

## CHANGES IN PHYSICO-CHEMICAL PROPERTIES OF BIOYOGHURT MADE FROM LACTOSE HYDROLYSED MILK

Abd El-Rahman, M.A.<sup>1</sup>; E. E. Kheadr<sup>2</sup> and T. M. El-Nemr<sup>2</sup>

<sup>1</sup>Dept. of Dairy Sci., Fac. of Agric., El-Minia, Univ., Egypt

<sup>2</sup>Dept. of Dairy Sci. and Technol., Fac. of Agric. Alex. Univ., Egypt

### ABSTRACT

Yoghurt made from lactose hydrolysed or unhydrolysed milks in presence of yoghurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus* LX and *Streptococcus thermophilus* S3) and four strains of bifidobacteria (*B. bifidum* DSM 20456, *B. bifidum* DSM BB12, *B. longum* DSM 20097 and *B. infantis* DSM 20090) were assessed during 15 days of refrigerated. A reduction in milk lactose of 42.3% was achieved using Maxilact, 20000. Milk was inoculated at a level of 2% (v/v) with a bacterial mixture of *Bifidobacterium* spp.: *Str. thermophilus* : *L. delbrueckii* ssp. *bulgaricus* using a ratio of 2:1:1 (v/v/v). During storage, hydrolysed yoghurt showed higher titratable acidity and lower pH values than unhydrolysed yoghurt. Hydrolysed yoghurt showed higher acetaldehyde and soluble tyrosine contents but lower lactose content and  $\beta$ -galactosidase activity than unhydrolysed yoghurt. Sensory assessment data showed that hydrolysed yoghurt containing *B. longum* or *B. bifidum* gained the highest flavour, texture and overall scores. While, standard yoghurt and yoghurt containing *B. infantis* scored lower because of the appearance of bitter off-flavour in the former and unpleasant flavour in the latter samples. In conclusion, *B. longum* proved to be a promising candidate to be used as dietary adjunct in fermented milk with probiotic properties.

**Keywords:** Bifidobacteria, Bioyoghurt, Lactose-hydrolysed, Refrigerated storage.

### INTRODUCTION

Bifidobacteria are the natural predominant microflora in the gut of breast-fed infants and offer resistance to enteropathogenics. Reduction of cholesterol in serum, activation of the immune system and anticarcinogenic activity, improving lactose utilisation in malabsorbers, deconjugation of bile acids are suggested health benefits attributed to bifidobacteria (Rasic and Kurmann, 1978; Simone *et al.*, 1992; Molder, 1994; Lankaputhra and Shah, 1995; Jiang *et al.*, 1996). Because of their health and therapeutic benefits, bifidobacteria are usually incorporated in yoghurt manufacture along with yoghurt organisms (Ballongue, 1998) and they are being incorporated in cheese manufacture (Dinakar and Mistry, 1994; Blanchette *et al.*, 1996). In addition, these organisms are considered essential for maintaining a healthy equilibrium between the population of beneficial and potentially harmful micro-organisms in the gastrointestinal tract (Sreekumar and Hosono, 1998), and for synthesising of B-complex vitamins and absorption of calcium (Hughes and Hoover, 1991). On the other hand, antimutagenic properties of live and killed cells of bifidobacteria have been recently reported by Lankaputhra and Shah (1998) and Shah *et al.* (1999). In order to attain the desired therapeutic and health benefits, bifidobacteria must be available in sufficient number in the food dietary. A minimum of  $10^6$  cfu/g of viable cells of

bifidobacteria should be present in a probiotic products at the time of consumption in order to have the probiotic effect (Blanchette *et al.*, 1996).

Bifidus milk has not been successfully commercialised because of its unacceptable flavour. On the other hand, it is well known that bifidobacteria grow poorly in milk unless it is supplemented with more readily available nutrients, and an incubation period of at least 14-16 hours is required to reach a pH 4.3-4.4 (Samona and Robinson, 1994).

In the present study four strains of bifidobacteria (two of *B. bifidum*, one of *B. longum* and one of *B. infantis*) were individually incorporated in yoghurt manufacture along with yoghurt bacteria. Yoghurt was made from lactose-hydrolysed or un-hydrolyzed milks. This was in order to determine the effect of lactose hydrolysis prior to fermentation on the chemical properties and sensory attributes of bioyoghurt during refrigerated storage.

## MATERIALS AND METHODS

### Bacterial strains

Four lyophilised strains of bifidobacteria (*B. bifidum* DSM 20456, *B. bifidum* DSM BB12, *B. longum* DSM 20097 and *B. infantis* DSM 20090) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. The cultures were reactivated by three consecutive subculturing in MRS-maltose broth containing 0.05% (w/v) L-cysteine-HCl (Hull and Roberts, 1984) and incubated anaerobically using an AnaeroGen system™ (Oxiod) at 37°C for 24 h. Cultures were transferred into 10% reconstituted non-fat dry milk supplemented with 0.05% L-cysteine-HCl (Lankaputhra *et al.*, 1996) and incubated at 37°C for 48 h. *Streptococcus thermophilus* (S3) and *Lactobacillus delbrueckii* spp. *bulgaricus* (LX) were provided by Dr. Joseph F. Frank, Department of Food Science and Technology, The University of Georgia, USA. S3 and LX cultures were maintained in sterile 10% (w/v) reconstituted non-fat dry milk. All cultures were successively transferred in milk at least three times prior to experimental use.

### Yoghurt manufacture

Yoghurt samples were prepared from cow's milk (3.5% fat, 8.7% SNF) at the dairy pilot plant of Department of Dairy Science and Technology, Alexandria University, Egypt. Raw milk was divided into two lots (30 kg each) and tempered to 37°C. The first lot received 300 mL of distilled water, while the second received 300 mL of a solution of 1% (w/v) of  $\beta$ -galactosidase. After 15 min at 37°C, lactose-hydrolysed lot was heated at 70°C for 5 min to inhibit the enzymatic reaction. For keeping on the similarity among treatments, unhydrolysed milk lot was also heated at 70°C for 5 min. Thereafter, both milk lots were cooled down at 45°C and fortified with 2% (w/v) low heat skim milk powder (Dairy Inc., Virginia-Alexandria, VA, USA). Milk lots were then homogenized at 150 kg/cm<sup>2</sup> using a Pierre Guerin S. A., France model homogenizer, heated at 85°C for 30 min and then cooled to 40-43°C and samples were taken in order to determine the lactose content in such milk lots. Such milk lot was divided into 10 batches of 2.5 kg and inoculated with the desired starter mixture at final level of 2% (v/v) as given in Table 1. Following inoculation, milk was distributed into plastic cups and

fermentation was carried out at 45°C till the pH of  $4.5 \pm 0.1$  was obtained. After fermentation, yoghurt samples were resulted from using different bacterial strains are presented in Table (1). Samples were transferred to a cold room at  $5 \pm 1^\circ\text{C}$  and were kept at this temperature for 15 days.

Table (1): Yoghurt samples prepared in this study.

Treatment cods*	Bacterial strains and their ratio
A	<i>B. longum</i> (DSM 20097): <i>Str. thermophilus</i> (S3): <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> (LX) (2:1:1)
B	<i>B. infantis</i> (DSM 20090): <i>Str. thermophilus</i> (S3): <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> (LX) (2:1:1)
C	<i>B. bifidum</i> (DSM BB12): <i>Str. thermophilus</i> (S3): <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> (LX) (2:1:1)
D	<i>B. bifidum</i> (DSM 20456): <i>Str. thermophilus</i> (S3): <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> (LX) (2:1:1)
E	<i>Str. thermophilus</i> (S3): <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> (LX) (1:1)
F	As A
G	As B
H	As C
I	As D
J	As E

\* Treatments A to E were made from lactose-unhydrolysed milk, while treatments F to J were made from lactose-hydrolysed milk (42.3% hydrolysis).

### Sampling

The '0' time represents the observations taken immediately after the addition of starter cultures to the milk tempered to  $40-43^\circ\text{C}$ . The 1-day period represents analysis carried out after an overnight storage of yoghurt samples at  $5 \pm 1^\circ\text{C}$ , and periods 3-15 day represent analysis carried out after 3, 7, 10 and 15 days of storage.

Changes in titratable acidity, pH, and acetaldehyde content were determined at 1, 3, 7, 10 and 15 days of storage. Lactose and soluble tyrosine contents were determined at 1, 7 and 15 days of storage. All yoghurt samples were organoleptically evaluated at 1-day storage.

### Chemical analysis

The titratable acidity was determined after mixing 10 g yoghurt sample with 10 mL of distilled water ( $40^\circ\text{C}$ ) and titrated with 0.1 N NaOH using 0.5% phenolphthalein as an indicator (Dave and Shah, 1997). The pH of milk and yoghurt samples were determined using pH meter (Cole-Parmer Co., Chicago, IL, USA). Acetaldehyde and soluble tyrosine contents of yoghurt samples were determined according to the methods of Robinson *et al.* (1977) and Vakalaris and Price (1959), respectively.

### Lactose and its hydrolysis products

Lactose and its hydrolysis products contents of milk and yoghurt samples were determined as described by El-Nemr (1990) using thin-layer chromatography technique. Sugars in the samples were identified by chromatography on silica gel G type 60 plates. The plates of silica gel (200 x 200 x 0.25 mm) were activated by heating at  $150^\circ\text{C}$  for one hour and cooled in a desecrator. The samples (2 $\mu$  liter) were spotted by a microsyring on the lower edge of the plates and developing were carried out using a solvent

system consisting of n-butanol; acetic acid; diethyl ether; water (9:6:3:1). After developing the plates were dried at room temperature and sprayed with 80% H<sub>2</sub>SO<sub>4</sub>. Visualization was performed by heating at 180° C and the area of charred spots were measured by reflectance scanning technique using a shimadzu CS-190 densitometer.

### **β-Galactosidase**

The β-galactosidase [Maxilact, 20000 ortho-nitrophenyl- β-D-galactosidase (ONPG) unit/g], Gist Brocades, Deft. Holland, was a gift from Dr. Attia, I., Department of Dairy Science and Technology, Alexandria University, Egypt. The enzymatic activity was determined according to the method of Shah and Jelen (1990) using 0.005M ONPG (Sigma, St. Louis, MO, USA) as substrate.

### **Sensory evaluation**

Consumers used for this study were faculty, staff members and untrained students from Department of Dairy Science and Technology, Alexandria University. Yoghurt samples were served in 120 mL plastic cups. The cups were labelled with random three digital codes. Water (21°C) was provided to cleanse the palate between samples. A nine-point hedonic scale was used to measure the acceptability in terms of flavour, texture and overall according to Bodyfelt *et al.* (1988).

### **Experimental design and statistical analysis**

A completely random 2x5 factorial design was used for statistical analysis. Two replications were used and analytical tests were performed in triplicate. Analysis of a variance (ANOVA) were performed on mean values. Estimated means were separated by Fisher's least significant difference procedure at level of  $\alpha = 0.05$  (Steel and Torrie, 1981) using a statistical software packages (SPW, 1995).

## **RESULTS AND DISCUSSION**

### **Changes in titratable acidity and pH values**

The changes in titratable acidity (TA) and pH values during refrigerated storage of different yoghurt samples are given in Table 2. The initial TA of milk (0.17%) increased, at 1-day storage, to 0.68 -0.72% in unhydrolysed samples containing bifidobacteria, which was significantly ( $P < 0.05$ ) lower than 0.75% for standard yoghurt. Bifidobacteria, in general, are widely differed in their intensity of milk acidification. Previous study has shown that different strains of bifidobacteria produced titratable acidities in the range of 0.60 to 1.40% lactic acid when grown individually in sterilized milk for 48 h at 37°C (Misra and Kuilla, 1991). In hydrolysed samples, TA of 0.83% was recorded for standard yoghurt at 1-day storage which was significantly ( $P < 0.05$ ) higher than those of 0.75-0.80% for bifidobacteria-containing batches. In general, lactose hydrolysed batches had significantly ( $P < 0.05$ ) higher titratable acidities compared to their corresponding unhydrolysed batches. During storage, there was significant ( $P < 0.05$ ) increase in TA in all batches, and TA in hydrolysed samples remained higher ( $P < 0.05$ ) than in unhydrolysed one.

Table (2): Changes in titratable acidity (TA) and pH during refrigerated storage of different yoghurt samples made from lactose hydrolyzed and lactose unhydrolysed milk.

Parameters	Storage period (days)	Without - galactosidase*					With - galactosidase <sup>†</sup>				
		A	B	C	D	E	F	G	H	I	J
Titratable acidity (%) as lactic acid	1	0.72 <sup>a</sup>	0.68 <sup>a</sup>	0.70 <sup>a</sup>	0.70 <sup>a</sup>	0.75 <sup>a</sup>	0.79 <sup>a</sup>	0.75 <sup>a</sup>	0.78 <sup>a</sup>	0.80 <sup>a</sup>	0.83 <sup>a</sup>
	3	0.78 <sup>a</sup>	0.71 <sup>a</sup>	0.76 <sup>a</sup>	0.78 <sup>a</sup>	0.80 <sup>a</sup>	0.84 <sup>a</sup>	0.82 <sup>a</sup>	0.83 <sup>a</sup>	0.83 <sup>a</sup>	0.90 <sup>a</sup>
	7	0.83 <sup>a</sup>	0.75 <sup>a</sup>	0.80 <sup>a</sup>	0.84 <sup>a</sup>	0.89 <sup>a</sup>	0.91 <sup>a</sup>	0.90 <sup>a</sup>	0.90 <sup>a</sup>	0.92 <sup>a</sup>	1.00 <sup>a</sup>
	10	0.93 <sup>a</sup>	0.89 <sup>a</sup>	0.90 <sup>a</sup>	0.91 <sup>a</sup>	0.96 <sup>a</sup>	1.02 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.05 <sup>a</sup>	1.15 <sup>a</sup>
	15	1.01 <sup>a</sup>	0.95 <sup>a</sup>	0.98 <sup>a</sup>	1.00 <sup>a</sup>	1.15 <sup>a</sup>	1.19 <sup>a</sup>	1.11 <sup>a</sup>	1.12 <sup>a</sup>	1.12 <sup>a</sup>	1.37 <sup>a</sup>
pH	1	4.45 <sup>a</sup>	4.60 <sup>a</sup>	4.55 <sup>a</sup>	4.50 <sup>a</sup>	4.40 <sup>a</sup>	4.40 <sup>a</sup>	4.49 <sup>a</sup>	4.40 <sup>a</sup>	4.37 <sup>a</sup>	4.25 <sup>a</sup>
	3	4.37 <sup>a</sup>	4.54 <sup>a</sup>	4.49 <sup>a</sup>	4.42 <sup>a</sup>	4.32 <sup>a</sup>	4.32 <sup>a</sup>	4.35 <sup>a</sup>	4.33 <sup>a</sup>	4.31 <sup>a</sup>	4.18 <sup>a</sup>
	7	4.22 <sup>a</sup>	4.45 <sup>a</sup>	4.40 <sup>a</sup>	4.31 <sup>a</sup>	4.22 <sup>a</sup>	4.22 <sup>a</sup>	4.20 <sup>a</sup>	4.19 <sup>a</sup>	4.17 <sup>a</sup>	4.05 <sup>a</sup>
	10	4.18 <sup>a</sup>	4.28 <sup>a</sup>	4.24 <sup>a</sup>	4.21 <sup>a</sup>	4.08 <sup>a</sup>	4.08 <sup>a</sup>	4.09 <sup>a</sup>	4.04 <sup>a</sup>	4.00 <sup>a</sup>	3.89 <sup>a</sup>
	15	4.05 <sup>a</sup>	4.14 <sup>a</sup>	4.07 <sup>a</sup>	4.05 <sup>a</sup>	3.90 <sup>a</sup>	3.90 <sup>a</sup>	3.92 <sup>a</sup>	3.90 <sup>a</sup>	3.87 <sup>a</sup>	3.72 <sup>a</sup>

<sup>a, b, c, d, e</sup> Means within a column followed by a different superscripts are significantly different (p<0.05).

\*A and F: made with a bacterial mixture of *B. longum* DSM 20097: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

B and G: made with a bacterial mixture of *B. infantis* DSM 20090: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

C and H: made with a bacterial mixture of *B. bifidum* DSM BB12: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

D and I: made with a bacterial mixture of *B. bifidum* DSM 20456: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

E and J: made with a bacterial mixture of *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 1:1(v/v), respectively.

Kneifel *et al.* (1993) observed an increase in TA of 14.9 to 22.3% during cold storage of yoghurt samples made with 44 different commercial starter cultures. Also, Kim *et al.* (1992) reported an increase in TA during cold storage of yoghurt made with commercial starter cultures containing *L. acidophilus* and bifidobacteria.

The initial pH of milk (6.6) decreased to 4.45- 4.60 in bifidobacteria containing batches made from unhydrolysed lactose milk versus 4.40 in standard yoghurt, at 1-day storage. For hydrolysed batches, pH values of 4.37 -4.49 were recorded for bifidobacteria-containing samples, while value of 4.25 was recorded for standard yoghurt. During storage there was a gradual decrease in the pH in all samples, and hydrolysed samples had significantly (P<0.05) lower pH values than unhydrolysed samples and significantly (P<0.05) higher values were recorded for bifidobacteria-containing samples compared to standard yoghurt batches. At 15-days storage, in the unhydrolysed yoghurt did the pH drop to ~ 4.0 which is known to be detrimental to the viability of bifidobacteria (Shah *et al.*, 1995). On contrary, all hydrolysed samples had pH below 4.0, this may explain the lower viable counts of bifidobacteria in hydrolysed than in unhydrolysed yoghurt at that stage (data not shown).

### β-Galactosidase activity

Data concerning the activity of bacterial β-galactosidase during refrigerated storage of different yoghurt samples are given in Table 3. For unhydrolysed samples, standard yoghurt demonstrated the highest (P<0.05) β-galactosidase activity throughout the storage period. At 1-day storage, the enzyme activity reached about 2.97 unit/g for unhydrolysed standard yoghurt versus 1.06-1.31 unit/g for yoghurt containing bifidobacteria. The β-

galactosidase activity of *Str. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* has been shown to be the highest among the lactic acid bacteria (Wierzbicki and Kosikowski, 1973). The  $\beta$ -galactosidase can survive passage through the gastrointestinal tract and contribute to improve lactose digestion (Wong et al., 1987).

**Table (3): Changes in  $\beta$ -galactosidase activity, lactose, soluble tyrosine and acetaldehyde contents during refrigerated storage of different yoghurt samples made from lactose hydrolysed and unhydrolysed milk.**

Treatment	Storage (days)	Without - galactosidase <sup>*1</sup>					With - galactosidase <sup>**2</sup>				
		A	B	C	D	E	F	G	H	I	J
$\beta$ -galactosidase (unit/g)	1	1.31 <sup>u</sup>	1.12 <sup>u</sup>	1.25 <sup>u</sup>	1.06 <sup>u</sup>	2.97 <sup>u</sup>	0.53 <sup>u</sup>	0.45 <sup>u</sup>	0.86 <sup>u</sup>	0.66 <sup>u</sup>	0.53 <sup>u</sup>
	7	1.71 <sup>u</sup>	1.32 <sup>u</sup>	1.65 <sup>u</sup>	1.98 <sup>u</sup>	3.04 <sup>u</sup>	1.39 <sup>u</sup>	1.12 <sup>u</sup>	1.58 <sup>u</sup>	0.86 <sup>u</sup>	0.53 <sup>u</sup>
	15	1.77 <sup>u</sup>	1.78 <sup>u</sup>	1.98 <sup>u</sup>	1.98 <sup>u</sup>	4.16 <sup>u</sup>	1.45 <sup>u</sup>	1.65 <sup>u</sup>	1.65 <sup>u</sup>	1.56 <sup>u</sup>	0.66 <sup>u</sup>
Lactose content (%)	1	4.05 <sup>u</sup>	4.36 <sup>u</sup>	4.17 <sup>u</sup>	4.01 <sup>u</sup>	3.81 <sup>u</sup>	3.60 <sup>u</sup>	3.60 <sup>u</sup>	3.60 <sup>u</sup>	3.60 <sup>u</sup>	3.60 <sup>u</sup>
	7	3.18 <sup>u</sup>	3.15 <sup>u</sup>	3.10 <sup>u</sup>	3.47 <sup>u</sup>	3.04 <sup>u</sup>	2.60 <sup>u</sup>	2.85 <sup>u</sup>	2.80 <sup>u</sup>	2.92 <sup>u</sup>	2.30 <sup>u</sup>
	15	2.53 <sup>u</sup>	3.01 <sup>u</sup>	2.70 <sup>u</sup>	2.70 <sup>u</sup>	2.36 <sup>u</sup>	2.04 <sup>u</sup>	2.23 <sup>u</sup>	1.95 <sup>u</sup>	2.17 <sup>u</sup>	1.22 <sup>u</sup>
Soluble tyrosine ( $\mu$ g/g)	1	3.2 <sup>a</sup>	2.9 <sup>d</sup>	2.2 <sup>e</sup>	2.1 <sup>d</sup>	2.5 <sup>f</sup>	3.5 <sup>u</sup>	3.1 <sup>u</sup>	2.9 <sup>u</sup>	2.5 <sup>u</sup>	3.0 <sup>u</sup>
	7	4.1 <sup>b</sup>	3.5 <sup>d</sup>	3.0 <sup>e</sup>	2.7 <sup>f</sup>	2.7 <sup>f</sup>	5.2 <sup>u</sup>	4.3 <sup>u</sup>	4.3 <sup>u</sup>	4.5 <sup>u</sup>	4.0 <sup>u</sup>
	15	5.4 <sup>e</sup>	5.0 <sup>f</sup>	4.1 <sup>g</sup>	4.1 <sup>g</sup>	3.9 <sup>u</sup>	7.6 <sup>u</sup>	5.8 <sup>u</sup>	6.7 <sup>u</sup>	6.2 <sup>u</sup>	6.2 <sup>u</sup>
Acetaldehyde (ppm)	1	10.9 <sup>u</sup>	8.3 <sup>u</sup>	11.5 <sup>u</sup>	11.0 <sup>u</sup>	10.2 <sup>u</sup>	14.2 <sup>u</sup>	10.4 <sup>u</sup>	13.5 <sup>u</sup>	11.5 <sup>u</sup>	12.5 <sup>u</sup>
	3	16.8 <sup>u</sup>	10.5 <sup>u</sup>	15.0 <sup>u</sup>	13.6 <sup>u</sup>	18.0 <sup>u</sup>	22.7 <sup>u</sup>	19.0 <sup>u</sup>	19.4 <sup>u</sup>	20.6 <sup>u</sup>	20.4 <sup>u</sup>
	7	24.3 <sup>u</sup>	23.4 <sup>u</sup>	21.5 <sup>u</sup>	24.4 <sup>u</sup>	24.5 <sup>u</sup>	28.8 <sup>u</sup>	26.4 <sup>u</sup>	25.6 <sup>u</sup>	24.3 <sup>u</sup>	26.2 <sup>u</sup>
	10	14.8 <sup>u</sup>	16.5 <sup>u</sup>	17.6 <sup>u</sup>	18.8 <sup>u</sup>	20.6 <sup>u</sup>	15.5 <sup>u</sup>	18.5 <sup>u</sup>	19.8 <sup>u</sup>	17.4 <sup>u</sup>	22.0 <sup>u</sup>
15	9.4 <sup>f</sup>	13.1 <sup>u</sup>	12.6 <sup>u</sup>	15.4 <sup>u</sup>	14.5 <sup>u</sup>	12.1 <sup>u</sup>	15.1 <sup>u</sup>	15.3 <sup>u</sup>	15.4 <sup>u</sup>	16.1 <sup>u</sup>	

<sup>c, d, e</sup> Means within a row followed by a different superscripts are significantly different ( $p < 0.05$ ). <sup>a, b</sup>.

- \*A: made with a bacterial mixture of *B. longum* DSM 20097: *Str. thermophilus*: *L. delbrueckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.
- B: made with a bacterial mixture of *B. infantis* DSM 20090: *Str. thermophilus*: *L. delbrueckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.
- C: made with a bacterial mixture of *B. bifidum* DSM BB12: *Str. thermophilus*: *L. delbrueckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.
- D: made with a bacterial mixture of *B. bifidum* DSM 20456: *Str. thermophilus*: *L. delbrueckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.
- E: made with a bacterial mixture of *Str. thermophilus*: *L. delbrueckii* ssp. *bulgaricus* at ratio of 1:1(v/v), respectively. <sup>\*\*2</sup>Treatments F to J made from lactose-hydrolysed milk (42.3% hydrolysis) using the same bacterial mixtures used, respectively, for yoghurt A to E.

It is obvious from Table 3 that lactose hydrolysis has resulted in a significant ( $P < 0.05$ ) reduction in  $\beta$ -galactosidase activity in all the samples. As hydrolysed yoghurt demonstrated the highest acid accumulation, the activity of  $\beta$ -galactosidase may thus be reduced due to acid denaturation of the enzyme (Shah and Jelen, 1990). The  $\beta$ -galactosidase in *Str. thermophilus*, *L. delbrueckii* ssp. *bulgaricus* and bifidobacteria showed optimum activity in the neutral pH range (Shah and Jelen, 1990; Desjardins et al., 1990 and 1991). Meanwhile, lactose hydrolysis prior to fermentation could provide sufficient concentration of simple sugars, particularly glucose, and may thus retard  $\beta$ -galactosidase production by the bacterial cells.

During storage, there were a gradual increase in  $\beta$ -galactosidase activity in all samples, and the activity remained lower ( $P < 0.05$ ) in hydrolysed than in unhydrolysed yoghurt. This increase may be due to rupturing of bacterial cells during storage which result in the release of their endoenzymes such as  $\beta$ -galactosidase (Jasewicz and Wasserman, 1961).

These results are coincided with the lactose content estimated in such yoghurt sample during storage period.

#### **Lactose content**

The changes in lactose content during refrigerated storage of different yoghurt samples are presented in Table 3. In unhydrolysed yoghurt, standard yoghurt had the lowest lactose content ( $P < 0.05$ ) throughout the storage period. At 1-day storage, reductions in lactose content in unhydrolysed samples of 18.95 to 25.46% were recorded for yoghurt samples supplemented with bifidobacteria versus 29.18% for standard yoghurt. Generally, about 34-35% of the initial lactose content is utilised during the fermentation process (Goodenough and Kleyn, 1976). The lower lactose hydrolysis obtained at 1-day storage could be due to the fortification of such yoghurt mixture with skimmed powder milk during yoghurt manufacture. Salama and Hassan (1994) reported that standard yoghurt and acidophilus yoghurt had lower lactose content compared to Bioyoghurt and Biogarde made with *B. bifidum* throughout 15 days of refrigerated storage.

There were minor differences in lactose content among yoghurt samples with added *B. longum* and *B. bifidum*. The major differences were between these samples and that containing *B. infantis*. The latter samples contained significantly ( $P < 0.05$ ) higher lactose content. A similar trend of results was recorded for lactose-hydrolysed samples. Similarly, Desjardins *et al.* (1990) reported that *B. bifidum* and *B. longum* hydrolysed twice as much as lactose as *B. infantis* when they grown individually in milk.

#### **Soluble tyrosine content**

Bifidobacteria have been shown to have a potential proteolytic system that exerts both dietetic and technological interests. These organisms are capable of producing several kinds of aminopeptidases, carboxypeptidases, dipeptidases and tripeptidases (Minagawa *et al.*, 1985; El Soda *et al.*, 1992). When added to fermented dairy products, bifidobacteria can improve milk protein digestibility (dietetic criterion) and contribute to flavour development (technological criterion).

The changes in soluble tyrosine content during refrigerated storage of different samples are given in Table 3. In general, all trials made from lactose hydrolysed milk accumulated significantly ( $P < 0.05$ ) higher soluble tyrosine compared to unhydrolysed samples. On the other hand, yoghurt samples with added bifidobacteria, prepared either from lactose hydrolysed or unhydrolysed milks, had significantly ( $P < 0.05$ ) higher tyrosine content compared to standard yoghurt, indicating the potential proteolytic system of bifidobacteria and/or a symbiotic relationship between yoghurt bacteria and bifidobacteria to hydrolyse milk proteins, especially caseins. Among trials made with bifidobacteria, *B. longum* appeared to liberate the highest ( $P < 0.05$ ) level of soluble tyrosine in both yoghurt types. At 1-day storage, the production of soluble tyrosine by bifidobacteria for both types of yoghurt was in the order *B. longum* > *B. infantis* > *B. bifidum* BB12 > *B. bifidum* 20456. When grown alone in sterilized skim milk for 24 h, bifidobacteria have been shown to liberate tyrosine in the range of 268 to 388  $\mu\text{g/g}$  (Misra and Kuilla, 1991). During storage, there were significant ( $P < 0.05$ ) increases in soluble tyrosine content in all trials with increasing storage time. Our results are in agreement with those reported by Salama and Hassan (1994) who pointed out that yoghurt samples supplemented with either *L. acidophilus* or *B.*

*bifidum* contained higher soluble nitrogen and non-protein nitrogen than standard yoghurt throughout 15 days of refrigerated storage. This was attributed to the limited hydrolysis of milk proteins by traditional yoghurt bacteria.

#### Acetaldehyde content

Acetaldehyde is the major flavour component in yoghurt. Thus it was necessary to determine the effect of the incorporation of such bifidobacterial strains on acetaldehyde content in the resultant yoghurt. The changes in acetaldehyde content during storage of different yoghurt samples are presented in Table 3. As shown, lactose-hydrolysed yoghurt accumulated significantly ( $P < 0.05$ ) higher quantities of acetaldehyde compared to unhydrolysed samples. This may be due to the fact that lactose hydrolysis provides simple sugars which supports growth and metabolic activities of the bacterial cultures (Kailasapathy and Supriadi, 1996). Among trials made with bifidobacteria, samples containing *B. longum* contained slightly higher acetaldehyde content, which may explain the sensorial acceptability of these samples. Salama and Hassan (1994) reported that Biogarde made with *B. bifidum* had higher level of acetaldehyde and achieved the highest total scores of sensory evaluation compared to standard, acidophilus and acidophilus-bifidus yoghurt.

During storage, acetaldehyde content, in general, increased significantly ( $P < 0.05$ ) and reached the maximum after one week. Thereafter, decreased gradually with increasing the storage length, these results are in agreement with the finding of Abd El-Rahman (2000). This may be due to the demonstrated ability of numerous microorganisms to reduce acetaldehyde to ethanol (Amer et al., 1991; Salama, 1993).

#### Sensory evaluation

Data concerning the sensory attributes of fresh yoghurt samples are given in Table 3. Results indicated that lactose-hydrolysed yoghurt supplemented with *B. longum* or *B. bifidum* obtained higher flavour, texture and overall scores. Whereas, no significant differences ( $P < 0.05$ ) were found among these samples. Hydrolysis of lactose in yoghurt mixes before fermentation has been shown to increase sweetness and flavour acceptance of the resultant yoghurt samples (Kailasapathy and Supriadi, 1998). Salama and Hassan (1994) reported that Biogarde made with *B. bifidum* had higher total scores of sensory evaluation compared to traditional yoghurt. On the other hand, significant differences ( $P < 0.05$ ) were found between the above samples and those made with traditional yoghurt culture alone or with the incorporation of *B. infantis*. Compared to their hydrolysed trials, lactose unhydrolysed yoghurt made with yoghurt culture alone or with the incorporation of *B. infantis* had slightly higher flavour and texture hedonic score. Samples with *B. infantis* exhibited unpleasant flavour defect, while standard yoghurt samples demonstrated bitter off-flavour. The intensities of such flavour defect were more pronounced in hydrolysed than in unhydrolysed samples. The bitter and unpleasant off flavours could be due to proteolytic activities of such starter cultures (Kailasapathy and Supriadi, 1998) rather than lactose hydrolysis. Lactose-hydrolysed yoghurt often gave a faintly bitter off-flavour which was not noticeable when hydrolysis was less than 80%. In this study, lactose hydrolysis did not exceed 43% which is within



the recommended limit of 40-50% for lactose-hydrolysed yoghurt mixes (Rasic et al., 1992).

Table (4): Sensory attributes of different yoghurt samples evaluated at 1-day of storage.

Treatment cods* <sup>1</sup>	Flavour (9)	Texture (9)	Overall(9)
A	8.2 <sup>a</sup>	8.0 <sup>a</sup>	8.2 <sup>a</sup>
B	7.2 <sup>*2c</sup>	7.5 <sup>c</sup>	7.5 <sup>c</sup>
C	8.0 <sup>a</sup>	7.9 <sup>a</sup>	8.0 <sup>a</sup>
D	7.8 <sup>b</sup>	7.7 <sup>b</sup>	7.8 <sup>b</sup>
E	7.5 <sup>*3d</sup>	8.0 <sup>a</sup>	7.7 <sup>b</sup>
F	8.5 <sup>a</sup>	8.2 <sup>a</sup>	8.4 <sup>a</sup>
G	7.0 <sup>*2c</sup>	7.0 <sup>d</sup>	7.0 <sup>d</sup>
H	8.2 <sup>a</sup>	8.2 <sup>a</sup>	8.4 <sup>a</sup>
I	8.1 <sup>a</sup>	8.0 <sup>a</sup>	8.0 <sup>a</sup>
J	7.2 <sup>*3c</sup>	7.5 <sup>b</sup>	7.4 <sup>c</sup>

A, b, c, d Means within a column followed by a different superscripts are significantly different (p<0.05).

\*<sup>1</sup>Treatments A to E were prepared from unhydrolysed-lactose milk, while treatments F to J were prepared from lactose-hydrolysed milk (42.3% hydrolysis).

A: made with a bacterial mixture of *B. longum* DSM 20097: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

B: made with a bacterial mixture of *B. infantis* DSM 20090: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

C: made with a bacterial mixture of *B. bifidum* DSM BB12: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

D: made with a bacterial mixture of *B. bifidum* DSM 20456: *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 2:1:1(v/v/v), respectively.

E: made with a bacterial mixture of *Str. thermophilus*: *L. delbreuckii* ssp. *bulgaricus* at ratio of 1:1(v/v), respectively.

<sup>2</sup>unpleasant-off flavour.

<sup>3</sup>bitter-off flavour.

## CONCLUSION

The lactose hydrolysed batches had higher titratable acidities compared to their corresponding unhydrolysed samples. During storage there was a gradual decrease in the pH in all samples, and hydrolysed samples had lower pH values than unhydrolysed samples and higher values were recorded for bifidobacteria-containing samples compared to standard yoghurt batches. At 15-days storage, in the unhydrolysed yoghurt did the pH drop to ~ 4.0 which is known to be detrimental to the viability of bifidobacteria.

The unhydrolysed samples, standard yoghurt demonstrated the highest ( $P<0.05$ )  $\beta$ -galactosidase activity throughout the storage period. At 1-day storage, the enzyme activity reached about 2.97 unit/g for unhydrolysed standard yoghurt versus 1.06-1.31 unit/g for yoghurt containing bifidobacteria. Lactose hydrolysis has resulted reduction in  $\beta$ -galactosidase activity in all the samples. The  $\beta$ -galactosidase in *Str. thermophilus*, *L. delbreuckii* ssp. *bulgaricus* and bifidobacteria showed optimum activity in the neutral pH range. Samples made from lactose hydrolysed milk accumulated higher soluble tyrosine compared to unhydrolysed samples. Among the samples

made with bifidobacteria, *B. longum* appeared to liberate the highest level of soluble tyrosine in both yoghurt types. During storage, there were increases in soluble tyrosine content in all trials with increasing storage time.

Lactose hydrolysed yoghurt had higher quantities of acetaldehyde compared to unhydrolysed samples. Yoghurt containing *B. longum* contained slightly higher acetaldehyde content. Acetaldehyde contents were reached the maximum after one week. Thereafter, decreased gradually with increasing the storage length.

Lactose-hydrolysed yoghurt supplemented with *B. longum* or *B. bifidum* obtained higher flavour, texture and overall scores. Compared to their hydrolysed trials, lactose unhydrolysed yoghurt made with yoghurt culture alone or with the incorporation of *B. infantis* had slightly higher flavour and texture hedonic score. Samples with *B. infantis* exhibited unpleasant flavour defect, while standard yoghurt samples demonstrated bitter off-flavour. The intensities of such flavour defect were more pronounced in hydrolysed than in unhydrolysed samples.

## REFERENCES

- Abd El-Rahman, A. M. (2000) Survival of probiotic bacteria in yoghurt and their antimicrobial effect. *Alex. J. Agric. Res.* 45 (1) 63-80.
- Amer, S.N.; A.E. Moussa; S.M. Anis and F.M. Salama (1991) Comparative study between Biogarde as a new product and Zabadi. *Egyptian J. Appl. Sci.*, 6:667.
- Ballongue, J. (1998) Bifidobacteria and probiotic action. Pp. 519-587. In "Lactic acid bacteria. Microbiology and Functional Aspects". S. Salmien and A. Von Wright (ed), Marcel Dekker, Inc., New York, N.Y.
- Blanchette; L., D. Roy; G. Belanger and S. Gauthier (1996) Production of Cottage cheese using dressing fermented by bifidobacteria. *J. Dairy Sci.*, 79: 8-15.
- Bodyfelt, F.W; J. Tobias and G.M. Trout (1988) Sensory Evaluation of Dairy Products. Van Nostrand Reinhold, NY, USA.
- Dave, R.I. and N.P. Shah (1997) Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurt made with commercial starter cultures. *Int. Dairy J.*, 7: 435-443.
- Desjardins, M.; D. Roy and C. Toupin (1990) Uncoupling of growth and acids production in *Bifidobacteria* ssp. *J. Dairy Sci.*, 73: 1478-1484.
- Desjardins, M.; D. Roy and J. Goulet (1991)  $\beta$ -Galactosidase and proteolytic activities of bifidobacteria in milk: A preliminary study. *Milchwissenschaft*, 46: 11-13.
- Dinaker, P. and V.V. Mistry (1994) Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. *J. Dairy Sci.*, 77: 2854-2864.
- El-Nemr, T. M (1990). Preparation of low lactose milk and whey. M. Sc. Thesis, Dairy Science and Technology Dept. Faculty of Agriculture, Alexandria University.
- El Soda, M.; A. Macedo and N.F. Olson (1992) The peptide hydrolase system of *bifidobacterium* species. *Milchwissenschaft*, 47: 87-90.
- Goodenough, E.R. and D.H. Kleyn (1976) Influence of viable yoghurt microflora on digestion of lactose by the rat. *J. Dairy Sci.*, 59:863.
- Hughes, D.B. and D.G. Hoover (1991). *Bifidobacteria*: their potential for use in American dairy products. *Food Technol.*, 45, 74, 76, 78-80, 83.

- Hull, R.R. and A.V. Roberts (1984) Differential enumeration of *Lactobacillus acidophilus* in yoghurt. *Aust. J. Dairy Technol.*, 3: 160-164.
- Jasewicz, L. and A.E. Wasserman (1961). Quantitative determination of lactase. *J. Dairy Sci.*, 44:393.
- Jiang, T.; A. Mustapha and D.A. Savaiano (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing *Bifidobacterium longum*. *J. Dairy Sci.*, 79: 750-757.
- Kailasapathy, K. and D. Supriadi (1998). Effect of partially replacing skim milk powder with whey protein concentrate on the sensory qualities of lactose hydrolysed acidophilus yoghurt. *Milchwissenschaft*, 53:385-389.
- Kailasapathy, K. and D. Supriadi (1996). Effect of whey protein concentrate on the survival of *Lactobacillus acidophilus* in lactose hydrolysed yoghurt during refrigerated storage. *Milchwissenschaft*, 51:565-569.
- Kim, E.R.; K.W. Lee; Y.H. Park and H.S. Kwak (1992). The study of lactic acid bacteria in yoghurt during delivery and storage. *Korean J. Dairy Sci.*, 14:260-263.
- Kneifel, W.; D. Jaros and F. Erhard (1993). Microflora and acidification properties of yoghurt and yoghurt related products fermented with commercially available starter cultures. *Int. J. Food Microbiol.*, 18:179-189.
- Lankaputhra, E.V. and N.P. Shah (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium* ssp. in the presence of acid and bile salts. *Cult. Dairy prod. J.*, 30: 2-7.
- Lankaputhra, W.E.; N.P. Shah and M.L. Britz (1996). Survival of bifidobacteria during refrigerated storage in the presence of acid and hydrogen peroxide. *Milchwissenschaft*, 51:65-70.
- Lankaputhra, W.E. and N.P. Shah (1998). Antimutagenic properties of probiotic bacteria and of organic acids. *Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis*, 397: 169-182.
- Minagawa, E., S. Kaminogawa; F. Tsukasaki; H. Motoshima and K. Yamauchi (1985). Exopeptidase profiles of bifidobacteria. *J. Nutr. Sci. & Vitaminology*, 31:599-606.
- Misra, A.K. and R.K. Kuilla (1991) The selection of bifidobacteria for the manufacture of fermented milks. *Aust. J. Dairy Technol.*, 46, 24-26.
- Molder, H.W. (1994) Bifidogenic factors-sources, metabolism and applications. *Int. Dairy J.*, 4:383-407.
- Rasic, J. L. and J. A. Kurmann (1978) The Nutritional-Physiological Value of Yoghurt. In: "Yoghurt Scientific Grounds, Technology, Manufacture and Preparations". Technical Dairy Publishing House, Copenhagen, Denmark.
- Rasic, J.L.; F.V. Kosikowski and Z. Bozic (1992) Nutrient yoghurt from low lactose milk using a combined lactase-UF retentate procedure. *Milchwissenschaft*, 47:32-35.
- Robinson, R.K.; A.Y. Tamime and L.W. Chubb (1977) Acetaldehyde as an indicator of flavour intensity in yoghurt. *Milk Industry*, 79:4-6.
- Salama, F.M. (1993) Chemical and organoleptical properties of Biogarde from lactose-hydrolysed milk. *Egyptian J. Dairy Sci.*, 21:273.
- Salama, F.M. and Hassan, A.M. (1994) Manufacture of new yoghurt-like products. *Egyptian J. Dairy Sci.*, 22:31-38.
- Samona, A. and R. Robinson (1994) Effect of yoghurt cultures on the survival of bifidobacteria in fermented milks. *J. Soci. Dairy Technol.*, 47: 58-60.

- Shah, N.P. and P. Jelen (1990) Survival of lactic acid bacteria and their lactases under acidic conditions. J. Food Sci., 55:506-509.
- Shah, N.P., W.E. Lankaputhra; M. Britz and W.S. Kyle (1995) Survival of *L. acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. Int. Dairy J., 5:515-521.
- Shah, N.P.; W. XiaoRong; N. Shah and W. XR (1999) Aflatoxin B1 binding abilities of probiotic bacteria. Biosci. Microflora, 18: 43-48.
- Simone, C.D.; A. Ciardi; A. Grassi; S.L. Gardini; S. Tzantzoglou; V. Trinchieri; S. Moretti and E. Jirillo (1992). Effect of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* of gutmucosa and peripheral blood B lymphocytes. Immunopharmacology and Immunotoxicology, 14:333-340.
- SPW (1995). Statgraphics Plus for Windows, Version 1.2, Manugistics, Inc., Rockville, MD, USA.
- Sreekumar, O. and A. Hosono (1998). The antimutagenic properties of a polysaccharide produced by *Bifidobacterium longum* and its cultured milk against some heterocyclic amines. Canadian J. Microbiol., 44:1029-1036.
- Steel, R.G. and J.H. Torrie (1981) Principles and Procedures of Statistics. McGraw Hill Book Co., Inc., NY, USA.
- Vakalaris, D. G. and W.V. Price (1959). A rapid spectrophotometric method for measuring cheese ripening. J. Dairy Sci., 42: 264.
- Wierzbicki, L.E. and F.V. Kosikowski (1973) Lactase potential of various microorganisms grown in whey. J. Dairy Sci., 56:26.
- Wong, N.P.; F.E. McDonough; P.A. Wells; A.D. Hitchins and C.E. Bodwell (1987) Stimulation of rat growth by yoghurt: A role of lactose and lactase. Nutr. Reports Int., 35:497.

### التغيرات في الصفات الطبيعية والكيميائية للبيويوغورت المصنع من لبن متحلل اللاكتوز

عبد الرحمن محمد عبد الرحمن\* - إيهاب عيسى خضر\*\* - طارق مراد النمر\*\*  
\* قسم علوم الألبان - كلية الزراعة - جامعة المنيا - مصر  
\*\* قسم علوم وتكنولوجيا الألبان - كلية الزراعة - جامعة الإسكندرية - مصر

تم في هذه الدراسة:

- تقييم الزبادى المصنع من لبن معاملة أو لبن غير معاملة بانزيم البيتا-جلكتوسيديز في وجود بكتيريا الزبادى

*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*

وزبادى مضاف إليه سلالة مفردة من البيفيدوبكتيريا الأربعة التالية:

(*B. bifidum* DSM 20456, *B. bifidum* DSM BB12, *B. longum* and *B. infantis*)

بالإضافة إلى بكتيريا الزبادى وذلك خلال ١٥ يوم تخزين في التلاجة. وتم إختزال ٤٢% من اللاكتوز الموجود باللبن. وإضيف البادئ إلى اللبن بنسبة ٢% (حجم/حجم) وكان خليط من

بنسبة ٢ : ١ : ١ : *L. bulgaricus* : *Str. thermophus* : *Bifidobacterium* ssp.

- وقد أظهر الزبادى المصنع من لبن متحلل اللاكتوز درجة حموضة أعلى وقيم pH أقل من تلك المصنع من لبن غير متحلل. وكذلك الزبادى المصنع من لبن متحلل اللاكتوز أظهر قيم أسيتالدهيد وتيروسين ذائب أعلى ومحتوى لاكتوز ونشاط بيثا-جالاكتوسيديز أقل من الزبادى المصنع من لبن غير متحلل اللاكتوز.

- وقد بين التقييم الحسى أن الزبادى المصنع في وجود *B. longum* أو *B. bifidum* أخذت درجات تحكيم أعلى لكل من النكهة والقوام والمظهر العام بينما عينات الزبادى الكنترول أو تلك المصنع في وجود *B. infantis* أخذت درجات تحكيم أقل حيث أظهرت عيوب للطعم (طعم مر وطعم غير مستساغ). وعموما فإن *B. longum* يمكن إضافتها إلى منتجات الألبان المتخمرة لما لها من صفات بروبيوتك مرغوبة.