis was measured as given by Mehanna and Mehanna (1989). For test of starter coagulation time during yoghurt making, milk was inculcated with starts and incubated at 40°C then coagulation was noticed at 30 min intervals.

Yoghurt samples were analyzed for the total viable bacterial count (TVBC), lactic acid bacteria (LAB), proteolytic, lipolytic, coliform bacteria, moulds and yeast counts according to the methods described by the American Public Health Association (1992). The count of bifidobacteria was determined according to Dinakar and Mistry (1994). A mixture of antibiotics, including 2 g of neomycin sulfate, 4 g of paromomycin sulfate, 0.3 g of nalidixic acid, and 60 g of lithium chloride (NPNL, Sigma Chemical Co.), was prepared in 1 L of distilled water, filter-sterilized (.22 pm), and stored at 4°C until use. The mixture of antibiotics (5 ml) was added to 100 ml of MRS agar medium. Cysteine-HC1 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.

Samples of yoghurt were organoleptically scored by the staff of the El-Serw Animal Production Research Station, Ministry of Agriculture. 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.

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 (± standard deviation) of 3 experiments.

RESULTS AND DISCUSSION

Changes in acidity during fermentation of yogurt mix for 180 min

Table 1 shows the development of acidity (as lactic acid percentages) of buffaloe's and cow's milk mixture inoculated with classic yoghurt and ABT cultures as indictor for the effect of adding honey and olive to buffaloe's and cow's milk mixture on starter activity. During 180 min of fermentation, acidity percentages of milk inoculated with classic yoghurt starter were higher than those inoculated with ABT culture. This may be attributed to the high activity of lactase in yoghurt starter (Tamime and Robinson 1985) splitting lactose into glucose and galactose as the first step of fermentation.

However addition of different concentrations of honey and olive oil to milk (treatments C, D, E and F) slightly increased ratios of titratable acidity at zero

time, but the rates of acidity development were slightly slower in these samples as compared with control (treatment B).

Changes in rheological properties of yoghurt

The effect of using various cultures and incorporation of honey and olive oil with buffaloe's and cow's milk mixture on coagulation time, curd tension and curd syneresis of yoghurt is illustrated in Table 2. The results revealed that coagulation time of ABT culture (treatment B) was longer by 11% than that of classic yoghurt starter (treatment A). This was in accordance with the findings of Damin *et al.,*(2008) who stated that the shortest fermentation time to reach pH 4.5 was obtained with milk fermented by *Streptococcus thermophilus* in co-culture with *Lactobacillus bulgaricus* (STLB) (5.4 h), *Bifidobacterium lactis* (STBL) (8.3 h) and *Lactobacillus acidophilus* (STLA) (9.3 h). Many other researchers have also reported that probiotic bacteria have a poor acidification performance in milk when compared to a yogurt starter culture (Saxelin *et al.,* 1999; Oliveira *et al.,* 2001; Sodini *et al.,* 2002; Damin 2003; Almeida *et al.,* 2008). The results of curd tension were similar in both types of yoghurt made by classic and ABT cultures. Curd syneresis values were determined as grams of whey separated from 15 g of yoghurt curd. Normally, as time of syneresis increased, grams of separated whey surely increased and in all treatments, but with varying degrees. ABT yoghurt had higher curd syneresis values than those of classic starter one. Conversely, Hussein (2010) stated that increased separation of whey was found from the infants' yoghurt-like fermented products (IYFP) made with traditional starter than that made with probiotic starter (ABT-2). Emara *et al.,* (2011) stated that there is no significant variation in whey syneresis and curd tension values among the treatments inoculated by yoghurt culture or ABT-3culture.

Table 1: Effect of adding honey and olive oil to buffaloe's and cow's milk mixture on activity of ABT culture (expressed as acidity percentage)

Treatments	Incubation time (min)						
	0	30	60	90	120	150	180
A	0.15	0.16	0.18	0.23	0.33	0.42	0.52
B	0.14	0.14	0.16	0.22	0.31	0.39	0.48
C	0.15	0.16	0.18	0.24	0.33	0.41	0.51
D	0.15	0.16	0.18	0.23	0.32	0.41	0.51
E	0.15	0.17	0.19	0.23	0.32	0.40	0.50
F	0.16	0.17	0.19	0.24	0.33	0.42	0.51

A-Yoghurt made using whole milk and *Streptococcus thermophillus* and *Lactobacillus bulgaricus* (control)

B-Yoghurt made using whole milk and ABT (*Lactobacillus acidophilus (A), bifidobacteria (B), and Streptococcus thermophilus (T))*

C-Yoghurt made using whole milk and ABT + 2% honey + 1% olive oil

D- Yoghurt made using whole milk and ABT + 4% honey +2% olive oil

E- Yoghurt made using whole milk and ABT + 6% honey +4% olive oil

F- Yoghurt made using skim milk and ABT + 6% honey +4% olive oil

It could be observed from Table 2 that supplementation of milk with honey and olive oil had no clear effect on coagulation time of yoghurt. There was considerable disparity in the curd tension values of the yoghurt made with honey and olive oil compared to the values obtained from control. With increasing the added levels of honey and olive oil, curd tension values raised. Also, this increase was noticed in yoghurt made from skim milk fortified with 6% honey + 4% olive oil (sample F). In contrary for curd tension increasing, curd syneresis values of yoghurt made with honey and olive oil were lower than those of control. This is may be due to the increasing viscosity of yoghurt by mixing olive oil with milk. Similar trend was found by Khalafalla and Roushdy (1996) who reported that the syneresis decreased with the increase in viscosity.

Table 2: Effect of using of ABT culture and adding of honey and olive oil to buffaloe's and cow's milk mixture on yoghurt rheological properties

Treatments	Coagulation time (hrs)	Curd tension (g)	Curd syneresis (gm/15 gm of curd)*			
			Time (min)			
			10	30	60	120
A	3.00	32.55	1.52	2.99	3.97	5.16
B	3.20	32.73	1.76	3.11	4.14	5.37
C	3.20	33.45	1.47	2.84	3.86	5.04
D	3.25	34.33	4.48	2.81	3.82	5.00
E	3.20	36.15	1.43	2.82	3.80	4.95
F	3.25	34.85	1.56	3.06	4.07	5.25

*** Whey excluded (grams) from 15 gm of curd kept at room temperature after 10, 30, 60 and 120min.**

Changes in chemical composition of yoghurt during refrigerated storage for 15 days

The chemical composition of yoghurt manufactured from whole and skim milk and using classic or ABT starters as affected by incorporation of honey and olive oil is shown in Table 3. It could be observed that the acidity varied according to the type of starter used. Yoghurt made with classic culture was characterized with the higher titratable acidity and lower pH values at zero time and during storage period as compared to ABT yoghurt. 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 throughout storage. Data in Table 3 showed that no significant differences in TS, fat and ash contents between classic and ABT yoghurts. Values of TS for samples A and B at the end of storage period were 15.70 and 15.72% respectively. These results are in agreement with those obtained by Hussein (2010) who mentioned that no significant differences in chemical composition between IYFP of different treatments.

Table 3: Effect of using ABT culture and adding of honey and olive oil on the chemical composition of yoghurt

Treatments	Storage Period (days)	Acidity %	pH values	TS %	Fat %	Fat/DM %	Ash %
A	0	0.79	4.70	15.47	5.9	38.14	0.88
	7	1.09	4.42	15.56	5.9	37.92	0.91
	15	1.25	4.21	15.70	6.0	38.22	0.95
B	0	0.62	4.98	15.51	5.8	37.40	0.86
	7	0.84	4.76	15.60	5.8	37.18	0.89
	15	1.01	4.53	15.72	6.0	38.17	0.93
C	0	0.65	4.91	17.44	6.3	36.12	0.90
	7	0.88	4.70	17.56	6.4	36.45	0.93
	15	1.05	4.47	17.67	6.5	36.79	0.95
D	0	0.66	4.89	20.11	7.2	35.80	0.92
	7	0.90	4.66	20.23	7.2	35.60	0.93
	15	1.08	4.42	20.34	7.3	35.90	0.96
E	0	0.68	4.84	23.89	7.8	32.65	0.95
	7	0.94	4.57	23.99	7.8	32.51	0.97
	15	1.14	4.34	24.15	8.0	33.13	0.98
F	0	0.73	4.75	20.34	4.6	22.62	0.93
	7	0.97	4.51	20.45	4.7	22.98	0.94
	15	1.19	4.29	20.57	4.7	22.85	0.96

Changes in TN,WSN and T.V.F.A of yoghurt during refrigerated storage for 15 days

On the other side, the acidity percentages of yoghurt samples contained honey and olive oil slightly was higher than that of control at zero time and within storage stage. values of pH had the opposite trend of acidity. This is may be due to the honey content of fructooligosacchrides (Akalin *et al.,* 2007) or the olive oil content of polyphenols. Petrotos *et al.,* (2012) reported that adding of olive fruit polyphenols to milk caused rapid drop of the pH value of yoghurt samples which was beneficial to yoghurt production as the residence time of yoghurt in the incubator at 41-42°C is determined by the pH value which has to reach the value of 4.60-4.65. On the whole, the acidity of yoghurt treatments supplemented with honey and olive oil was in acceptable limit according to Mehanna *et al.,* (2013). As shown in Tables 3 and 7, yoghurt acidity and pH value were affected ($P<0.001$) by treatments and the interaction of treatment × age. As storage period progressed, there were noticeable and gradual increases in the titratable acidity ratios and decreases in the pH values of all yoghurt samples, due to the slow metabolic activity of the starter cultures (Barrantes *et al.,* 1994). It could easily be observed from Table 3 that there is a substantial effect of the presence of honey and olive oil on TS and fat contents of yoghurt. Significant increases in TS and fat contents were obtained with incorporation of honey and olive oil in yoghurt which was positive proportion with the concentrations added of honey and olive oil. Also, adding of honey and olive oil to skim milk (sample F) increased TS content of the resultant yoghurt while fat percentage remained lower than control (sample B) but it stayed in acceptable levels as described by Egyptian Standards (2005). Ash contents were slightly higher in yoghurt treatments contained honey and olive oil. Ash values were 0.86, 0.90, 0.92 and 0.95% for fresh B, C, D and E samples.

The changes in TN, TN/DM, WSN, WSN/TN and TVFA contents of yoghurt during storage are illustrated in Table 4. It is evident that both types of yoghurt manufactured by classic or ABT cultures possessed nearly the same TN contents at zero time and during cold storage. values TN of were 0.722 and 0.724% for samples A and B after 15 days of storage. These findings are similar to those reported by Ayad, *et al.,* (2010). The degrees of nitrogen and lipids analysis as measured by WSN and TVFA contents respectively was more noticeable in yoghurt made using classic starter than that made by ABT-5 culture. Contents of WSN of treatments A and B after seven days of refrigerated preservation were 0.173 and 0.166% respectively.

Table 4: Effect of using ABT culture and adding of honey and olive oil on TN and some repining indices of yoghurt

Treatments	Storage Period (days)	TN %	TN/ DM %	WSN %	WSN/TN %	TVFA*
A	0	0.694	4.40	0.151	21.75	6.0
	7	0.701	4.50	0.173	24.67	6.7
	15	0.722	4.59	0.185	25.62	7.7
B	0	0.697	4.49	0.143	20.51	5.8
	7	0.704	4.51	0.166	23.57	6.4
	15	0.724	4.60	0.178	24.58	7.3
C	0	0.695	3.98	0.145	20.86	6.8
	7	0.703	4.00	0.168	23.89	7.4
	15	0.723	4.09	0.179	24.75	8.6
D	0	0.698	3.47	0.144	20.63	7.3
	7	0.705	3.48	0.166	23.54	8.1
	15	0.726	3.56	0.180	24.79	9.2
E	0	0.694	2.90	0.145	20.89	7.6
	7	0.704	2.93	0.169	24.00	8.8
	15	0.726	3.00	0.180	24.79	9.6
F	0	0.703	3.45	0.158	22.47	8.2
	7	0.715	3.49	0.180	25.17	9.3
	15	0.735	3.57	0.191	25.98	10.4

*** expressed as ml 0.1 NaOH 100 g^{-1} cheese**

Table 4 shows the effect of fortification of yoghurt with various levels of honey and olive oil on TN, WSN and TVFA contents. There were no significant differences between TN and WSN values of yoghurt made with or without adding of honey and olive oil. In contrary, the highest TVFA values were observed in yoghurt treatments contained honey and olive oil. Increasing the ratio of both honey and olive oil concentrations significantly raised the TVFA values of the samples. This effect was more pronounced for yoghurt made from skim milk and containing honey and olive oil (treatment F). General spiking, TS, fat, ash, TN, WSN and TVFA contents gradually increased in different yoghurt samples as storage period advanced. The obtained results were in accordance with that found by Badawi *et al.,*(2008) who noticed that the acidity and TVFA of stirred yoghurts increased while pH decreased all through storage. El-Sayed *et al.,*(2013) reported that the increasing in TS content of yoghurt during storage might be attributed to slight evaporation. The yoghurt cups were covered by plastic lids which may allow for some evaporation and increases in TS contents.

Changes in microbial counts of yoghurt during refrigerated storage for 15 days

Table 5 illustrates the total viable bacterial counts (TVBC) and the viable counts of lactic acid bacteria, bifidobacteria, coliform, proteolytic, lipolytic bacteria, moulds and yeasts of yoghurt made using classic and ABT-5 cultures and supplementation of various concentrations of honey and olive oil. Coliform bacteria were found only in fresh samples A and B with counts not exceed $1x10^2$ cfu.g^{-1} and disappeared during storage period. Abd El-Salam *et al.,* (2011) stated that the addition of honey or/and *Bifidobacteria lactis* Bb.12 in yoghurt had significant effect against *E. coli* and *S. aureus*. On the other hand, no moulds and yeasts growth was observed in different yoghurt treatments at zero time but they appeared after 7 days and increased till the end of storage. Also, TVBC for all samples increased through refrigerated storage while numbers of lactic acid bacteria, bifidobacteria, proteolytic and lipolytic bacteria gradually lowered during storage of yoghurt. This reduction in the counts of the different microorganisms may be attributed to the high acidity produced by microbial fermentation (Dave and Shah, 1997). These results are consistent with previous findings (Hussein 2010 and El-Sayed *et al.,* 2013)

Table 5: Effect of using ABT culture and adding of honey and olive oil on some microbial groups of yoghurt

Treatments	Storage Period (days)	TVBC (x 10^5)	Lactic acid bacteria (x 10^3)	Bifido-bacteria (x 10^5)	Coliform bacteria (x 10^2)	Proteolytic bacteria (x 10^2)	Lipolytic bacteria (x 10^2)	Moulds & Yeast (x 10^3)
A	0	42	18	-	1	6	5	0
	7	128	14	-	0	5	3	38
	15	437	8	-	0	3	1	115
B	0	51	21	28	1	4	4	0
	7	131	18	23	0	3	3	31
	15	445	13	17	0	1	1	107
C	0	43	28	38	0	4	4	0
	7	119	24	34	0	2	2	22
	15	421	17	28	0	1	1	91
D	0	39	32	46	0	4	5	0
	7	107	27	42	0	3	3	18
	15	408	21	36	0	1	2	87
E	0	30	41	54	0	3	4	0
	7	92	34	47	0	2	3	14
	15	387	27	41	0	1	0	79
F	0	32	43	53	0	3	4	0
	7	90	35	48	0	2	2	16
	15	390	26	40	0	0	0	78

It could be observed form Table 5 that ABT yoghurt had slightly higher numbers of TVBC and lactic acid bacteria than those of control for fresh samples and during storage. Nevertheless, the numbers of proteolytic, lipolytic bacteria, moulds and yeasts were lower in the former than that the later. These outcomes contradicted with Emara *et al.,* (2011) who stated that type of culture had an obvious correlation with the counts of organisms during storage. The counts of yoghurt culture were higher than that found in ABT

treatments along the storage period. They explained these results according to the symbiosis theory between *Streptococcus thermophillus* and *Lactobacillus bulgaricus*, where *L. bulgaricus* stimulates *Str. Thermophilus* by releasing glycin and histidine into the growth media and the latter organism produces formic acid, pyrovic acid and carbon dioxide compounds which promote the growth of *L. bulgaricus*.

Mixing of honey and olive oil with milk significantly decreased the TVBC, moulds and yeasts numbers of the produced yoghurt. These results refer to honey or olive oil has antibacterial or antifungal activities. Petrotos *et al.,*(2012) demonstrated that the polyphenol (from olive fruit) enriched yogurt samples had a better self life while the control was spoiled by moulds in less than 24 days of storage. Counts of proteolytic and lipolytic bacteria were not affected by addition of honey and olive oil to yoghurt. In contrary, the counts of lactic acid bacteria and bifidobacteria increased in yoghurt samples fortified with honey and olive oil as compared with control. Ustunol and Gandhi (2001) found that the honey promotes of *Bifidobacterium bifidium* growth. 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.

The viability of bifidobacteria in fermented dairy products is a cause for concern. However acidity of yoghurt and storage at 5°C, the viable counts of bifidobacteria in all bio-yoghurt treatments were within the recommended minimum daily intake numbers (10^6- 10^7 cfu/ml) needed for probiotic to exhibit their claimed health benefits. These results were in agreement with FAO/WHO (2001) and Akin *et al*. (2007). Matto *et al.,* (2006) indicated that the developing probiotic food products (ABC product) with multiple probiotic strains *Lactobacillus* F19, *Lb. acidophilus* NCFB 1748 & *B. animalis* ssp. *lactis* Bb-12 is feasible.

Changes in sensory evaluation of yoghurt during refrigerated storage for 15 days

Table 6 shows the organoleptic properties of yoghurt fortified with honey and olive oil during storage for 15 days at 4°C. According to the Table, no statistical significant difference was observed in all the samples' attributes – color and appearance evaluated on the first day and during storage period. While the scores of body, texture and flavour varied between different treatments. Generally, no undesirable flavour was detected in all samples. Also, fresh treatments ranked the highest scores of color, appearance, body, texture and flavour. They were described as good flavour, rich taste, normal body and texture and good appearance. 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 (El-Sayed *et al.,* 2013). These trends are similar to other works in literature. Badawi *et al.,*(2008) mentioned that scores for sensory properties were almost unchanged during the first 6 days of storage and then decreased. In their study, Routray and Mishra (2011) found that the storage time had a negative impact on the flavour scores of yoghurt which they attributed to changes in the aroma compounds.

It was observed from Table 6 that the effect of starter type was not so much pronounced in the organoleptic properties of the resultant yoghurt. Samples A and B (classic and ABT cultures respectively) gained approximately the same scores for color, appearance, body, texture and flavour. El-Sayed *et al.,* (2013) reported a similar 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.,* (2011), 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.

Table 6: Effect of using ABT culture and adding of honey and olive oil on organoleptic properties of yoghurt

Treatments	Storage Period (days)	Color& Appearance (15)	Body& Texture (35)	Flavor (50)	Total (100)
A	0	13	31	45	89
	7	13	31	45	89
	15	12	29	42	83
B	0	13	31	44	88
	7	13	31	44	88
	15	12	28	42	82
C	0	13	32	45	90
	7	13	32	45	90
	15	12	31	43	86
D	0	13	34	47	94
	7	13	34	47	94
	15	12	32	46	90
E	0	13	34	47	94
	7	13	34	47	94
	15	12	33	46	91
F	0	13	31	46	90
	7	12	31	46	89
	15	11	30	43	84

Supplementation of yoghurt with honey and olive oil significantly improved the sensory qualities. On other words, color, appearance, body, texture and flavour of yoghurt samples containing honey and olive oil, when compared to plain (control) yoghurt sample were preferred by the panelists that tasted the samples. Samples D (4% honey +2% olive oil) and E (6% honey +4% olive oil) received the highest scores in body, texture and flavour on zero day and through cold storage. Improvement of body and texture of yoghurt by adding honey and olive oil may be due to the increasing of TS and fat contents (Table 3) which raised curd tension and lowered syneresis (Table 2). Of course, improvement of yoghurt flavour is related to the lovely sweet taste of honey. Regarding yoghurt made from skim milk (treatment F), improvement of sensory quality was more noticeable in flavour but no difference was observed in body and texture. 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, where as *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. Marco *et al.*, (2012) stated that together with their bioactivity, olive oil phenols have a significant role on the flavour and the bitter taste of olive oil.

CONCLUSION

The present results of this part demonstrated that the fortification of yoghurt with honey and olive oil stimulated the growth and viability of lactic acid bacteria and bifidobacteria. For healthy food commercial production, honey and olive oil (2+1, 4+2, and 6+4% respectively) could be used as prebiotic in bio-yoghurt manufacturing. Also, the addition of honey and olive oil may improve the dietetic value of such fermented milk.

REFERENCES

Abd El-Salam, B. A.; Zayan, A. F. and Mailam, M. A. (2011). Effect of fortification with honey and Bifidobacterium strain on the characteristics of yoghurt. Egyptian J. Dairy Sci., 39:65-74.

Akalin, A.S.; Gonc, S.; Unal, G. and 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.

Akin, M. B.; Akin, M. S. and Kirmaci, Z. (2007). Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chem., 104:93-101.

Al Jamal, A. and Ibrahim, A. (2011). Effects of olive oil on lipid profiles and blood glucose in type2 diabetic patients. Int J Diabetes & Metab 19:19-22.

Almeida, K. E., Tamime, A. Y. and Oliveira, M. N. (2008). Acidification rates of probiotic bacteria in Minas frescal cheese whey. LWT – Food Sci. Technol. 41, 311–316.

American Public Health Association (1992). Standard methods for the examination of dairy products. Amer. Publ. Health Assoc. Inc.12th ed., New York, USA.

Amiri, Z. R.; Khandelwal, P. and Aruna, B. R. (2010). Development of acidophilus milk via selected probiotics & prebiotics using artificial neural network. Advances in Bioscience and Biotechnology. 1: 224-231.

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

Ayad, H. E.; Darwish, A. M. G.; Darwish, S.M. and El-Soda, M. (2010). Production of novel functional yoghurt-like products. Egyptian J. Dairy Sci., 38:183-199.

Badawi, R.M.; Hamed, A.I.; Kebary, K. M. K. and Hweda A. El-Sayed (2008) Effect of replacing milk fat with fat replacers on the quality of stirred yoghurt. Egyptian J. Dairy Sci., 36 (2), 71-83.

Barrantes, E.; Tammime, A. Y.; Muir, D. D. and Swoed, A. M. (1994). The effect of substitution of fat by microparticulated whey protein on the quality of set-type natural yoghurt. J. Society Dairy Techn. 47:61-66.

Chandrasekhara, M. R., Bhagawan, R. K., Swaminathan, M., Subrahmanyan, V. (1957). The use of mammalian milk and processed milk foods in the feeding of infants. Indian J. Child. Health, D, 70L.

Damin, M. R. (2003). Utilization of casein hydrolysate and whey protein concentrate in probiotic fermented milk manufacture. MSc Thesis, p. 116, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil.

Damin, M. R.; Minowa, E.; Alcantara, M. R. and Oliveira, M. (2006). Chemical and viability changes during fermentation and cold storage of fermented milk manufactured using yogurt and probiotic bacteria. XIIIth World Congress of Food Science and Technology “FOOD IS LIFE”, Nantes, France, September, 17–21. doi:10.1051/IUFost:2006635

Damin, M. R.; Minowa, E.; Alcantara, M. R. and Oliveira, M. N. (2008), Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yoghurt and probiotic bacteria. J. Texture Studies, 39: 40–55.

Dave, R. I. and Shah, N. P. (1997). Viability of probiotic bacteria in yoghurts made from commercial starter culture. Int. Dairy J., 7:31-38.

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

Douglas, L. C. and Sanders, M. E. (2008). Probiotics and prebiotics in dietetics practice. J. of the American Dietetic Association, 108(3), 510-521.

Duncan, D. B. (1955). Multiple Range and Multiple F-test. Biometrics,11:1–42.

Egyptian Standards for Zabady, (2005). ES:1000/2005. Ministry of Industry and Technological Development. Egypt.

El-Sayed, S. M.; Hagrass, A. E.; Asker, A. A.; Malhat, F. M.; El-Sayed, M. M. and Abd El-Salam, M. H. (2013). Effect of using some vitamin B producing microorganisms as adjunct cultures in the manufacture of yoghurt. Egyptian J. Dairy Sci., 41:127-136.

Emara, E. A. Gh.; Abdel-Malek, F. A.; El-Zawahary, A. and El-Gabri, E. (2011). Effect of milk types and probiotic bacteria on quality and cholesterol content of certain fermented milks. Egyptian J. Dairy Sci., 39:219-228.

FAO/WHO, (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotic in Food Including Powder milk with Live Lactic Acid Bacteria. Cordoba, Argentina. 1-4 October. ftp://ftp.fao.org. Accessed 1 March 2007.

Hussein, G. A. M. (2010). Preparation of yoghurt and probiotic yoghurt- like fermented products for bottle-fed infants. Egyptian J. Dairy Sci., 38:171-182.

Jan Mei, S.; Mohd Nordin, M. S.; Norrakiah, A. S. (2010). Fructooligosaccharides in honey and effects of honey on growth of *Bifidobacterium longum* BB 536. Int. Food Res. J., 17: 557-561.

Khalafalla, S. M. and Roushdy, I. M. (1996). Effect of stabilizer on rheological and sensory properties of low fat buffalo's yoghurt. Egyptian J. Food Sci.,24:199-207.

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

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

Marco, D. R.; Silva, G.; Freitas, A. M. C.; Cabrita, M. J. B. and Garcia, R. (2012). Olive Oil Composition: Volatile Compounds, Olive Oil - Constituents, Quality, Health Properties and Bioconversions. InTech. Rijeka,Croatia**.**

Matto, J.; Fonden, R.; Tolvanen, T.; Wright, A.V.; Vilpponen-Salmela, T.; Satokari, R. and Saarela, M. (2006). Intestinal survival and persistence of probiotic *Lactobacillus* and *Bifidobacterium* strains administered in triple-strain yoghurt. Int. Dairy J., 16:1174-1182.

Mehanna, N. M.; Eman M. Ibrahim and Lamiaa I. El-Nawasany (2013). Minimizing whey separation from non-fat yoghurt. Egyptian J. Dairy Sci., 41:59-68.

Mehanna, N.M. and Mehanna, A. S. (1989). Studies on the use of stabilizer for improving some properties of cow's milk yoghurt. *Egyptian J. Dairy Sci.,* 17:289.

Meile, L. Blay, G. L. and Thierry, A. (2008) Safety assessment of dairy microorganisms: Propionibacterium and Bifidobacterium. Int. J of Food Microbiology, 126(3), 316-320.

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

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

Niness, K.R. (1999). Inulin and oligofructose. What are they? J. of Nutrition, 129(7), 1402-1406.

Oliveira, M. N., Sodini, I., Remeuf, F. and Corrieu, G. (2001). Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. Int. Dairy J. 11, 935–942.

Parmar, R. (2003). Incorporation of acid whey powders in probiotic yoghurt. M. Sc. Thesis, Major in Biological Sciences, Specialization in Dairy, South Dakota State University, U.S.A.

Petrotos, B. K.; Karkanta, F. K.; Gkoutsidis, P. E.; Giavasis, I.; Papatheodorou, K. N. and Ntontos, A. X. (2012). Production of novel bioactive yoghurt enriched with olive fruit polyphenols. World Academy of Sci., Eng. and Tech. 64:867-872.

Routray, W. and Mishra, H. N. (2011). Scientific and technical aspects of yoghurt aroma and taste: A Review. Comprehensive Rev. Food Sci., Food Safety. 10:208-215.

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

Saxelin, M., Grenov, B., Svensson, U., Fonden, R., Reniero, R. and Mattila-Sandholm, T. (1999). The technology of probiotics. Trends Food Sci. Technol. 10, 387–392.

Sodini, I., Lucas, A., Oliveira, M. N., Remeuf, F. and Corrieu, G. (2002). Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. J. Dairy Sci. 85, 2479–2488.

Tamime, A. Y. and Robinson, R. K. (eds.) (1985). Yoghurt: Science and Technology. (1st Ed.), pp.295-327. Pergamon Press Inc., New York, USA.

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

Verma, S.V.S. and Singh, R. (1995). Probiotic and their role in broiler chicken production: An overview. J. of Animal Sci., 10, 359-363.

استخدام العسل وزيت الزيتون فى انتاج الزبادى الحيوى

الطاهرة محمد أحمد عمار* , عبد الوهاب الشاذلى خليل* , مجدى محمدا سماعيل و محمد زكى عيد****

*** قسم الألبان , كلية الزراعة , جامعة المنصورة.**

****قسم تكنولوجيا الألبان , معهد بحوث الانتاج الحيوانى , مركز البحوث الزراعية .**

تم دراسة تأثير اضافة زيت الزيتون وعسل النحل على جودة الزبادى الحيوى حيث تم تصنيع المعاملة الكنترول باستخدام بادىء الزبادى العادى والأربع معاملات الأخرى من الزبادى باستخدام خليط من اللبن البقرى والجاموسى الخام بنسبة (١: ١) وبادىء ABT واضافة العسل زيت الزيتون الخام بنسبة (٢% +١% و ٤% + ٢% و ٦% + ٤%).ى والمعاملة السادسة تم تصنيعها من لبن فرز طازج وبادىء ABT و خليط من العسل وزيت الزيتون الخام بنسبة(٦% + ٤%).التغييرات التى حدثت فى الخواص الريولوجية والكيماوية والميكروبيولوجية وكذلك الحسية تم دراستها خلال فترة التخزين على درجة حرارة ٤°م ولمدة ١٥ يوم كالآتي :-

أظهرت نتائج خلط زيت الزيتون الخام وعسل النحل مع الزبادى الحيوى انخفاض طفيف فى الحموضة. أدت اضافة زيت الزيتون الخام وعسل النحل الى الزبادى الحيوى لعدم ظهور تأثير واضح على وقت التجبن فى حين أدت الى زيادة فى قوة جذب الخثرة وانخفاض فى التشريش.

اضافة زيت الزيتون الخام و عسل النحل الى الزبادى الحيوى أدت الى حدوث زيادة فى المادة الصلبة والدهن والرماد وكذلك الأحماض الدهنية الطيارة بينما لم يكن هناك اختلاف فى قيم كل من النيتروجين الكلى والنيتروجين الذائب.

عينات الزبادى الحيوى المحتوية على زيت الزيتون الخام وعسل النحل احتوت على اعداد منخفضة من العدد الكلى للبكتيريا والفطريات والخمائر مقارنة بالمعاملة الكنترول.

اضافة زيت الزيتون الخام وعسل النحل أدى الى تحفيز نمو البيفيدوبكتيريا والعمل على المحافظة على أعداد بكتيريا البيفيدوبكتيريا أعلى من الأعداد الموصى بها واللازمة لاحداث تأثير البروبيوتيك. تدعيم الزبادى الحيوى بعسل النحل وزيت الزيتون الخام أدى الى تحسن كبير فى درجات التقييم الحسى.