EFFECT OF SUPPLEMENTING HONEY TO MILK ON STARTER VIABILITY AND SOME CHEMICAL AND ORGANOLEPTIC PROPERTIES OF ZABADY-BIFIDUM FERMENTED MILK DURING COLD STORAGE

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ABSTRACT

Buffalos' milk was modified to 3% fat, fortified with 1, 2 or 3% of honey and fermented with a mixed culture of *S. thermophilus, L. delbrueckii subsp bulgaricus* (Zabady culture) and *B. bifidum*. The effect of honey on the viability of the culture strains and some chemical and organoleptic properties of the resultant fermented milk products were investigated during 8 days of cold storage at 7°C±2. The fresh fermented milk added with honey resulted in higher acidity values than the control. The acidity gradually increased during storage with slightly higher values for the cultured product with 1 and 2 % of honey. Pronounced gradual increase in the total volatile fatty acids (TVFA) was found in all fermented milk during storage, moreover its acetaldehyde content increased during the first 2 days of storage with a gradual reduction till the end of the storage period. Lactic acid and bifidobacterial counts gradually increased during storage up to the fourth day of storage in all samples then gradually decreased. However the addition of honey increased the viability of *L. bulgaricus, S.*

thermophilus and *B. bifidum* compared to the control with a maximum count with 2 % honey for Zabady culture and 2 or 3% for bifidobacteria. Sensory evaluation of the fermented milk showed no significant differences between the control samples and the milk added with 1 or 2% of honey. However the addition of 3% of honey negatively affected the flavour and appearance of the fermented milk with no effect on body and texture.

Key words: Yoghurt, Bifidobacteria, Probiotics, Prebiotics, Honey.

INTRODUCTION

Zabady is the Egyptian traditional fermented milk. It is a form of yoghurt as they made by the assistance of a mixture of *S. thermophilus* and *L. bulgaricus* as a starter culture. Zabady made from buffalos' milk or its mixture with cow's milk however that made from buffalos' milk considered to be the best. Many studies demonstrated the beneficial health promoting effects of Zabady microorganisms. They were shown to reduce plasma cholesterol and increase the antioxidant activity of different tissues (Rao, *et al.* 1981; Beena & Prasad, 1997, Kawase *et al.* 2000 and Zommara, 2002), enhance the gastrointestinal tract health and lactose digestion (Martini *et al.* 1991, Chen *et al.* 2000; Drouault & Corthier, 2001), stimulate the immune system (Perdigón *et al.* 1994) and reduce the diet-associated risk of carcenogenesis (Goldin, 1990 and Perdigón *et al.* 1998).

During the past two decades probiotic (health promoting) microorganisms have been increasingly included in various types of food products, especially fermented milks. Probiotics are live microbial food supplements, which benefit the health of consumers by maintaining or improving their intestinal microbial balance (Fuller, 1989). Among the probiotics, bifidobacteria are known as "friendly" bacteria

that begin to occupy the large intestine immediately following birth. Many recent reports reveal both a natural and therapeutic benefits of bifidobacteria. These benefits include antimicrobial effect (Ogata *et al.* 1997; Romond *et al.* 1998; Bruno and Shah, 2002), anticarcinogenic properties (Pahwa & Mathur, 1987; Rowland *et al.* 1998), reduction of serum cholesterol (Hawkins, 1993; Kheadr *et al.* 2000), synthesis of B. complex vitamins (Hawkins, 1993) and improving lactose tolerance (Vijayendra & Gupta, 1992). Due to their perceived health benefits bifidobacteria, especially *B. bifidum* and *B. longum* have been increasingly included in yoghurt and other fermented milk products in the past two decades. Driessen and de Boer (1989) and Laroia and Martine (1990) classified the fermented milk according to their bacterial composition to several kinds. Yoghurt (*S. thermophilus* and *L. delbruckii var. bulgaricus*), special yoghurt (*B. bifidum, L. acidophilus, S. thermophillus and L. delbruckii var. bulgaricus*, bifighurt (*B. bifidum* and *S. thermophilus*), biogarde (*B. bifidum, L. acidophilus*, and *S. thermophilus*), progurt (*S. lactis subsp. diacetylactis, S. lactis subsp. cremoris, L. acidophilus and B. bifidum*), biokys: (*B. bifidum, L. acidophilus* and *P. acidilactici*), cultura (*B. bifidum* and *L. acidophilus*) bifidus milk (*B. bifidum* or *B. longum*) and acidophilus milk (*L. acidophilus*).

However, in order to produce the health benefits of these microorganisms, the suggested minimum level for their numbers in fermented milk should be about 10^5- 10^7 viable cells per ml or g of the final product (Kurman & Rasic, 1991 and Ishibashi & Shimamura, 1993). Despite the importance of viability of these microorganisms, surveys have shown poor viability of it in the market preparations (Kailasapathy & Rybka, 1997 and Shah *et al.* 1997). Kneifel, *et al.* (1993) reported that the viability of bifidobacteria in yoghurt depends on the used strain, interaction between species present, culture chemical composition of the fermentation medium (e.g. carbohydrate source), final acidity, milk solids content, availability of nutrients, present of growth

promoters and inhibitors, concentration of sugars (osmotic pressure), dissolved oxygen, level of inoculation, incubation temperature, fermentation time and storage temperature.

The term prebiotic was introduced by Gibson and Roberfroid (1995) as a non-digestible food ingredient that selectively stimulating the growth and/or activity of the probiotics in the colon. Bifidobacteria may be promoted by the ingestion of substances such as polysacchararides, fructooligosaccharides and inulin (Mitsuoka, 1992; Gibson, *et al.* 1995; Kaplan & Hutkins, 2000 and Shin, *et al.* 2000). Honey contains various sugars and oligosaccarides, which may enhance the lactic and acetic acids production by yoghurt bacteria and *B. bifidum* (Mitsuoka, 1992; National Honey Board, 1996; Chick, *et al.*, 2001 and Ustunol & Ganhi, 2001). When a product contains both probiotics and prebiotics, it defined as a synbiotic product (Schrezenmeir & De vrese, 2001). In The present study a synbiotic Zabady was made by fermentation of buffaloes' milk supplemented with honey by a mixed culture of *S. thermophilus*, *L. bulgaricus* and *B. bifidum*. The viability of starter culture and some chemical and organoleptic properties of the resultant product were investigated during cold storage for 8 days.

MATERIALS AND METHODS

***Preparation of fermented milk*:** A modified buffaloes' milk (3 %, fat and about 11.5%, TS) was used for preparing the fermented milk products. The milk was divided into 4 portions. The control was prepared without any additives however honey (obtained from the local market at Kafr El-sheik city) was added in 3 levels (1, 2, 3 % w/v) to the other 3 milk portions. A mixed culture (1:1) of Zabady starter *(S. thermophilus* and *L. bulgaricus*) and *B. Bifidum* were added to milk (3%w/v), divided in small plastic cups, (50 g each) and incubated at 40°C ± 2 until complete coagulation. The resultant milk products were stored for 8 days at 5°C and

analyzed at zero time (after overnight cold storage) and every other day for acidity (%), pH value, concentration of total volatile fatty acids (TVFA) and acetaldehyde content. Organoleptic properties and viability of starter microorganisms during storage period were also examined.

Determination of titratable acidity (%): The titratable acidity (%) was determined in the fermented milk samples according to Ling (1963).

Determination of total volatile fatty acids (TVFA): TVFA in the fermented milk products was determined by a direct distillation method according to Kosikowski (1978), and the results were expressed as ml 0.1N-NaOH/100g sample.

Determination of acetaldehyde: Acetaldehyde content in the fermented milk products was estimated according to the Conway microdiffusion semicarbazide method as described by Less and Jago (1969). In this method acetaldehyde reacts with semicarbazide to form semicarbazone, which has an absorption peak at 224 nm. Three ml of 1µmol semicarbazide solution was pipetted in the inner wall of Conway microdiffusion cell. Five grams of fermented milk were mixed rapidly in the outer compartment, and the cell was covered and placed in an incubator at 30°C for 90 min. The solution in the inner well was transferred into a cuivette to measure absorbency at 224 nm. The concentration of acetaldehyde was calculated using a standard curve prepared from serial solutions of acetaldehyde ranging from 1 to 30 µmol/100 ml.

Microbiological analysis: One gram of fermented milk sample was diluted with 9 ml of 0.15% of peptone water solution and gently mixed uniformly with a vortex mixer. Subsequent serial dilutions were prepared and viable numbers enumerated using the pour plate technique. The results were expressed as cfu /gm fermented milk according

to Dave and Shah (1997). The counts of *S. thermophilus* and *L. bulgaricus* were enumerated on bromocresol green whey agar medium and incubated at 43°C for 48 hrs as described by Yamani, *et al.*, (1996). Two liters of 15% (w/w) re-constituted non-fat dry milk were prepared and held at room temperature for 30 min. The pH of the milk was lowered to 5.7 by 1N hydrochloric acid, and milk was then divided equally into four flasks and autoclaved at 121°C /15 min. This resulted in the formation of a curd and separation of whey. When cooled, the whey was transferred carefully under aseptic conditions into sterile conical flasks and kept in the refrigerator. Directly before use, 100 ml of the whey was warmed to 45°C and mixed with 50 ml of sterile agar solution (121°C /15 min) contained 1.5 gm yeast extract, 0.6 gm K_2HPO_4, 1 ml bromocresol green solution (0.2% in 50% ethanol) and 2 gm agar. The agar solution was cooled to 45°C before mixing with whey. *Bifidobacteria* was determined on a double layer of deMan, Rogosa and Sharpe medium (MRS) according to Colins and Hull (1984).

Sensory evaluation: The sensory evaluation of Zabady was assessed according to El-Shibiny *et al.* (1979) using the following points for different properties: Appearance (10 points), body & texture (30 points), and flavor (60 points).

Statistical Analysis: Data are expressed as mean ± SE and significant variations were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Changes in acidity: Table (1) shows changes of acidity during refrigerator storage of Zabady-bifidum fermented milk with different levels of honey. The fermented milk with honey resulted in slightly higher acidity values at the beginning of the analysis (fresh) than the control sample.

This acidity elevation may be attributed to the organic acid content of honey. Honey contains a variety of organic acids such as acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic and succinic acids, which give the product an average pH of 3.9 (National Honey Board, 1996). The acidity gradually increased during the 8 days of storage period in all samples with slightly higher values for the cultured product with 1 and 2 % of honey. Honey contains various sugars and oligosaccharides, which may enhance the lactic and acetic acids production by Zabady bacteria and *B. bifidum* (Ustunol and Ganhi, 2001; Chick, *et al.*, 2001 and Mitsuoka, 1992).

***Changes of total volatile fatty acids (TVFA) in the cultured milk products*:** The effect of storage period on total volatile fatty acids content of the fermented milk products is shown in table (2). The data shows pronounced gradual increase in the total volatile fatty acids (TVFA) in all fermented milk during eight days of cold storage. The use of honey by 2 and 3% in the preparation of Zabady-bifidum fermented milk resulted in elevation of TVFA in the fresh products. This effect may be attributed to the fact that *bifidobacteria* fermentation is unique in that it produces 1.5 moles of acetic acid as well as 1 mole of lactic acid as the end products of the fermentation process of 1 mole of glucose (Tamime, *et al.*, 1995). The liberation of TVFA during Zabady processing may be responsible for their present in the fresh products. In this respect, Rasic and Kurmann (1978), demonstrated that the weak lipolytic action of yoghurt starter should be taken in mined to explain the changes in fatty acid pattern and free fatty acid in the resultant fresh yoghurt.

Changes of acetaldehyde content of the resultant cultured milk products: As shown in table (3), addition of honey to Zabady-bifidum fermented milk significantly increased the acetaldehyde content in the fermented milk during the first 2 days of storage period. After 2 days of cold storage all fermented milk samples showed a gradual reduction of its acetaldehyde content till the end of the storage period.

Table (1) Effect of storage period on acidity (%) of yoghurt-bifidum fermented milk with different levels of honey.

Storage period (day)	Control	Honey		
		1%	2%	3%
Fresh*	0.78 ± 0.03^{a}	0.86 ± 0.02^{b}	0.90 ± 0.01^{b}	0.90 ± 0.03^{b}
2	0.86 ± 0.04	0.90 ± 0.04	0.93 ± 0.03	0.94 ± 0.01
4	0.94 ± 0.06	0.96 ± 0.06	0.96 ± 0.03	0.94 ± 0.01
6	1.05 ± 0.03^{ab}	1.05 ± 0.06^{a}	1.06 ± 0.04^{ab}	1.14 ± 0.02^{b}
8	1.11 ± 0.01	1.10 ± 0.06	1.13 ± 0.06	1.13 ± 0.04

*After overnight cooling.
Data are means for 3 replicates.
[a, b]Means within a row with different superscripts are significantly different ($p<0.05$).

Table (2) Effect of storage period on total volatile fatty acids (ml 0.1N NaOH/100g) of yoghurt-bifidum fermented milk with different levels of honey.

Storage period (day)	Control	Honey		
		1%	2%	3%
Fresh*	4.17 ± 0.10^{a}	4.17 ± 0.08^{a}	4.27 ± 0.13^{ab}	4.57 ± 0.17^{b}
2	4.93 ± 0.12^{a}	4.73 ± 0.09^{ab}	4.35 ± 0.12	4.62 ± 0.18^{ab}
4	5.08 ± 0.18^{a}	4.88 ± 0.04^{ab}	4.73 ± 0.07^{b}	4.92 ± 0.04^{ab}
6	6.30 ± 0.26^{a}	5.07 ± 0.07^{b}	5.75 ± 0.25^{a}	5.80 ± 0.04^{a}
8	6.48 ± 0.25^{ac}	5.62 ± 0.14^{b}	5.83 ± 0.19^{ab}	6.37 ± 0.26^{c}

*After overnight cooling.
Data are means for 3 replicates.
[a, b, c]Means within a row with different superscript are significantly different at ($p<0.05$).

El-Shibiny, *et al.*,(1979b) mentioned that the acetaldehyde content increases with advancing cold storage period. On the other hand, Rasic and Kurman (1978) demonstrated that the rate of acetaldehyde production is highly dependent on the acidity level during yoghurt processing. Its formation begins at pH 5, and rapidly increased by decreasing the pH values to 4.4- 4.3, thereafter, it increases very slowly and stabilizes at pH of about 4.0. In the present study, the reduction of acetaldehyde content in the fermented milk products may be due to the transformation of this component to other components by oxidation or by the action of microorganisms. Also, it may be attributed to the reduction of the bacterial count in the fermented milk by time as mentioned above.

***Viability of lactic acid bacteria and bifidobacteria during cold storage*:** The recorded data in table (4) shows the log bacterial count of lactic acid bacteria during cold storage of Zabady-bifidum fermented milk manufactured with different levels of honey. The obtained results demonstrated a continual increase in lactic acid bacterial count during storage up to the fourth day in all samples. Addition of honey increased the viability of lactic acid bacteria in the cultured milk products, mainly, *L. bulgaricus* and *S. thermophilus*. The maximum bacterial counts were found with 2 % honey in the cultured milk products. Table (4) also shows that the bacterial count decreased by increasing the storage period at refrigerator. This decrease in viability may be attributed to the reduction of pH value, secretion of antimicrobial substances and accumulation of bacterial metabolites in the cultured products during cold storage. The decrease of Zabady bacterial count during storage was demonstrated by Hamann and Marth (1984) and Kehagias and Dalls (1984). They reported that the numbers *S. thermophilus* and *L. bulgaricus* decreased during storage of yoghurt at low temperature. Also, honey was found to increase the viability of different lactic acid bacteria including Zabady culture, Chick, *et al.* (2001).

Table (3) Effect of storage period on acetaldehyde content (µmol/ 100g) of yoghurt-bifidum fermented milk with different levels of honey.

Storage period (day)	Control	Honey		
		1%	2%	3%
Fresh*	53.6 ± 5.52^{a}	85.7 ± 1.76^{b}	88.2 ± 3.75^{b}	97.5 ± 3.82^{b}
2	68.7 ± 5.38^{a}	86.5 ± 2.10^{b}	95.7 ± 3.09^{b}	99.6 ± 5.42^{b}
4	54.5 ± 4.45	54.9 ± 0.93	65.8 ± 2.93	62.9 ± 5.56
6	40.7 ± 2.50^{a}	54.1 ± 1.54^{b}	63.9 ± 1.84^{c}	61.0 ± 2.62^{ab}
8	39.2 ± 4.04	44.1 ± 2.68	39.6 ± 3.44	42.7 ± 1.93

*After overnight cooling.
Data are means for 3 replicates.
$^{a, b}$Means within a row without unlike superscripts are significantly different ($p<0.05$).

Table (4) Effect of storage period on lactic acid bacterial count (log cfu/g) of yoghurt-bifidum fermented milk with different levels of honey.

Storage period (day)	Control	Honey		
		1%	2%	3%
Fresh*	9.12 ± 0.03^{a}	9.37 ± 0.02^{a}	9.95 ± 0.18^{b}	9.87 ± 0.19^{b}
2	10.4 ± 0.04^{a}	10.8 ± 0.13^{b}	10.6 ± 0.13^{ab}	10.8 ± 0.13^{b}
4	10.8 ± 0.15	10.9 ± 0.13	10.8 ± 0.14	10.9 ± 0.17
6	10.0 ± 0.13^{a}	10.4 ± 0.04^{ab}	10.3 ± 0.10^{ab}	10.4 ± 0.03^{b}
8	9.65 ± 0.16	9.65 ± 0.20	9.78 ± 0.11	9.85 ± 0.13

*After overnight cooling.
Data are means for 3 replicates.
$^{a, b}$Means within a row without unlike superscripts are significantly different ($p<0.05$).

Data in table (5) shows the log bacterial count of *bifidobacteria* in Zabady-bifidum fermented milk with different levels of honey during cold storage for 8 days. Addition of honey to the fermented milk significantly increased the viability of *B. bifidum* bacteria. However, the addition of 2 and 3% of honey to the milk were more effective than the addition of 1% throughout the storage period. All fermented milk samples showed a gradual increase in the viability of *bifidobacteria* up to 4 days of cold storage and declined till the end of the storage period. These results are in consistent with the results reported by Chick, *et al.* (2001), they obtained higher cell numbers of *bifidobacteria* when grown in skim milk supplemented with honey. The growth promoting effect of honey may be attributed to its content of oligosaccharides. It is reported that honey has approximately 4-5 % of its composition of various oligosaccharides (National Honey Board, 1996). Ustunol and Gandhi (2001), reported that honey appears to be the preferred sweetener by *bifidobacteria*. Fructose and oligosaccharides present in honey may be the primary components contributing to enhanced growth, acid production and viability of these microorganisms. Honey and oligofructose or inulin reported to have a supporting effect and increased the viability of *bifidobacteria* and yoghurt cultures (Chick, *et al.*, 2001; and Gibson, *et al.*, 1995). Other studies demonstrated the growth promotion effect of yoghurt bacteria on *bifidobacteria*. Shah and Lankaputhra (1997), found that yoghurt with ruptured or whole starter cells promoted the viability of *bifidobacteria*. Dave and Shah (1997), and Ishibashi and Shimamura, (1993), demonstrated the promotion effect of bifidobacterial growth by yoghurt starter. This promoting effect may be explained by the reduction of pH and oxygen contents in the cultured products that enhances the growth of the acid tolerant-anaerobic bifidobacterial strains.

The influence of honey on the organoleptic properties of Zabady-bifidum fermented milk: The scores given for the

Table (5) Effect of storage period on bifidobacterial count (log cfu/g) of yoghurt-bifidum fermented milk with different levels of honey.

Storage period (day)	Control	Honey		
		1%	2%	3%
Fresh*	8.05 ± 0.24^{a}	9.17 ± 0.10^{b}	9.22 ± 0.16^{b}	9.53 ± 0.18^{b}
2	9.18 ± 0.11^{a}	9.35 ± 0.10^{a}	9.80 ± 0.12^{b}	9.93 ± 0.13^{b}
4	9.40 ± 0.06^{a}	9.48 ± 0.03^{a}	9.94 ± 0.17^{b}	9.92 ± 0.06^{b}
6	9.24 ± 0.03^{a}	9.44 ± 0.01^{b}	9.46 ± 0.06^{b}	9.50 ± 0.03^{b}
8	8.60 ± 0.18^{a}	9.14 ± 0.16^{ab}	9.35 ± 0.14^{b}	9.30 ± 0.15^{b}

*After overnight cooling.
Data are means for 3 replicates.
$^{a, b}$Means within a row without unlike superscripts are significantly different ($p<0.05$).

Table (6) Effect of honey on the organoleptic properties of fresh* yoghurt-bifidum fermented milk with different levels of honey.

Properties & Scores	Control		Honey		
	(1)	(2)	1%	2%	3%
Flavour (60)	56.8 ± 0.76^{a}	55.2 ± 0.64^{a}	56.7 ± 0.71^{a}	53.8 ± 1.33^{a}	43.7 ± 2.17^{b}
Body &Texture (30)	26.1 ± 1.01	27.9 ± 0.66	26.4 ± 0.46	26.7 ± 0.47	26.0 ± 0.50
Appearance (10)	9.17 ± 0.24^{a}	8.78 ± 0.17^{a}	9.28 ± 0.25^{a}	9.11 ± 0.31^{a}	7.67 ± 0.41^{b}
Total (100)	92.1 ± 1.46^{a}	91.9 ± 1.06^{a}	92.3 ± 0.51^{a}	89.6 ± 1.72^{a}	77.3 ± 2.01^{b}

*After overnight cooling.
Data are means for 3 replicates.
$^{a, b}$Means within a row with unlike superscripts are significantly different ($p<0.05$).
Control (1): fermented milk made by yoghurt starter only.
Control (2): fermented milk made by a mixed culture of yoghurt starter and *B. bifidum*.

organoleptic properties of fresh Zabady and Zabady-bifidum milk with different levels of honey (1, 2, 3%) are shown in Table (6). No significant sensory differences were found between the control samples and the cultured milk added with 1 or 2% of honey. However the addition of 3% of honey resulted in lowered scores for flavour and appearance with no effect on body and texture of the resultant product. In conclusion, the obtained results may recommend the use of honey by 1 or 2% for fortification milk for the production of Zabady or Zabady-bifidum fermented milk in order to enhance the viability of the bacterial cultures and increase its nutritional value without affecting its sensory properties.

REFERENCES

Beena, A. and Prasad, V. (1997). Effect of yoghurt and bifidus yoghurt fortified with skim powder, condensed whey and lactose-hydrolyzed condensed whey on serum cholesterol and triacylglycerols concentrations in rats. J. Dairy Res., 64: 453-457.

Bruno, F. A. and Shah, N. P (2002). Inhibition of pathogenic and putrefactive microorganisms by *Bifidobacterium sp.* Milchwissenschaft, 57: 617-621.

Chen, R. M.; Wu, J. J.; Lee, S. C.; Huang, A. H.; and Wu, H. M. (2000). Increase of intestinal Bifidobacterium and suppression of coliform bacteria with short- term yoghurt ingestion. J. Dairy Sci., 83: 931-939.

Chick, H.; Shin, H. S. and Ustunol, Z. (2001). Growth and acid production by lactic acid bacteria and bifidobacteria in skim milk containing honey. J. Food Sci., 66: 41-45.

Collins, S. B. and Hull, B. J. (1984). Growth of bifidobacteria in milk and preparation of Bifidobacteria infantis for a dietary adjunct. J. Dairy Sci., 67: 1376-1380.

Dave. R. I. and Shah, N. P. (1997). Viability of yoghurt bacteria and probiotic bacteria in yoghurts made from commercial starter cultures. Int. Dairy J., 7: 31-41.

Dave, R. I. and Shah, N. P. (1998). Ingredient

supplementation effects on viability of probiotic bacteria in yoghurt. J. Dairy Sci., 81: 2804-2816.

Driessen, F. M. and De Boer, R. (1989). Fermented milks with selected intestinal bacteria: a healthy trend in new products. Neth. Milk Dairy J., 43: 360-382 .

Drouault, S. and Corthier, G. (2001). Health effects of lactic acid bacteria ingested in fermented milk. Vet. Res., 32: 101-117.

Duncan, D. B. (1955). Multiple range and Multiple F tests. Biometrics, 11: 1-42.

El-Shibiny, S.; El-Dien, H. F. and Hofi, A. A. (1979). Effect of storage on the chemical composition of zabady. Egyptian J. Dairy Sci.7: 1-7.

Fuller, R. (1989). Probiotics in man and animals. J. Appl. Bacteriol. 66: 365-378.

Gibson, G. R, Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 125: 1401-1412

Gibson, G. R.; Beatty, E. R.; Wang, X.; and Cummings, J. H (1995). Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology, 108: 975-982.

Goldin, B. R. (1990). Intestinal microflora: metabolism of drugs and carecinogens. Anal. Med., 22: 43-48.

Hawkins, S. M. (1993). Bifidobacteria in dairy products. Cultured Dairy products J., 28: 16-20.

Hamann, W. T. and Marth, E. H. (1984). Survival of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in commercial and experimental yoghurt. J. Food Protection, 47: 781-786.

Ishibashi, N. and Shimamura, S. (1993) Bifidobacteria: Research and development. In Japan. Food Technol. 47: 126-135.

Kailasapathy, K. and Rybka, S. (1997). *Lb. acidophilus* and *Bifidobacteria spp.* Their therapeutic potential and survival in yoghurt. Australian J. Dairy Technol. 52: 28-82.

Kaplan, H. and Hutkins, R. W. (2000). Fermentation of

fructo-oligosaccharides by lactic acid bacteria and bifidobacteria. Appl. Enivron Microbiol. 66: 2682-2684.

Kawase, M.; Hashimoto, H.; Hosoda, M.; Morita, H. and Hosono, A. (2000). Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. J Dairy Sci., 83: 255-263

Kehagias, C. H. and Dalles, T. N. (1984). Bacteriology and biochemical from sheep's and cow's milk. J. Food Protection, 47: 760-761.

Kheadr, E. E.; Abd El-Rahman, A. M.; El-Soukary, F. A. (2000) Impact of yoghurt and probiotic strains on serum cholesterol and lipoprotein profile in rats. Alexandria J. of Agricultural Research 45: 81-100.

Kneifel, W.; Jaros, D. and Erhard, F. (1993). Microflora and acidification properties of yoghurt and yoghurt-related products fermented with commercially available starter cultures. Int. J. Food Microbiol. 18: 179-189.

Kosikowiski, F.V. (1978) Cheese and Fermented Milk Food .2nd Ed., Published by the author, Cornell Univ., Ithaca, New York, USA.

Kurmann, J. A. and Rasic, J. L. (1991). The health potential of products containing bifidobacteria. Pages, 117-158, In Therapeutic Properties of Fermented Milks. Robinson, R. K. (ed.), Elsevier Applied Food Science series, London, United Kingdum.

Laroia, S. and Martin, J. H. (1990). Bifidobacteria as possible dietary adjuncts in cultured dairy products "A review". Cultured Dairy products J., 11: 18-22.

Lees, G. J and Jago, G. R. (1969). Methods for the estimation of acetaldehyde in cultured dairy products. Australian J. Dairy Technol., 24:181.

Ling, E.R. (1963) A text Book of Dairy Chemistry. Vol.11, Practical, 3rd Ed. Chapman and Hall, London., UK.

Martini, M. C.; Lerebours, E.; Lin, W. J.; Harlander, S. k.; Berrada, N. M.; Antoine, J. M. and Savaiano, D. A. (1991). Strains and species of lactic acid bacteria in fermented milks (yoghurts): effect on in vivo lactose

digestion. Am. J. Clin. Nutr., 54: 1041-1046.

Mistuoka, T. (1992). Role of intestinal flora in health with special reference to dietary control of intestinal flora. Microbiology Application in Food Biotechnology. Eds. B.H. Hga and Y. K. Lee. Elsevier, Appl. Sci., London, pp135-148.

National Honey Board (1996). Honey information kit of the food and beverage industries. National Honey Board, Longmont, Colo. (www.nhb.org)

Ogata,T.; Nakamura, T.; Anjitsu., k.; Yaeshina, T.; Takahashi, S.;Fukuwatari, Y.; Ishibashi, N.; Hayasawa, H.; Fujisawa, T. and Lino, H. (1997). Effect of *Bifidobacteria longum* BB536 administration on the intestinal environment, defection frequency and fecal characteristics of human volunteers. Bioscience Microflora, 16: 53-58.

Pahwa, A. and Mathur, B.N. (1987). Assessment of a bifidus containing infant formula. Part: II- Implantation of *Bifidobacterium bifidum*. Indian J. Dairy Sci., 40: 364-367.

Perdigón, G.; Rachid, M., DE Budeguer, M. V. and Valdez, J. C. (1994). Effect of yoghurt feeding on the small and large intestine associated lymphoid cells in mice. J. Dairy Res. 61: 553-562.

Perdigón, G.; Valdez, J. C. and Rachid, M. (1998). Antitumour activity of yoghurt: study of possible immune mechanisms. J. Dairy Res. 65: 129-138.

Rao, D. R.; Chawen, C. B. and Plusani, S. R. (1981). Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. J. Food Sci., 46: 1339-1341.

Rasic, J. L. and Kurmann, J. A. (1978). Yoghurt: Scientific Ground, Technology, Manufacture and preparation, P. 117. Tech. Dairy Publ. Hous, Copenhagen, Denmark.

Romond, M. B.; Ais, A.; Guillemot, F.; Bounouader, R.; Cortot, A. and Romond, C. (1998). Cell-free whey from milk fermented with Bifidobacterium breve C50 used to modify the colonic microflora of healthy subjects. J

Dairy Sci., 81: 1229-1235.

Rowland, I. R.; Rumney, C. J.; Coutts J. T. and Lievense, L. C. (1998). Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis, 19: 281-285.

Schrezenmeir, J and De Vrese, M. (2001). Probiotics, prebiotics and synbiotics-approaching a definition. Am. J. Clin. Nutr., 73 (suppl): 361S-364S.

Shah, N. P and Lankaputhra, W. E. V. (1997). Improving viability of *Lactobacillus acidophilus* and *Bifidobacteria spp.* in yoghurt. Int. Dairy J., 7: 349-356.

Shin, H. S.; Lee, J. H.; Pestka, J. J. and Ustunol, Z. (2000). Viability of bifidobacteria in the commercial dairy products during refrigerated storage. J. Food Protection, 63: 327-331.

Tamime, A. Y.; Marshall, V. M. and Robinson, R. K. (1995). Microbiological and Technology aspects of milks fermented by bifidobacteria. J. Dairy Res., 62: 151-187.

Ustunol, Z. and Gandhi, H. (2001). Growth and viability of commercial *bifidobacteria spp.* in Honey sweetened skim milk. J. Food Protection, 64 : 1775-1779.

Vijayendra, S. V. and Gupta, R.C. (1992). Therapeutic importance of bifidobacteria and *Lb. acidophilus* in fermented milks. Indian Dairyman 44 (12): 595-599.

Yamani, M. I. and Ibrahim, S. A. (1996). The differential enumeration of *Lactobacillus delbruekii subspecies bulgaricus* and *Streptococcus salivarius subspecies thermophilus* in yoghurt and labneh using an improved whey medium. J. Society of Dairy Technol., 49: 103-108.

Zommara, M. (2002). In vitro studies on the antioxidant effect of Zabady microflora. Egyptian J. Dairy Sci., 30: 53-61.

الملخص العربى

تأثير إضافة تركيزات مختلفة من عسل النحل على اللبن المتخمر المحضر بواسطة خليط من بادئ الذبادى والبكتريا *B. bifidum* أثناء التخزين فى الثلاجة.

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أدى إضافة عسل النحل إلى حدوث زيادة طفيفة فى حموضة الألبان المتخمرة الطازجة (بعد التبريد طول الليل) مقارنة بعينة المقارنة ثم ازدادت الحموضة تدريجيا طول فترة التخزين خاصة فى الألبان المتخمرة المضاف إليها العسل بنسبة ١% و٢%. لوحظ زيادة تركيز الأحماض الدهنية الطيارة (TVFA) فى كل عينات الألبان المتخمرة أثناء فترة التخزين وقد احتوت العينات الطازجة من الألبان المتخمرة المضاف إليها العسل بنسبة ٢% و٣% على أعلى قيم من هذه الأحماض مقارنة بباقى العينات، كما حدثت زيادة ملحوظة فى تركيز الأسيتالدهيد خلال اليومين الأولين من التخزين، بينما انخفض هذا التركيز تدريجيا فى كل عينات اللبن بعد ذلك وحتى نهاية فترة التخزين.

أدى إضافة العسل إلى زيادة تدريجية فى أعداد بكتريا حمض اللاكتيك (بادئ الذبادى) وكذلك *B. bifidum* فى كل عينات الألبان المتخمرة حتى اليوم الرابع من التخزين تبع ذلك انخفاض فى أعدادها حتى نهاية فترة التخزين. وأوضحت النتائج أن أعلى قيم لبكتريا الذبادى حدثت عند إضافة العسل بنسبة ٢% إلى اللبن المتخمر بينما وجد أن تركيزات ٢% و٣% كانت الأفضل فى زيادة حيوية الـ Bifidobacteria. لقد أوضحت اختبارات التحكيم الحسى عدم وجود أى فروق معنوية بين الخواص الحسية لعينة المقارنة والعينات المضاف إليها العسل بنسبة ١% أو ٢%، بينما أثر إضافة العسل بنسبة ٣% أثر سلبيا على الخواص الحسية للبن المتخمر.