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IMPROVING VIABILITY OF BIFIDOBACTERIA BY MICROENTRAPMENT AND THEIR EFFECT ON SOME PATHOGENIC BACTERIA IN STIRRED YOGHURT

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Fifteen batches of stirred yoghurt were made to study the effect of microentrapment on the viability of bifidobacteria and their ability to inhibit the growth of *E. coli* and *Staph. aureus*. Entrapped cells of *Bifidobacterium bifidum* and *Bifidobacterium infantis* were able to produce antimicrobial agents which inhibited *E. coli* and *Staph. aureus* used as test organisms. Viable counts of unentrapped bifidobacteria decreased sharply, while entrapped cells of bifidobacteria were quite stable during refrigerated storage of stirred yoghurt. *Bif. infantis* was more tolerant to storage conditions than *Bif. bifidum*. Microentrapment of bifidobacteria improved their survival during storage of stirred yoghurt, especially *Bif. bifidum*, whose viability was not significantly ($P>0.05$) different from entrapped *Bif. infantis*. Viable counts of *E. coli* decreased during storage of stirred yoghurt. Addition of bifidobacteria caused a sharp decrease in the viability of *E. coli*. *E. coli* growth was not dectected at the 5th day, when entrapped cells of bifidobacteria were added to stirred yoghurt, while *E. coli* growth was not detected at the 7th day of storage in yoghurt containing unentrapped bifidobacteria. Addition of *Bif. bifidum* inhibited the growth of *E. coli* more effectively than *Bif. infantis*. *Staph. aureus* showed similar patterns to *E. coli*, except that *Staph. aureus* was more tolerant to storage conditions. The counts of total bacteria, lactobacilli and *Streptococcus salivarius* subsp. *thermophilus* increased up to the third day then declined till the end of storage. Titratable acidity increased gradually during the first 3 days of storage then increased slightly up to the end of storage, while pH values dropped during storage. Adding of bifidobacteria, *E. coli* and *Staph. aureus* did not affect significantly ($P>0.05$) the counts of lactobacilli and *Str. salivarius* subsp. *thermophilus*, acidity and pH value.

Keywords: viability, bifidobacteria, microentrapment, stirred yoghurt, pathogenic bacteria

Yoghurt is the most popular fermented milk produced in Egypt and worldwide. Its consumption in Egypt has almost doubled in the past three years. The value of yoghurt in human nutrition is based, not only on the nutritive effect of the milk from which it is made and increased digestibility, but also on the beneficial effect of intestinal microflora, prophylactic and healing effects (RASIC & KURMANN, 1978;

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MARSHALL, 1984; GURR, 1987; AGERBAEK et al., 1995; SCHAAFSMA, 1996; TVEDE, 1996; YAESHIMA, 1996; BADAWI & EL-SONBATY, 1997 and BUTTRISS, 1997).

The majority of yoghurt brands are made in modern dairy plants but still some brands are made in dairy stores under poor hygienic conditions. Yoghurt is very sensitive to changes in temperature and could be exposed to spoilage. Also yoghurt could be contaminated during processing and handling. Coliform bacteria, *Escherichia coli* and staphylococci have been found in yoghurt collected from retail markets (ABOU-DONIA et al., 1975; SAAD et al., 1987; IBRAHIM et al., 1989 & YOUSEF, 1996).

Bifidobacteria are the predominant gut flora in breast-fed infant (RASIC & KURMANN, 1983). These bacteria are becoming recognized worldwide because of their health and nutritional benefits such as potential beneficial roles in the human intestinal tract (KURMANN & RASIC, 1991; ROBINSON & SAMONA, 1992; VIJAYENDRA & GUPTA, 1992; HOOVER, 1993). Antitumorigenic activity, improvement of lactose-tolerance, reduction of serum cholesterol levels, reduction of ammonia and free serum phenol in patients with liver disease, synthesis of vitamins, increased immunocompetence and antagonistic effects towards enteropathogenic bacteria have all been deviled (POUPARD et al., 1973; ANAND et al., 1984; KAGEYAMA et al., 1984; OKAMURA et al., 1986; UEDA, 1986; YAMAZAKI et al., 1985; HUGHES & HOOVER, 1991; HOOVER, 1993; ISHIBASHI & SHIMAMURA, 1993; DAVIDE, 1995; KEBARY, 1995; SUZUKI, 1995; JIANG et al., 1996; ZOMMARA et al., 1996; BADAWI & EL-SONBATY, 1997; KAILASAPATHY & RYBKA, 1997; ATHRAYILKKALATHIIL & PRASAD, 1997). It is estimated that over 70 products containing bifidobacteria are produced worldwide (HUGHES & HOOVER, 1991; SHAH, 1997). They include fermented milk, butter milk, sour cream, frozen dessert, cheese, baby foods, pharmaceutical preparations and livestock feed supplements (KURMANN & RASIC, 1991; HEKMAT & MCMAHON, 1992; HUNGER & PEITERSEN, 1992; DINAKAR & MISTRY, 1994; GOMES et al., 1995; TAMIME et al., 1995; BLANCHETTE et al., 1996; FORBES et al., 1996; HAYES et al., 1996; KEBARY, 1996; MARTIN, 1996; SEZGING et al., 1996; KEBARY et al., 1998).

It has been speculated that the minimal number of viable cells of bifidobacteria in a product should be more than 10^5 g^{-1} to achieve the therapeutic effects (SAMONA & ROBINSON, 1991; HUNGER & PEITERSEN, 1992; LEE et al., 1996). Survival of bifidobacteria in fermented dairy products depends on the strain, the species, fermentation conditions and storage temperature (MARTIN & CHOU, 1992; LANKAPUTHRA et al., 1996; BLANCHETTE et al., 1996; MARTIN, 1996; SHIN et al., 1996; BADAWI & EL-SONBATY, 1997; MICANEL et al., 1997; DAVE & SHAH, 1997). Even the most resistant strains of bifidobacteria are likely to decrease during storage of fermented milks. Although *Bifidobacterium bifidum* is commonly used by manufacturers, it appears to be less tolerant to pH than *Bifidobacterium infantis*

(MARTIN, 1996). Microencapsulation has been used to protect bifidobacteria in cheese and frozen ice milk (DINAKAR & MISTRY, 1994; KEBARY et al., 1998).

The objectives of this study were to investigate the effect of microentrapment of bifidobacteria on the production of antimicrobial substances, to study the behaviour of different strains of bifidobacteria during storage of stirred yoghurt, to study the effect of microentrapment on the survival of bifidobacteria and to study the effect of adding bifidobacteria to stirred yoghurt on the growth of some pathogenic bacteria.

1. Materials and methods

1.1. Bacterial strains

Streptococcus salivarius subsp. *thermophilus* EMCC1043 and *Lactobacillus delbrueckii* subsp. *bulgaricus* EMCC1102 were obtained from Cairo Mircen (Ain Shams University, Cairo, Egypt). *Bifidobacterium bifidum* DI was provided by Diversitech Inc (Gainesville, FL), while *Bifidobacterium infantis* 4038 was provided by Prof. Morsi El-Soda (Dairy Sci. Department, Faculty of Agriculture, Alexandria University, Egypt). *Escherichia coli* K12 and *Staphylococcus aureus* K12C600 were obtained from the Department of Microbiology, College of Pharmacy, Tanta University, Egypt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were activated by three successive transfers in sterile 10% reconstituted non-fat dry milk. Bifidobacteria strains were activated by three successive transfers in modified lactobacilli MRS broth and incubated under anaerobic conditions (VENTLING & MISTRY, 1993). *E. coli* and *Staph. aureus* were activated by two successive transfers in MacConkey and Staphylococcus Medium 110 broth, respectively.

1.2. Preparation of entrapped bifidobacteria

Five milliliters from each active bifidobacterial strain was inoculated separately into flasks containing 95 ml of modified MRS and incubated for about 18 h at 37 °C under anaerobic conditions. Cells were harvested by centrifugation at 1500×g for 15 min and washed twice with sterile saline solution. Bifidobacterial cells from each strain were suspended individually in sterile saline to about 1.0×10^{10} CFU ml^{-1}. Cells from each strain were microentrapped individually in calcium alginate gels according to the method described by SHEU and MARSHALL (1993). One part of cell suspension was mixed with four parts of 3.0% sodium alginate solution (BDH Chemicals Ltd Poole, England). One part of the alginate-cell mixture was then added dropwise by a syringe to five parts of corn oil containing 0.2% Tween 80 (in a 1000 ml beaker), which was magnetically stirred at 200 r.p.m. A uniform turbid emulsion was obtained within 10 min. Calcium chloride (0.05 mol) was added quickly at the side of the beaker, while it

was magnetically stirred until the water-oil emulsion was broken. Calcium alginate beads were formed within 10 min. The beads were collected by centrifugation at 350×g for 10 min and washed with sterile distilled water. Beads were kept at 5–8 °C in a small volume of sterile distilled water until usage to prevent their collapse and adherence.

1.3. Production of antimicrobial substances

This test was carried out to study the effect of microentrapment on the production of antimicrobial substances. Tubes containing 10.0 ml of sterile modified MRS broth were inoculated with about 1.0×10^6 viable cells of *Bifidobacterium bifidum* or *Bifidobacterium infantis* either entrapped or unentrapped separately. Tubes were incubated anaerobically at 37 °C for 48 h. The fermented cultures were centrifuged at 8000 r.p.m. for 30 min to obtain the cell free broth, which was used to measure the inhibitory activity by the disc assay procedure (PULUSANI et al., 1979). *Staph. aureus* K12C600 and *E. coli* K12 were seeded on Staphylococcus Medium 110 and MacConkey agar C and used as test organisms.

1.4. Manufacture of yoghurt

Fresh buffaloe's milk was obtained from the herd of the Faculty of Agriculture, Shibin El-Kom, Egypt. Yoghurt was made as illustrated in Fig. 1. The amount of entrapped and unentrapped bifidobacteria were calculated to get approximately 1.5×10^7 CFU g^{-1} of stirred yoghurt. Also *E. coli* and *Staph. aureus* were added to levels of about 1.0×10^7 CFU g^{-1} stirred yoghurt. Stirred yoghurt was distributed into 120 ml plastic containers and stored in the refrigerator for 7 days. The experiment was performed in triplicate. All treatments were sampled at zero time and 1st, 3rd, 5th and 7th day of storage for bacteriological, pH and acidity analysis. Sample designations are shown in Fig. 1.

1.5. Bacteriological analysis

Total viable bacterial counts were enumerated on standard plate count agar (MESSER et al., 1985). MRS medium was used to enumerate *L. delbrueckii* subsp. *bulgaricus* (DEMAN et al., 1960). *Str. salivarius* subsp. *thermophilus* was enumerated on yeast-lactose agar (SKINNER & QUESNEL, 1978). *E. coli* and *Staph. aureus* were enumerated on violet red bile agar and Staphylococcus Medium 110 agar, respectively (DIFCO, 1984). Bifidobacterium counts were enumerated on modified MRS agar (VENTLING & MISTRY, 1993) with NPNL solution (neomycin sulfate 0.2%, paromomycin sulfate 0.2%, nalidixic acid 0.03% and lithium chloride 6.0%) (SAMONA & ROBINSON, 1991). Samples containing beads were suspended in 9.0 ml of sterile phosphate buffer (1 mol, pH 7.5) followed by gentle shaking at room temperature for 10 min to release bifidobacteria from beads (SHEU et al., 1993).

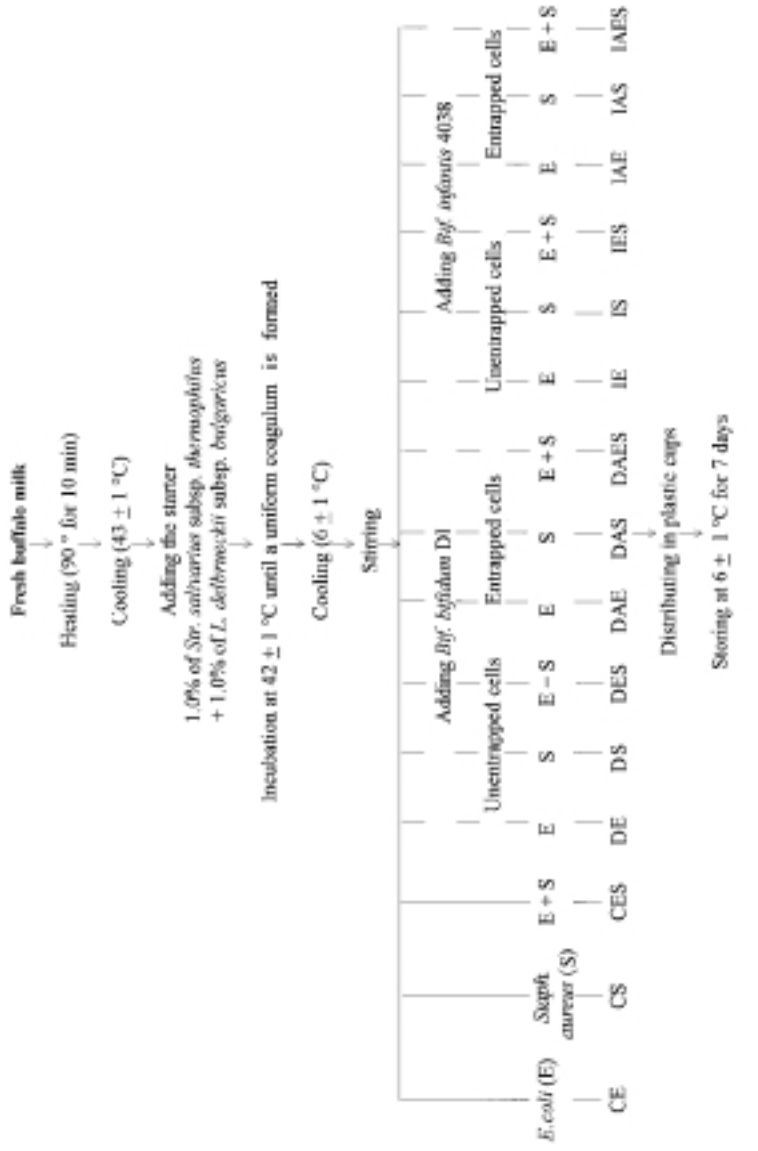

Fig. 1. Manufacturing steps of stirred yoghurt. E: *E. coli*, S: *Staphylococcus aureus*

1.6. Chemical analysis

Titratable acidity and pH value were determined according to LING (1963).

1.7. Statistical analysis

A two factors ×3 replicates factorial design was used to analyze the data for each effect and Duncan's test was used to make the multiple comparisons (STEEL & TORRIE, 1960). Significant differences were determined at $P \leq 0.05$ level.

2. Results and discussion

2.1. Production of antimicrobial agent by bifidobacteria

Inhibitory activity of bifidobacteria against gram-positive and gram-negative-bacteria has been reported (HOOVER, 1993; ISHIBASHI & SHIMAMURA, 1993; GIBSON & WANG, 1994; DAVIDE, 1995; KEBARY, 1995; BADAWI & EL-SONBATI, 1997). Antagonistic effects of *Bif. bifidum* DI and *Bif. infantis* 4038 against *Escherichia coli* K12 and *Staphylococcus aureus* K12C600 are shown in Figs 2 and 3. It is obvious that entrapped cells of *Bif. bifidum* or *Bif. infantis* were able to produce antimicrobial substances and inhibit the growth of *E. coli* or *Staph. aureus* (Figs 2 and 3). Supernatant of *Bif. bifidum* culture was more effective at inhibiting the growth of *E. coli* or *Staph. aureus* than that of *Bif. infantis*, which might be due to the differences in the amount and/or the kind of antimicrobial substances produced by each strain (GIBSON & WANG, 1994; BADAWI & EL-SONBATY, 1997).

2.2. Survival of bifidobacteria

Product should contain levels of bifidobacteria higher than 10^5 CFU ml^{-1} to produce therapeutic benefits. Therefore this work is concerned with improving viability of bifidobacteria during storage of yoghurt. The viability of both *Bif. bifidum* and *Bif. infantis* increased up to the first day of storage then declined during the storage of stirred yoghurt (Fig. 4 and Table 5) (MARTIN & CHOU, 1992; LANKAPUTHRA et al., 1996; KEBARY et al., 1996; MARTIN, 1996; BADAWI & EL-SONBATY, 1997; DAVE & SHAH, 1997; MICANEL et al., 1997). SHAH (1997) reported that the growth of most strains of *Bifidobacterium* is significantly retarded below pH 5.0. Unentrapped *Bif. infantis* showed better survival than unentrapped *Bif. bifidum* during storage of stirred yoghurt (Fig. 4 and Table 5). *Bif. bifidum* appeared to be less tolerant to storage conditions (LANKAPUTHRA et al., 1996; MARTIN, 1996; SHAH, 1997).

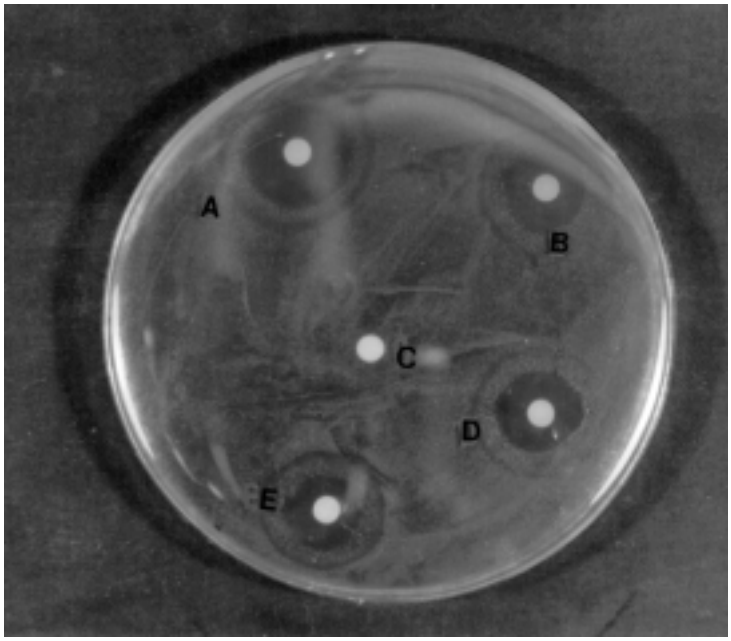

Fig. 2. Photography showing the inhibitory effect of supernatant fluid of bifidobacteria on the growth of *Escherichia coli* K12. A: Supernatant of unentrapped *Bif. bifidum*; E: Supernatant of entrapped *Bif. bifidum*; C: Free disk; B: Supernatant of unentrapped *Bif. infantis*, D: Supernatant of entrapped *Bif. infantis*

These results revealed that tolerance of bifidobacteria to storage conditions of stirred yoghurt is species dependent. The counts of unentrapped cells of *Bif. bifidum* and *Bif. infantis* decreased almost one log cycle, while counts of entrapped cells of both strains were almost stable during refrigerated storage of stirred yoghurt (Fig. 4 and Table 5). These results indicate that microentrapment of *Bif. bifidum* cells increased their viability about one log cycle during storage for only 7 days (Fig. 4 and Table 5). Entrapped cells of *Bif. bifidum* survived well and became more tolerant to storage conditions which might be due to the protection provided by microencapsulation of bifidobacterial cells. Survival of entrapped *Bif. bifidum* and *Bif. infantis* was not significantly different ($P>0.05$) from each other (Fig. 4 and Table 5). The counts of bifidobacteria, even unentrapped cells, after 7 days of refrigerated storage remained higher than the recommended level ($>10^5$ CFU ml^{-1}) that should be present in yoghurt to achieve the therapeutic benefits (Fig. 4).

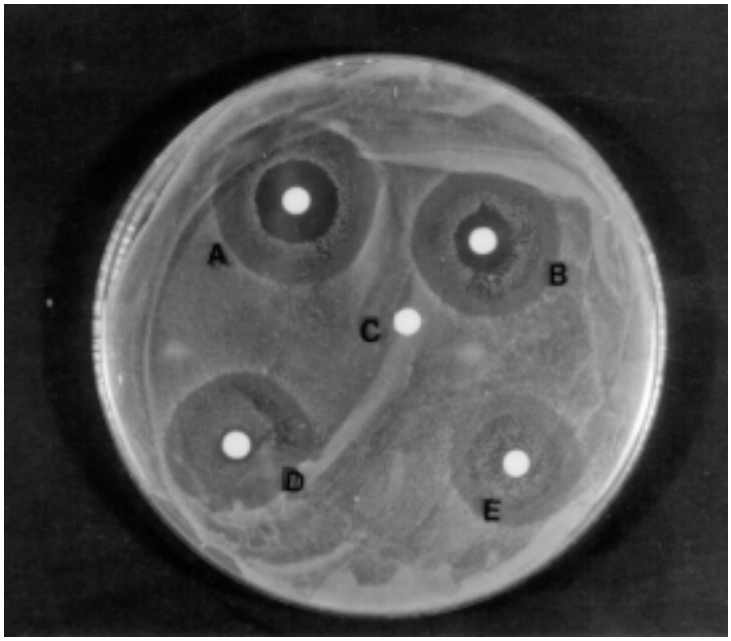

Fig. 3. Photography showing the inhibitory effect of supernatant fluid of bifidobacteria on the growth of *Staphylococcus aureus* K12 C 600. A: Supernatant of unentrapped *Bif. bifidum*; B: Supernatant of entrapped *Bif. bifidum*; D: Supernatant of unentrapped *Bif. infantis*; E: Supernatant of entrapped *Bif. infantis*; C: Free disk

2.3. Fate of pathogenic bacteria

Changes in the counts of *E. coli* during refrigerated storage of stirred yoghurt are presented in Table 1. The cold storage and acidity had an adverse effect on the viability of *E. coli*. Although, the counts of *E. coli* decreased markedly about 5 log cycles during storage in yoghurt without added bifidobacteria, *E. coli* showed faster decay in the presence of bifidobacteria (Tables 1 and 5). This sharp decrease might be due to the production of antimicrobial agents by bifidobacteria (GIBSON & WANG, 1994; KEBARY, 1995; BADAWI & EL-SONBATY, 1997). The viable count of *E. coli* dropped sharply in yoghurt samples containing bifidobacteria and no *E. coli* growth was detected at the 7th day of storage (Tables 1 and 5). *E. coli* growth was not observed in yoghurt samples containing entrapped bifidobacteria at the 5th day of storage (Table 1), which could be attributed to the higher populations of bifidobacteria and subsequently higher production of antimicrobial agents. Samples containing *Bif. bifidum* showed more effective inhibition of the growth of *E. coli* than those containing *Bif. infantis*, which

might be due to the differences in the amount and kind of antimicrobial agents produced. The counts of *Staph. aureus* showed similar patterns to those of *E. coli* except that staphylococci were more tolerant to storage conditions than *E. coli* (Tables 2 and 5). *Staph. aureus* survived until the 7th day of storage in yoghurt samples without bifidobacteria and with *Bif. infantis,* while no *Staph. aureus* growth was detected at the 7th day in yoghurt samples containing *Bif. bifidum* and entrapped cells of *Bif. infantis* (Tables 2 and 5). This means that adding bifidobacteria to yoghurt could inhibit the growth of some pathogenic and undesirable bacteria, which could reach yoghurts by post contamination.

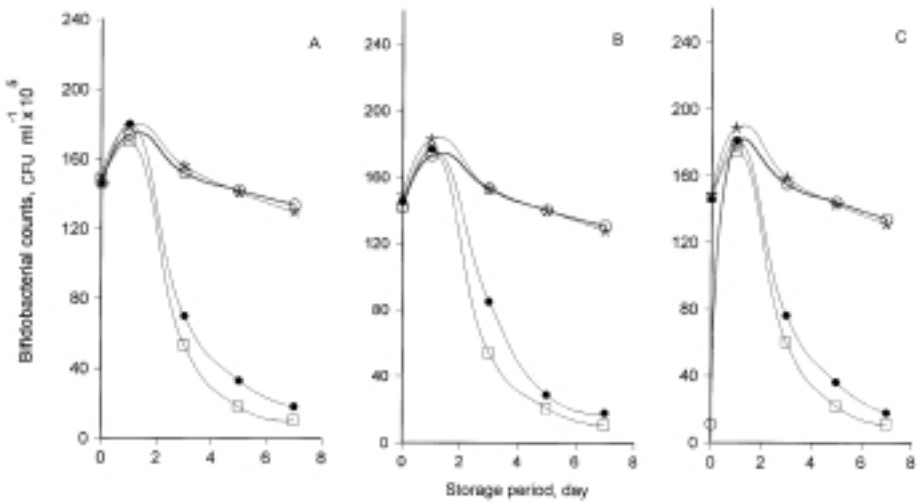

Fig. 4. Survival of bifidobacteria during storage of stirred yoghurt to which bifidobacteria, *E. coli* and *Staph. aureus* were added after manufacturing (each value in the Figure is the mean of three replicates). A: ● IE* ★IAE ⊟ DE ⊖ DAE; B: ● IS* ★IAS ⊟ DS ⊖ DAS; C: ● IES* ★IAES ⊟ DES ⊖ DAES; *See Fig. 1

Table 1

Changes in viable counts (CFU ml^{-1}) of Escherichia coli *during storage of stirred yoghurt to which bifidobacteria,* E. coli *and* Staph. aureus *were added after manufacturing*

Samples	Storage period (days)				
	0	1	3	5	7
CE[a]	96×10^5	31×10^5	73×10^3	28×10^2	17×10
CS	NG	NG	NG	NG	NG
CES	94×10^5	46×10^5	64×10^3	21×10^2	13×10
IE	97×10^5	98×10^3	70×10^2	13×10	NG
IS	NG	NG	NG	NG	NG
IES	96×10^5	86×10^3	63×10^2	9×10	NG
IAE	94×10^5	93×10^3	58×10^2	NG	NG
IAS	NG	NG	NG	NG	NG
IAES	97×10^5	86×10^3	52×10^2	NG	NG
DE	97×10^5	77×10^3	47×10^2	6.3×10	NG
DS	NG	NG	NG	NG	NG
DES	98×10^5	72×10^3	45×10^2	4.5×10	NG
DAE	94×10^5	83×10^3	35×10^2	NG	NG
DAS	NG	NG	NG	NG	NG
DAES	96×10^5	72×10^3	32×10^2	NG	NG

Each value in the table is the mean of 3 replicates.
[a] See Fig. 1.
NG: no growth.

2.4. Behaviour of total bacterial, lactobacilli and streptococci counts

Changes in total bacterial counts are illustrated in Fig. 5. Counts of total bacteria increased up to the third day of storage of all yoghurt samples, which could be due to the residual activity during this period (KEBARY et al., 1996; BADAWI & EL-SONBATY, 1997). Total bacterial counts decreased after the third day to the 7th day by about 40–50% of the maximum survival at the third day of storage (Fig. 5 and Table 5). The decrease in total bacterial counts was more obvious in yoghurt samples containing bifidobacteria (Fig. 5 and Table 5), which might be due to the inhibitory effects of antimicrobial agents produced by bifidobacteria (GIBSON & WANG, 1994; KEBARY, 1995; KEBARY et al., 1996).

Table 2

Changes in viable counts (CFU ml^{-1}) of Staphylococcus aureus *during storage of stirred yoghurt to which bifidobacteria,* E. coli *and* Staphylococcus aureus *were added after manufacturing*

Samples	Storage period (days)				
	0	1	3	5	7
CE[a]	NG	NG	NG	NG	NG
CS	95×10^5	80×10^5	41×10^4	53×10^3	81×10
CES	97×10^5	73×10^5	31×10^4	38×10^3	58×10
IE	NG	NG	NG	NG	NG
IS	93×10^5	90×10^4	28×10^3	37×10^2	8.7×10
IES	94×10^5	75×10^4	16×10^3	22×10^2	4.8×10
IAE	NG	NG	NG	NG	NG
IAS	97×10^5	86×10^4	20×10^3	10.1×10^2	NG
IAES	98×10^5	74×10^4	11×10^3	9.5×10^2	NG
DE	NG	NG	NG	NG	NG
DS	95×10^5	79×10^4	10.8×10^3	9.2×10^2	NG
DES	94×10^5	69×10^4	10.4×10^3	8.5×10^2	NG
DAE	NG	NG	NG	NG	NG
DAS	96×10^5	79×10^4	9.3×10^3	6.0×10^2	NG
DAES	93×10^5	68×10^4	9×10^3	5.7×10^2	NG

Each value in the table is the mean of 3 replicates.
[a] See Fig. 1.
NG: no growth.

Viable counts of lactobacilli and *Streptococcus salivarius* subsp. *thermophilus* (yoghurt bacteria) showed similar patterns of increase or decrease during storage of stirred yoghurt (Figs 6, 7 and Table 5). Counts of yoghurt bacteria increased and reached their maximum survival at the third day of storage then declined up to the end of storage (Figs 6, 7 and Table 5) (KEBARY et al., 1996). Adding bifidobacteria and/or *E. coli* and *Staph. aureus* did not affect significantly ($P>0.05$) the count of lactobacilli and *Str. salivarius* subsp. *thermophilus* (Figs 6, 7 and Table 5).

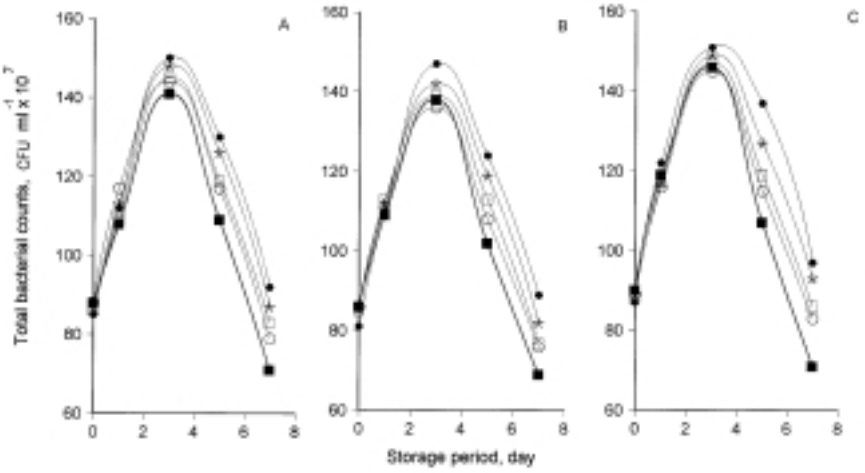

Fig. 5. Total bacterial counts during storage of stirred yoghurt to which bifidobacteria, *E. coli* and *Staph. aureus* were added after manufacturing (each value in the figure is the mean of three replicates). A: ● CE* ★IE ⊟ IAE ○ DE ■ DAE; B: ● CS* ★IS ⊟ IAS ○ DS ■ DAS; C: ● CES* ★IES ⊟ IAES ○ DES ■ DAES; *See Fig. 1

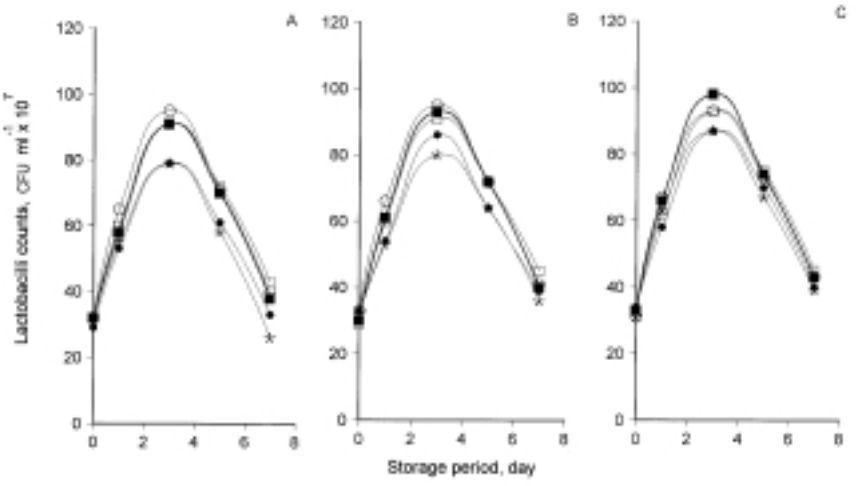

Fig. 6. Lactobacilli counts during storage of stirred yoghurt to which bifidobacteria, *E. coli* and *Staph. aureus* were added after manufacturing (each value in the figure is the mean of three replicates). A: ● CE* ★IE ⊟ IAE ○ DE ■ DAE; B: ● CS* ★IS ⊟ IAS ○ DS ■ DAS; C: ● CES* ★IES ⊟ IAES ○ DES ■ DAES; * See Fig. 1

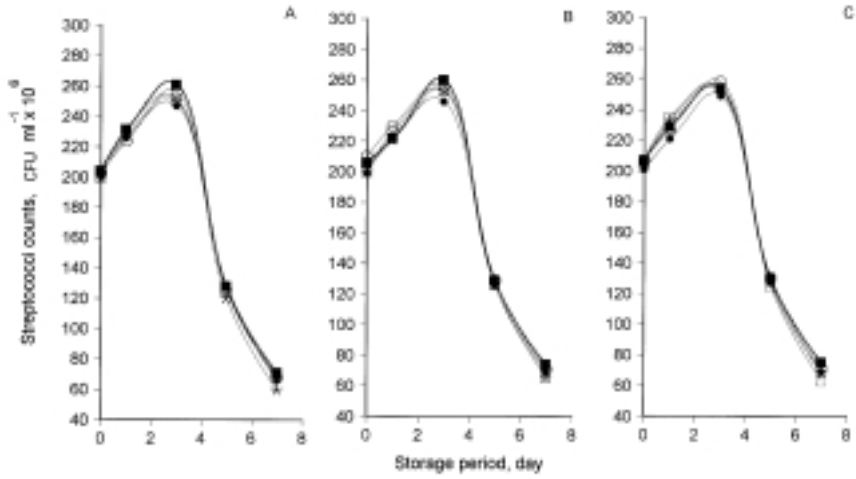

Fig. 7. *Streptococcus salivarius* subsp. *thermophilus* counts during storage of stirred yoghurt to which bifidobacteria, *E. coli* and *Staph. aureus* were added after manufacturing (each value in the figure is the mean of three replicates). A: ● CE* ✶IE ⊟ IAE ⊖ DE ■ DAE; B: ● CS* ✶IS ⊟ IAS ⊖ DS ■ DAS; C: ● CES* ✶IES ⊟ IAES ⊖ DES ■ DAES; * See Fig. 1

Table 3

Changes in titratable acidity (%) during storage of stirred yoghurt to which bifidobacteria, E. coli *and* Staph. aureus *were added after manufacturing*

Samples	Storage period (days)				
	0	1	3	5	7
CE[a]	0.93	1.09	1.14	1.18	1.20
CS	1.00	1.16	1.16	1.19	1.21
CES	1.03	1.12	1.17	1.20	1.24
IE	1.04	1.15	1.17	1.22	1.26
IS	1.02	1.17	1.18	1.21	1.23
IES	1.04	1.15	1.18	1.23	1.26
IAE	1.01	1.13	1.15	1.17	1.23
IAS	0.95	1.12	1.15	1.19	1.24
IAES	1.01	1.12	1.16	1.20	1.24
DE	0.98	1.17	1.19	1.24	1.27
DS	1.05	1.18	1.22	1.26	1.29
DES	0.98	1.17	1.18	1.27	1.31
DAE	0.95	1.16	1.17	1.19	1.21
DAS	0.95	1.16	1.16	1.18	1.20
DAES	0.99	1.18	1.19	1.23	1.26

Each value in the table is the mean of 3 replicates.
[a] See Fig. 1.

2.5. Changes in acidity

Titratable acidity increased gradually during the first 3 days of storage, then increased slightly up to the end of the storage period (Tables 3 and 5). The gradual increase coincides with the increase in yoghurt bacterial counts. Addition of bifidobacteria and pathogenic bacteria did not affect significantly ($P>0.05$) the titratable acidity. Conversely, pH values dropped during the storage period of all yoghurt samples (Tables 4 and 5) (KEBARY et al., 1996, DAVE & SHAH, 1997). Neither bifidobacteria nor pathogenic bacteria affected significantly ($P>0.05$) the pH values of yoghurt (Tables 4 and 5).

Table 4

Changes in pH value during storage of stirred yoghurt to which bifidobacteria, E. coli *and* Staph. aureus *were added after manufacturing*

Samples	Storage period (days)				
	0	1	3	5	7
CE[a]	4.86	4.43	4.13	4.03	3.88
CS	4.83	4.53	4.23	4.00	3.80
CES	4.73	4.33	4.16	3.93	3.73
IE	4.80	4.33	4.06	3.88	3.63
IS	4.80	4.36	4.13	3.96	3.73
IES	4.76	4.33	4.10	3.88	3.60
IAE	4.86	4.46	4.16	4.10	3.88
IAS	4.90	4.43	4.13	4.00	3.73
IAES	4.83	4.43	4.13	3.96	3.73
DE	4.83	4.30	4.03	3.83	3.60
DS	4.76	4.23	4.00	3.80	3.53
DES	4.86	4.30	4.06	3.73	3.43
DAE	4.93	4.43	4.16	4.03	3.76
DAS	4.90	4.40	4.10	4.10	3.83
DAES	4.83	4.35	4.10	4.00	3.73

Each value in the table is the mean of 3 replicates.
[a] See Fig. 1.

Table 5

Statistical analysis of yoghurt properties

Properties of yoghurt	Effect of treatments																Effect of storage period (days)					
	Mean squares	Multiple comparisons[a]															Mean squares	Multiple comparison[a]				
		CE[b]	CS	CES	IE	IS	IES	IAE	IAS	IAES	DE	DS	DES	DAE	DAS	DAES		0	1	3	5	7
Bifidobacterial counts	12305*	NG	NG	NG	C	C	C	A	AB	A	D	D	D	A	A	A	9176*	B	A	C	D	E
E. coli counts	2.299*	A	NG	A	B	NG	B	D	NG	D	C	NG	C	E	NG	E	0.632*	A	B	C	D	E
Staphylococci counts	3.988*	NG	A	A	NG	B	B	NG	D	D	NG	C	C	NG	E	E	0.6719*	A	B	C	D	E
Total bacterial counts	3.388*	A	A	A	B	B	B	B	B	B	B	B	B	B	BC	B	2957*	D	BC	A	B	D
Lactobacilli counts	1076	AB	A	A	ABC	AB	A	A	A	A	A	A	A	A	A	A	18561*	DE	BC	A	B	D
Str. salivarius subsp. *thermophilus*	4.054	A	A	A	AB	A	A	A	A	AB	A	A	A	A	A	A	13687*	C	B	A	D	E
Titratable acidity	0.0103	ABCD	ABCD	ABCD	AB	ABCD	ABC	ABC	ABCD	ABCD	ABC	AB	AB	ABCD	ABCD	ABC	0.430*	E	D	C	AB	A
pH value	0.0936	ABC	AB	ABC	ABC	ABC	ABC	A	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	8.321*	A	B	C	D	E

[a] For each effect, different letter (in the same row) means the multiple comparisons are different from each other, letter A is highest means followed by B, C,... E.

[b] See Fig. 1.

* Significant at 0.05.

NG: No growth.

3. Conclusion

It could be concluded that unentrapped cells of bifidobacteria decreased one log cycle during storage for 7 days, but entrapped cells were stable during storage. *Bif. infantis* was more tolerant to storage conditions than *Bif. bifidum*. Entrapment of bifidobacteria increased their viability by one log cycle. Viable counts of *E. coli* and *Staph. aureus* declined during storage, while in the presence of bifidobacteria the decline was greater. *Bif. bifidum* was more effective for inhibiting the growth of *E. coli* and *Staph. aureus*. Adding bifidobacteria and pathogenic bacteria did not affect the acidity, pH value, the counts of lactobacilli and *Str. salivarius* subsp. *thermophilus*. These results revealed that microentrapment of *Bif. bifidum* and *Bif. infantis* is a promising method to improve the viability of bifidobacteria especially that of *Bif. bifidum*, which became more stable and tolerant to storage conditions of yoghurt. Therefore *Bif. bifidum* will be the most likely choice to use as dietary adjuncts in yoghurt because it is preferred by the manufacturers and, in addition, it has a higher inhibitory activity on some pathogenic bacteria.

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