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ENHANCEMENT OF PROBIOTIC VIABILITY IN BIO-YOGHURT

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ABSTRACT

The growth behavior and acid production of *Lactobacillus casei* -01 and *Bifidobacterium bifidum* Bb-12 in bio-yoghurt made with three different yoghurt starter cultures (YC- Fast 1, YC-380 and YC-180) were investigated. The titratable acidity was increased rapidly in yoghurt samples made with YC fast-1, compared wih the other yoghurt strains. The coagulation time was 3, 3.5 and 4 h for bio-yoghurt made with YC fast-1, YC-380 and YC-180 respectively. The total viable counts of *L*. *casei* and *B*. *bifidum* were the highest in bio- yoghurt samples made with YC-180 at the end of fermentation period (4h). Effect of some techniques on the viability of probiotics bacteria was also studied. Microencapsulation of probiotics, addition of heat shocked yoghurt starter or nonviable *K*. *lactis* NRRL Y-8279 had an enhancement effect on the viability of probiotics. Microbiologically, all fresh and stored yoghurt samples were free from coliform and molds & yeasts. Organoleptically, bi-yoghurt made with microencapsulated bacteria or with the addition of nonviable cells had higher scores for acceptability compared with the others.

INTRODUCTION

Lactic acid bacteria have been used since long for the production of a variety of foods , such as fermented milks, cheeses etc. In addition to their general usage in the food industry for fermentation purposes. Quiet recently some strains belonging to the genus *Lactobacillus* or *Bifidobacterium* have attracted a great deal of attention due to probiotic properties attributed to them.

To produce the beneficial effect, a sufficient number of viable microorganisms must be present throughout the entire shelf life of the product. In this regard, minimum levels for probiotic bacteria in fermented milks ranging from 10^6 to 10^7 cfu/ml have been suggested (Perea -Velez *et al*., 2007). However, these organisms grow slowly in milk and often show a loss in viability during refrigerated storage. Their viability is affected by pH as well as the presence of lactic acid. Persistent acid production by *Lactobacillus delbrueckii* subsp. *bulgaricus* is a major cause of the reduction in their viability as well as the presence of hydrogen peroxide and dissolved oxygen in fermented milks (Godward *et al*; 2000). One way to improve probiotic cell counts in fermented milks is to use cultures devoid of *Lactobacillus delbrueckii* subsp. *bulgaricus* (Sodini *et* al., 2002). However, fermentation time can be two – three times longer if the starter culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* is devoid (Sodini *et al.*, 2002). Many other methods have been applied to enhance the probiotics survival during manufacture and refrigerated storage of fermented milks such as

microencapsulation of probiotics (Shah and Ravula, 2000; Adhikeri *et al*.; 2003, Augustine, 2003 and Picot and Locriox, 2004). As micro- encapsulation using gelatin or vegetable gum , which provides protection to acid sensitive *Bifidobacterim Sp*. Encapsulation of *L. rhamnosus* in alginate improved survival at pH 2.0 up to 48 h, while the free cells were destroyed completely (Goderska *et al.*, 2003). Also, addition of baker's yeast improved the survival of *B. longum* in milk (Kailaspathy and Rybka, 1997).

This study was undertaken to examine the growth behavior of probiotics in bio- yoghurt during fermentation, using three yoghurt cultures with different rate of acid production and to enhance the viability of probiotic bacteria during refrigeration storage by using the followings techniques: a) microencapsulation of *L.casei* -01 and *B. bifidum* Bb-12, b) heat shock of yoghurt culture, c) inoculation of yoghurt milk with *L. casei* - 01 and *B. bifidum* Bb-12 one hour before heat shocked yoghurt starter addition, and d) addition of nonviable yeast strains (*K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449) as growth promoters for probiotic bacteria , and it might play a beneficial role in human health in some activities e.g. Improving digestion of lactose (De Vrese *et al*., 2001), or some immune system modulation activities (Marin *et al*., 1997 and Hosono *et al*., 1997). So, the beneficial effects of two nonviable yeast strains were also evaluated.

MATERIALS AND METHODS

Fresh whole cows' milk was obtained from the herd of the Faculty of Agriculture, Cairo University and skim milk powder was obtained from Arla Foods, Sweden.

L. casei – 01, *B. bifidum* Bb-12 and three different types of yogurt cultures (YC-180, YC-380 and YC- fast-1) were obtained from Chr. Hansens' Laboratories, Copenhagen, Denmark. *Kluveromyces lactis* NRRL Y- 8279 was obtained from Northern Regional Research Laboratory, USA. *Saccharomyces cerevisiae* DSMZ 70 449 was obtained from Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH, German.

All microbiological media (MRS M17, malt extract broth & agar, nutrient agar, MacConkey agar) used were obtained from Oxoid Division of Oxiod LTD, London.

Water soluble cholesterol (polyethanyl cholesteryl sebecate) and sodium tauroglycocholate were obtained from Sigma Chemical Co., USA. Anaerogen shachets were obtained from Oxoid Ltd., Basingstoke, Hampshire, England. Vancomycin was obtained from Merck Co., Germany. Sodium alginate was obtained from MIFAD Co., for food industries, Egypt. Cholesterol, glucose,and total antioxidant capacity kits were pursched from Biodiganostc Co. Egypt. Iodine and potassium iodide were obtained from Adwic Co. Egypt.

Direct Vat Set (DVS) *Bifidobacterium bifidum* Bb-12 and *Lactobacillus casei* -01 strains were transferred at rate of 2 % into MRS broth supplemented with L-cystein hydrochloride 0.05% (MRS-C) and incubated aerobically for lactobacilli or anaerobically for bifidobacteria at 37 ºC for 18 h. However, lyophilized yeast strains were subcultured two times consequently prior to use in sterile malt extract (ME) broth and incubated aerobically at 37 ºC for 18 h.

The activated cultures were centrifuged at 3000 ×g for 5 min at 4˚C then pellets were harvested, washed twice and suspending in normal saline (Mandal *et al*., 2006).

Bifidobacterium bifidum Bb-12 and *Lactobacillus casei* -01 were microencapsulated in 3% sodium alginate matrix as described by Sheu and Marshall (1993). Cells were microentrapped by mixing one part culture concentrate with four parts sodium alginate (3%). One part of the mixture was then added dropwise to 5 parts vegetable oil (250 ml in an 800 ml beaker) containing Tween 80 (0.2%), which was stirred at 200 rpm by magnetic stirring. Within 10 min, an uniformly turbid emulsion was obtained with no evidence of a free aqueous phase. Calcium chloride (~500 ml 0.05M) was added quickly but gently (20 ml/sec) down the side of the beaker until the water/oil emulsion was broken. Calcium alginate beads were formed within 10 min. The beads were collected by gentle centrifugation (350 ×g for 10min at 4˚C) and washed with sterile water.

Nonviable *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 cells were prepared by autoclaving the viable cells at 120˚C / 15 min (Zhang and Ohta, 1991).

For detecting the cholesterol removal activity , freshly malt extract broth was supplemented with water soluble cholesterol to a final concentration of 200 mg/100 ml and 0.2% sodium taurocholate. The broth media were inoculated with 2% of culture in free, microencapsulated or non viable cells and incubated aerobically at 37 ºC for 24 h, these experiments were carried out at 37 ºC to simulate the conditions of the intestine. After incubation, cells were removed by centrifugation for 7 min at 5400 ×g at 4 ºC (Kimoto *et al*., 2000) and the free amount of cholesterol was determined calorimetrically by Bio-diagnostic kit (Allain *et al*., 1974).

As with the β-galactosidase activity,all tested microorganisms in free, microencapsulated, and non viable cells were inoculated (2% w/v) in peptone yeast extract broth with lactose (10 g/L) as carbon source, and incubated at 37 ºC for 24 h under aerobic conditions. Cultures were centrifuged at 20.000 ×g for 30 min and the supernatant was used to determine the β. galactosidase activity by monitoring the librated glucose (Rabiu *et al*., 2001). The free amount of glucose was determined by Bio-diagnostic kit (Trinder, 1974) and β. galactosidase activity was calculated according to the equivalent of Dahlqvist (1968) as follows:

Disaccharidase activity/ ml = a.d / n. 1080

a = µl glucose liberated after 24 h incubation.

d = dilution factor for the enzyme solution.

n = number of glucose molecule per molecule of disaccharide (n = 1).

The antioxidant activity of free, microencapsulated and nonviable cells was measured by the following methods:

a. Iron (III) to iron (II) reducing activity

The ability of the free, microencapsulated and non viable cells to reduce iron (III) was assessed by the method of Oyaiza (1986). One gram of free, capsules, and nonviable cells was mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% aqueous potassium hexacyanoferrate ($K_3Fe(CN)_6$) solution. After 30 min incubation at 50˚ C, 2.5 ml of 10%

trichloroacetic acid were added, and the mixture was centrifuged at 4000 rpm for 10 min. A 2.5 ml aliquot of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1 % aquesus $FeCl_3$, and the absorbance was recorded at 700 nm using spectrophotometer type SHIMADZU. Iron (III) reducing activity was determined as ascorbic acid equivalents (mmol ascorbic acid /g).

b. 1, 1. Diphenyl. 2. Picrythyrazyl assay

The 1, 1. diphenyl. 2. picrythyrazyl (DPPH) test was carried out as described by Cuendet *et al*. (1997) . 50 µg of each form of microorganisms were mixed with 5 ml of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm.

c. Total antioxidant capacity

Free, microencapsulated and non viable cells were homogenated on ice in 1-2 ml of cold buffer (5 mM potassium phosphate, pH 7.4 containg 0.9 % sodium chloride and 0.1 % glucose), then centrifuged at 10.000 ×g for 15 min at 4ºC. The supernatants were used immediately for assay by Bio-diagnostic kit (Koracevic *et al*., 2001)**.**

d. Antitumor bioassay

Crown gall tumorigensis on discs of potato tubers (*Solanum tuberosum* L) was proposed as an ideal system for investigating the antitumor bioassay (Ferrigni *et al*., 1982). Potatoes were washed with running water for 30 min, then sterilized by immersion in sodium hypochlorite (Clorox) for 20 min, a core of the tissue was extracted from each potatoes with a sterilized 1.5 cm cork borer, 2 cm pieces are removed from each end of tuber and discarded, and the reminder of the cylinder is cut into 0.5 cm discs. The discs were transferred to 1.5% agar plates. Each plate contains 5 discs, and 3-5 petri dishes were used for each experimental sample. One gram of each tested microorganisms was mixed with 2 ml of broth culture of *A*. *tumefacium*. 0.05 ml of this mix was inoculated into potato disc. The plates were incubated at room temperature for 12 d. The tumors were counted after staining with Lugol's solution (I_2-KI). The results are expressed as + or – percentages versus the number of tumors on the control discs; inhibition is expressed as a negative percentage and stimulation expressed as a positive percentage. Significant activity is indicated when two or more independent assays give consistent negative value of ca. 20 % or greater inhibition.

The mechanism of tumor induction by *A*. *tumefacium* is during the infection process, the bacterium attaches to plant cells and transfers the Ti plasmid into the plant cells genome resulting over production of plant growth regulators (auxins) which are responsible for tumor formation (Kado, 1991).

Standardized cows' milk (fat 3.5%, protein 3.8% and TS 14%) was heated at 90˚C/10min then cooled to 37˚C , then inoculated with 1% *L*. *casei* and *B*. *bifidum* (1:1).The inoculated milk was divided into three equal portions. The first portion was inoculated with yoghurt culture YC- fast-1 (high acid producer), the second inoculated with YC- 380 (medium acid producer), while the third inoculated with YC- 180 (slow acid producer). All yoghurt cultures were added at level of 1% of the milk weight. The different mixes were then dispended into 120 ml polystyrene cups and incubated at 37˚ C

until pH reaches to 4.6. The titrable acidity (TA) %, pH, and starter cultures count were determined during incubation period periodically.

For enhancement of probiotic viability in bio-yoghurt , cows' milk (TS 14%) was heated at 90°C /10min and cooled to 37 °C. Then the milk was divided into three parts. The first part (TI) was inoculated with yoghurt culture YC-180 at 1% of milk weight. The second part (TII) was inoculated with heat shocked (58°C/5 min) YC- 180 yoghurt culture at the same rate (Marshall, 1992). In these two treatments a mixture of probiotics bacteria (1:1) was added (1%) immediately after the addition of YC-180.The milk in the third part (TIII) was inoculated with probiotic bacteria one hour prior to the addition of the heat shocked YC -180.

Within each treatment, the milk was divided into four portions the first portion (1) was inoculated (1%) with free cells of *L. casei* and *B. bifidum* (1:1) while the second portion was inoculated (1%) with microencapsulated *L. casei* and *B. bifidum* (1:1) , the third portion was inoculated (1%) with free cells of *L.casei* and *B.bifidum* and nonviable *K.lactis* (1:1:1) .The fourth portion was inoculated (1%) with microencapsulated *L.casei* and *B,bifidum* and nonviable *K.lactis* (1:1:1). *K.lactis* exhibited higher beneficial effects than *Sacch. cerivisiae* ,so it was selected, added and used as a source of nitrogen.

Yoghurt samples were analyzed for total solids (T.S.), fat (Gerber method), and titratable acidity (T.A. %) according to Ling (1963). The pH measurements were carried out using a laboratory pH meter type (3305) Jenway Co., England.

Yoghurt samples were examined microbiologically for *S. thermophilus* using M17 agar (Terzaghi and Sandine, 1975), *L*. *delbrueckii* subsp. *bulgaricus* using MRS galactose agar (Vinderola and Reinheimer, 1999), *L. casei* using MRS agar with vancomycin (Tharmaraj and Shah, 2003), *B. bifidum* using MRS agar supplemented with 0.05% L- cystein hydrochloride and 0.3 % lithium chloride (Dave and Shah, 1996), moulds & yeasts using malt extract agar (Oxoid Manual, 1982), and Coliform using MacConky agar (A.P.H.A, 1992).

The viable count of microencapsulated *B*. *bifidum* and *L*. *casei* in yoghurt samples were also determined according to Adhikari *et al*., (2000) method as follows : 10 g of sample was added to 90 ml of 0.05 M EDTA in 0.1 M sodium phosphate buffer at pH 7and incubated at 42° C for 20 min before making serial dilution in saline.

The plates of *S. thermophilus* and *L*. *delbrueckii* subsp. *bulgaricus* were incubated aerobically at 37 ºC for 48 h. The plates of *B*. *bifidum* and *L*. *casei* were anaerobically incubated at 37 ºC for 72 h. The plates of single yeast strains and mould & yeasts were aerobically incubated at 25 ºC for 5 days.

Yoghurt samples were organoleptically scored for flavor (45 points), acidity (10 points), body and texture (30 points), and appearance (15 points) with a total acceptance of 100 points according to the score card suggested by EL- Senaity (1999). Samples were judged by the staff members of the Dairy Science Department, Faculty of Agriculture, Cairo University.

The values of all experiments are presented as the means of triplicate analysis. Statistical analysis for obtained data was carried out using the Statistical Analysis System (SAS, 1994).

RESULTS AND DISCUSSION

The most important issue to the development and consumption of probiotic – containing products is the concept of "active principle". For the most part, it is assumed that the active component of probiotic products is viable microorganisms. However the literature suggests several situations in which viability is not required for some activities (Maeno *et al*., 1996; Marin *et al*., 1997; Hosono *et al*., 1997 and De Vrese *et al*., 2001). Results obtained illustrate the growth behavior and acid production of *L.casei* –01 and *B. bifidum* Bb.12 in bio-yoghurt made with three different yoghurt starter cultures on one hand and the effect of the previously mentioned techniques on the viability of probiotic bacteria on the other hand.

In recent years probiotics have increasingly been incorporated into foods as dietary adjuncts. One of the most popular dairy products for delivery of viable probiotics is bio-yoghurt. Therefore bio-yoghurt samples were manufactured by using *L.casei* –01 and *B. bifidum* Bb.12 with one of the following yoghurt starter cultures YC fast -1, medium YC-380 or slow YC-180 starter cultures. Changes in the pH and titratable acidity values as well as the viabilty of L *L.casei* –01 and *B. bifidum* Bb.12 in different treatments were followed and the results obtained are tabulated in Tables (1) and (2).

Table 1: Changes in pH and TA values of bio- yoghurt samples during fermentation(*).

Treatments	Fermentation period (hours)											
	0		1		2		3		3.5		4	
	pH	TA	pH	TA	pH	TA	pH	TA	pH	TA	pH	TA
YC- Fast 1	6.6	0.17	5.70	0.30	5.6	0.40	4.60	0.70	ND	ND	ND	ND
YC- 380	6.6	0.17	5.90	0.27	5.7	0.40	5.00	0.63	4.60	0.70	ND	ND
YC-180	6.6	0.17	6.0	0.20	5.9	0.27	5.60	0.50	5.00	0.56	4.6	0.65

(*): fermentation at 37°C (Kneifil *et al.* 1993).
ND: Not determined.
TA $LSD_{0.05}$ of experimental treatments= 0.12 ($P<0.05$)
pH $LSD_{0.05}$ of experimental treatments= 0.20 ($P<0.05$)

Table 2: The total viable counts (log cfu/g) of *L. casei*-01, *B. bifidum* Bb-12 and yoghurt starter bacteria in bio-yoghurt made with three different yoghurt cultures

Treatments	Fermentation period (hours)											
	0				2				End of fermentation period(*)			
	Lb	St	Lc	Bb	Lb	St	Lc	Bb	Lb	St	Lc	Bb
YC Fast- 1	4.00	3.90	3.80	4.00	6.60	6.80	6.90	4.77	8.30	8.00	8.20	6.00
YC- 380	4.00	3.60	3.80	4.00	6.20	6.00	7.30	6.00	8.20	8.00	8.00	7.20
YC-180	3.80	3.77	4.00	4.10	5.20	5.10	6.00	5.77	7.30	7.60	8.30	8.10

Lb: *L. bulgaricus*, St: *St. thermophilus*, Lc: *L. casei*-01, Bb: *B. bifidum* Bb-12.
(*): End of fermentation period for YC-Fast-1 was 3h, YC-380 was 3.5 h and YC-180 was 4h.
$LSD_{0.05}$ of experimental treatments= 0.70 ($P<0.05$)

As expected, titratable acidity (TA) of all samples increased, while pH values decreased during the incubation period as shown in Table (1). Such changes might be due to continuation of fermentation of lactose and hydrolysis of protein and fat with the formation of volatile substances (Abd El Salam *et al*., 1996). The coagulation time was 3, 3.5 and 4 h for bio-yoghurt made with YC fast-1, YC-380 and YC-180, respectively.

The total viable counts of yoghurt starter cultures and probiotics (*L. casei* -01 and *B. bifidum* Bb-12) in bio- yoghurt samples during incubation period at 37°C are shown in Table (2). The count of all starter cultures increased significantly after 2 h incubation at 37°C, compared with their initial count. The count of both probiotic strains was the highest in yoghurt samples made with YC-180 at the end of incubation period. It is also noticed that the count in *B. bifidum* Bb-12 was affected by the type of yoghurt cultures used. As at the end of fermentation period, there was a significant difference in the total count of *B. bifidum* Bb.12 by using different yoghurt culture. The increase in the growth rate was higher in YC- 180 being 4 log when compared with YC fast –1 or YC –180 being 2 and 3.2 log cycles, respectively ,with non significant difference between the latest two culture. The slow rate of growth might be due to the high rate of acid development during the fermentation period in case of YC fast – 1 and YC 380 cultures. On the contrary, the type of yoghurt culture used had no effect on the total count of *L.casei* 01. As at the end of fermentation period there was no significant difference between the three different cultures used. The increase in the growth rate ranged between (4.2 - 4.4 log). This result confirmed the impact of acidity on the growth and proliferation of *Bifidobacterium* Spp. These results are in accordance with Adhikari *et al*. (2000) , who reported that *B. bifidum* can grow well in milk inoculated with low acid producer yoghurt strains.

Enhancement of survival of probiotic viability in bio-yoghurt was carried out by using the following techniques: a) microencapsulation of *L.casei* -01 and *B. bifidum* Bb-12, b) heat shock of yoghurt culture, c) inoculation of yoghurt milk with *L. casei* - 01 and *B. bifidum* Bb-12 one hour before heat shocked yoghurt starter addition, and d) addition of nonviable yeast strain (*K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70449). Regarding the last technique, the beneficial effects of viable and nonviable yeast cells (cholesterol removal ability, β-galactosidase, antioxidant and antitumor activities) were investigated. Data obtained (Table 3) show that both yeast strains used exhibited a beneficial effects for all the tested activities. Although the viable form exhibited higher values than the nonviable, however the beneficial effects of nonviable could not be neglected. Furthermore, the antioxidant activity was significantly higher in the nonviable cells , compared with the viable cells. These results are in agreement with those reported by Psmos *et al.* (2003) , who examined the ability of *Sacch. cerevisiae* 832, *Sacch. cerevisiae* KK1 and *Issatchenkia orintalis* KK5.Y.1 to assimilate cholesterol from their growth media and showed that *Saccharomyces* strains were able to remove cholesterol without degradation after 24 h of growth at 37ºC. Also Tavan *et al*. (2003) showed that some LAB had antimutagenic activity against food mutagens, and Orrhage *et al.* (1994)

found that the non viable cells bind mutagens with equal efficiency as viable cells.In comparison between the two studied yeast strains, *K. lactis* showed higher beneficial effects in all activities tested. So it was selected for the preparation of bio-yoghurt.

Table 3: The beneficial effects of viable and nonviable yeast cells.

Beneficial activities	Strains				$LSD_{0.05}$ (P<0.05)
	K. lactis NRRL Y-8279		*Sacch. cerevisiae* DSMZ 70449		
	Viable	Nonviable	Viable	Nonviable	
Cholesterol removal ability (%)	59.93	29.50	59.75	29.10	15.07
β-Galactosidase activity (unit/ml)	0.33	0.20	0.22	0.10	0.11
Antioxidant activity TA	0.73	0.94	0.60	0.82	0.15
ARP	1.47	1.95	1.48	1.83	0.28
DPPH Scavenging	37.26	38.35	36.30	37.00	0.60
Antitumor activity	++	++	++	++	++

TA: Total antioxidant; ARP: Antireducing power; DPPH: 1, 1. Diphenyl. 2. Picrythyrazyl

Tables (4) and (5) show changes in pH, titratable acidity values and *L. casei* - 01 and *B. bifidum* Bb-12 counts during storage for 15 days at 6 ± 1°C. In general, pH values decreased and TA values increased as the storage period progressed. At the end of storage period the acid development was the highest and the total count of *L.casei* -01 and *B. bifidum* Bb-12 was the lowest in treatment I , compared with the other treatments . The loss in the count of *B. bifidum* Bb-12 was higher than in *L.casei* -01 which might be due to the higher sensitivity of *B. bifidum* Bb-12 towards acidity.

Table (5) shows that the addition of heat shocked yoghurt starter (Treatment II) or addition of *L.casei* -01 and *B. bifidum* Bb-12 one hour before heat shocked yoghurt starter addition (Treatment III) had a stimulatoty effect on the viability of probiotic bacteria. At the end of storage period there were no significant differences in the total count of *L.casei* -01 and *B. bifidum* Bb-12 between the subtreatments ($T_{II:1}$ & $T_{III:1}$ or $T_{II:2}$ & $T_{III:2}$……etc) in treatments II and III or between the subtreatments in the same treatment. Regarding the effect of storage period Table (5), results showed positive effect of using heat shocked yoghurt starter culture or addition of *L.casei* -01 and *B. bifidum* Bb-12 one hour before heat shocked yoghurt starter addition on the viability of *L.casei* -01 and *B. bifidum* Bb-12 ,as there were no significant difference in their counts after 15 days storage at 6 ± 1°C.The only significant loss in the count was observed in treatment II subtreatment $T_{II:1}$, which inoculated with *B. bifidum* Bb-12being 0.85 log after 15 days.

Table 4: Changes in pH and titratable acidity (TA) values of different bio-yoghurt treatments during storage at 6± 1ºC.

Treatments	Subtreatments*	Storage period , days							
		0		5		10		15	
		pH	TA	pH	TA	pH	TA	pH	TA
T_I (Normal yoghurt starter)	1	4.60	0.70	4.50	0.79	4.40	0.84	4.28	0.98
	2	4.60	0.69	4.58	0.74	4.48	0.79	4.41	0.81
	3	4.60	0.69	4.52	0.78	4.45	0.77	4.44	0.79
	4	4.60	0.69	4.60	0.70	4.59	0.73	4.56	0.77
T_{II} (Heat shocked yoghurt starter)	1	4.60	0.70	4.59	0.72	4.58	0.74	4.45	0.76
	2	4.60	0.70	4.60	0.70	4.59	0.72	4.59	0.73
	3	4.60	0.70	4.59	0.73	4.58	0.75	4.45	0.76
	4	4.60	0.68	4.59	0.70	4.59	0.72	4.59	0.72
T_{III} (Addition of probiotics one hour before heat shocked yoghurt starter addition)	1	4.60	0.69	4.60	0.69	4.60	0.70	4.58	0.74
	2	4.60	0.70	4.60	0.71	4.60	0.71	4.60	0.72
	3	4.60	0.70	4.60	0.72	4.59	0.74	4.45	0.76
	4	4.60	0.69	4.60	0.69	4.60	0.71	4.59	0.73

(*) 1: Free probiotics *L. casei*-01 & *B. bifidum*-12.
2: Microencapsulated probiotics *L. casei*-01 & *B. bifidum*-12.
3:Free probiotics *L. casei*-01 & *B. bifidum*-12 with nonviable *K. lactis* NRRL Y-8279.
4: Microencapsulated probiotics *L. casei*-01 & *B. bifidum*-12 with nonviable *K. lactis* NRRL Y-8279.
TA $LSD_{0.05}$ of experimental treatments= 0.14 (*P*<0.05)
pH $LSD_{0.05}$ of experimental treatments= 0.21 (*P*<0.05)

These results are in agreement with those reported by Marshall (1992), who reported that heat Shock of yoghurt culture (58ºC/ 5 min) before the addition of the probiotics might prevent the over acidification and enhance their viability. Also Dinaker and Mistry (1994) who reported that microencapsulation was an effective method for maintaining the vaiblity of probiotics in food products. Kailaspathy and Rybka (1997) showed that addition of baker's yeast improved the survival of *B. longum* in milk. Initial fermentation might be carried out with probiotic cultures followed by completion of fermentation with yoghurt starter bacteria. This allows the probiotic organisms to be in their final stage of lag phase or early stage of log phase, resulting in higher counts of probiotic organisms at the end of fermentation time (Shah and Lankaputhra, 1997) Furthermore Dave and Shah (1998) observed the decline in population of bifidobacteria during the refrigerated storage. Adhikarii *et al*. (2000) reported that yoghurt made with microencapsulated *Bifidobacterium* had lower acidity than yoghurt made with free *Bifidobacterium*. Donker *et al*. (2006) studied the effect of acidification on the viability of probiotics (*L. acidophilus* LAFI L-10, *B. Lactis* LAFTI B94 and *L. paracasei* LAFI L26) and found that the cell counts of *B. lactis* decreased one log cycle at the end of storage period.

Table 5: Changes in *L. casei*-01 and *B. bifidum* Bb-12 counts (log cfu/g) of different bio-yoghurt treatments during storage at 6± 1ºC.

Treatments	Subtreatments*	Storage period , days							
		0		5		10		15	
		Lc	Bb	Lc	Bb	Lc	Bb	Lc	Bb
T_I (Normal yoghurt starter)	1	8.30	8.00	8.00	7.30	7.30	6.30	6.30	5.30
	2	8.15	8.00	8.00	7.90	7.90	7.00	7.00	6.00
	3	8.30	8.00	8.15	7.90	8.00	7.30	7.30	6.65
	4	8.27	8.07	8.07	8.00	8.05	7.90	7.65	7.00
T_{II} (Heat shocked yoghurt starter)	1	8.30	8.15	8.15	8.07	8.07	7.65	7.90	7.30
	2	8.25	8.15	8.05	8.07	8.00	7.90	8.00	7.65
	3	8.30	8.15	8.07	8.10	8.00	8.00	7.90	7.75
	4	8.20	8.25	8.10	8.20	8.05	7.90	7.95	7.85
T_{III} (Addition of probiotics one hour before heat shocked yoghurt starter addition)	1	8.40	8.15	8.20	8.07	8.00	8.00	7.90	7.75
	2	8.40	8.20	8.30	8.00	8.20	7.90	8.00	7.90
	3	8.40	8.20	8.30	8.05	8.20	8.00	7.90	7.95
	4	8.44	8.20	8.30	8.15	8.20	8.00	8.00	7.95

$LSD_{0.05}$ of experimental treatments= 0.57 (*P*<0.05)
(*) : as mentioned in Table (4).
Lc: *L. casei*-01, Bb: *B. bifidum* Bb-12.

Microbiological quality of bio-yoghurt samples of all treatments when fresh or during storage show that they were free from coliform and molds & yeasts. Results obtained indicated that all yoghurt samples were produced and stored under good sanitary and hygienic conditions.

The sensory evaluation of fresh and stored bio-yoghurt samples as well as control are given in Table (6). Results show that there were non significant differences between fresh and stored samples. The differences in acidity, flavor and appearance scores among samples were not significant. It means that all treatments had an excellent taste and appearance when fresh or during storage. However, a non significant difference between control, $T_{I:1}$ and $T_{II:1}$ and significant differences between them and $T_{I:2}$, $T_{I:4}$,$T_{II:2}$ and $T_{III:4}$ were observed concerning body and texture. This is attributed to formation of exopolysaccharides (EPS) by the starter and an increase in viscosity combined with a better mouth feel (Kailaspathy e*t al* ., 2006).

Generally, data obtained showed that bio- yoghurt made with microencapsulated bacteria or with the addition of nonviable cells had higher scores for acceptability ,compared with the others suggests that consumer was influenced by their addition.

Table 6: Sensory evaluation of bio-yoghurt during storage period at 6±1ºC/14 days.

Treatments	Treatments	Storage period (days)	Acidity (10)	Floavor (45)	Appearance (15)	Body & texture (30)	Total (100)
Control	**---**	0	10	43	15	27	95
		7	10	43	15	27	95
		14	9	43	15	27	94
T_I Normal yoghurt starter	1	0	10	44	15	27	96
		7	10	44	15	27	96
		14	8	44	15	26	93
	2	0	10	44	15	29	98
		7	10	44	15	29	98
		14	10	44	15	28	97
	3	0	10	43	15	28	96
		7	10	43	15	28	96
		14	10	43	15	28	96
	4	0	10	43	15	29	97
		7	10	43	15	29	97
		14	10	43	15	29	97
T_{II} Heat shocked yoghurt starter	1	0	10	43	15	27	95
		7	10	43	15	27	95
		14	10	43	15	27	95
	2	0	10	43	15	29	97
		7	10	43	15	29	97
		14	10	43	15	29	97
	3	0	10	43	15	28	96
		7	10	43	15	28	96
		14	10	43	15	28	96
	4	0	10	43	15	28	96
		7	10	43	15	28	96
		14	10	43	15	28	96
T_{III} (Addition of probiotics	1	0	10	43	15	28	96
		7	10	43	15	28	96
		14	10	43	15	28	96
one hour before heat shocked yoghurt starter addition	**2**	0	10	43	15	28	96
		7	10	43	15	28	96
		14	10	43	15	28	96
	3	0	10	44	15	28	97
		7	10	44	15	28	97
		14	10	44	15	28	97
	4	0	10	43	15	29	97
		7	10	43	15	29	97
		14	10	43	15	29	97
$LSD_{0.05}$	---	---	1.50	1.70	1.20	1.46	--

CONCLUSION

Results obtained showed that nonviable *K. lactis* NRRL Y- 8279 had higher beneficial effects than *Sacch*. *cerevisiae* DSMZ 70 449 and yoghurt strain YC-180 had the lowest effect on the viability of *L. casei* -01 and *B.*

bifidum Bb-12. So *K. lactis NRRL* Y- 8279 ,and yoghurt strain 180 were selected for the preparation of bio- yoghurt. Microencapsulation of probiotics, addition of heat shocked yoghurt starter or nonviable *K*. *lactis* NRRL Y-8279 all had a stimulatory effect on the viability of probiotics.

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تحسين حيوية البكتريا الداعمة للحيوية فى الزبادى.

فوزيــة حســن رجــب عبــد ربــه ، ســامية محمــود الــديب ، ســناء محمــد بــدران ، علاء محمد عبد الفتاح و فؤاد محمود فؤاد الشغبى .
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تم دراسة نمو كل من *B. bifidum Bb*-12 و*L.casei* 01 فى الزبادى الحيوى المصنع بأستخدام ثلاث أنواع مختلفة من بادىء الزبادى من حيث إنتاجها للحموضة. كما تم ايضا دراسة تأثير بعض التقنيات على حيوية هذه البكتريا . وكانت اهم النتائج المتحصل عليها كما يلى: زادت الحموضة زيادة سريعة فى الزبادى المصنع بإستخدام بادىء YC fast -1 مقارنة بالبادئات الاخرى وأختلفت مدة التحضين حيث كانت ٣ ، ٣,٥ ، ٤ ساعة فى الزبادى المصنع بإستخدام YC fast-1، YC-380 وYC-180 على التوالى. وتميز الزبادى المصنع ببادىء YC-180 بإرتفاع العدد الكلى لكل من *B.bifidum Bb*-12 و*L.casei* 01 مقارنة بأنواع الزبادى الأخرى . ووجد أن كبسلة البكتريا الداعمة للحيوية وإضافة بادىء الزبادى المعرض للصدمة الحرارية وإضافة خلايا غير حية من خميرة*K.Lactis NRRL-Y*-8279 كل كان له تأثير على تحسين حيوية البكتريا الداعمة للحيوية . ومن الناحية الحسية فقد وجد أن الزبادى المصنع بإستخدام البكتريا المكبسلة او الزبادى المصنع بخلايا الخميرة الغير حية قد حصل على أعلى القيم مقارنة بأنواع الزبادى الأخرى. ومن الناحية الميكروبيولوجية فقد وجد أن كل عينات الزبادى الطازجة او المخزنة كانت خالية من بكتريا القولون والخمائر والفطريات.