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Stability of free and encapsulated *Lactobacillus acidophilus* ATCC 4356 in yogurt and in an artificial human gastric digestion system

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ABSTRACT

The objective of this study was to determine the effect of encapsulation on survival of probiotic *Lactobacillus acidophilus* ATCC 4356 (ATCC 4356) in yogurt and during artificial gastric digestion. Strain ATCC 4356 was added to yogurt either encapsulated in calcium alginate or in free form (unencapsulated) at levels of 8.26 and 9.47 log cfu/g, respectively, and the influence of alginate capsules (1.5 to 2.5 mm) on the sensorial characteristics of yogurts was investigated. The ATCC 4356 strain was introduced into an artificial gastric solution consisting of 0.08 *N* HCl (pH 1.5) containing 0.2% NaCl or into artificial bile juice consisting of 1.2% bile salts in de Man, Rogosa, and Sharpe broth to determine the stability of the probiotic bacteria. When incubated for 2 h in artificial gastric juice, the free ATCC 4356 did not survive (reduction of >7 log cfu/g). We observed, however, greater survival of encapsulated ATCC 4356, with a reduction of only 3 log cfu/g. Incubation in artificial bile juice (6 h) did not significantly affect the viability of free or encapsulated ATCC 4356. Moreover, statistically significant reductions (~1 log cfu/g) of both free and encapsulated ATCC 4356 were observed during 4-wk refrigerated storage of yogurts. The addition of probiotic cultures in free or alginate-encapsulated form did not significantly affect appearance/color or flavor/odor of the yogurts. However, significant deficiencies were found in body/texture of yogurts containing encapsulated ATCC 4356. We concluded that incorporation of free and encapsulated probiotic bacteria did not substantially change the overall sensory properties of yogurts, and encapsulation in alginate using the extrusion method greatly enhanced the survival of probiotic bacteria against an artificial human gastric digestive system.

Key words: probiotic, encapsulation, gastric, yogurt

INTRODUCTION

The ability of probiotic bacteria to provide various health benefits (Krasaekoopt et al., 2003; Singh et al., 2011) depends on their ability to survive passage through the gastrointestinal tract (Champagne et al., 2005; Stanton et al., 2005) in sufficient numbers (10^8; Lourens-Hattingh and Viljoen, 2001). According to Antoine (2011), probiotic delivery in food faces dual challenges of surviving refrigerated storage (~4°C), followed by transition to mouth temperature (~25°C), and then to body temperature (~37°C) in the gastrointestinal tract, followed by the chemical challenge as the bacteria enters the hyper-acidic (fasting pH of around 1 to 2) environment of the stomach, and finally by rapid neutralization to pH 7.5 and the detergent bile salts in the duodenum.

In order for probiotics to provide a metabolic function in the gut of host, they must be delivered to the human gut (Mattila-Sandholm et al., 2002; Krasaekoopt et al., 2003; Champagne et al., 2005; Stanton et al., 2005). Losses of 6 to 8 log cfu/g of probiotic bacteria using artificial gastric digestion have been reported (Hansen et al., 2002; Muthukumarasamy et al., 2006; Sabikhi et al., 2010; Brinques and Ayub, 2011), which implies that the recommended 10^7 cfu/g of bacteria in a probiotic food system throughout shelf life (Ouwehand and Salminen, 1998) may not be sufficient to exert health beneficial effects. Although it is well known that the food matrix itself protects bacteria during gastric passage, Sharp et al. (2008) have shown that this may not be the case if no buffering occurs. In other words, yogurt alone is not a good protector of probiotic bacteria in artificial gastric conditions when the pH maintained at pH 2.3. Providing increased protection to probiotics during gastric passage positively influences their health-promoting effects.

Encapsulation as a technology for improving the survival of probiotics in in vitro gastric solutions and during storage has been studied in several dairy foods, including yogurt (Sultana et al., 2000; Krasaekoopt et al., 2006), Turkish kasar and white cheeses (Ozer et al., 2008, 2009), and ice cream (Homayouni et al., 2008). For example, Krasaekoopt et al. (2006) reported a loss

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of approximately 1 log of encapsulated *Lactobacillus acidophilus* compared with a 2-log loss of free bacteria over 4 wk of refrigerated storage of yogurts. Moreover, Kailasapathy (2006) reported losses of 4 and 2 log of free and encapsulated *Lb. acidophilus* DD910, respectively, over 6-wk refrigerated storage of yogurts.

Survival in gastric conditions has also been studied, and increased survival via encapsulation varies depending on capsule size and gastric juice conditions. Better survival occurs with larger capsules and higher pH of the gastric juice. Kim et al. (2008) observed 3-log reductions in the numbers of *Lb. acidophilus* ATCC 43121 encapsulated in alginate via an extrusion method and no survival of free ATCC 43121 in artificial gastric juice (**AGJ**; pH 1.2 and 1.5). Mandal et al. (2006) found that encapsulated *Lb. casei* NCDC-298 had better survival (2 log) than free NCDC-298 during a 3-h incubation in AGJ (pH 1.5). Likewise, Sabikhi et al. (2010) reported that encapsulated (using alginate and starch) cells of *Lb. acidophilus* LA1 survived the artificial gastric conditions (pH 1.5 for 2 h) better than the free form of the same strain. They observed 2- and 4.5-log losses of encapsulated and free LA1 during 2 h incubation at pH 1.5, respectively. Ding and Shah (2009) reported better survival of alginate-encapsulated *Lb. acidophilus* after incubation at pH 2 for 2 h. They observed that free *Lb. acidophilus* cells had a ~7-log reduction (10.7 to 3.8) compared with alginate-encapsulated *Lb. acidophilus* cells, which had only a 3.5-log reduction after 2 h of incubation in high acid conditions.

Although larger capsules provide better protection (Muthukumarasamy et al., 2006), they are more likely to influence the textural properties of the food. However, we have not found any reports in the literature on the effect of large calcium alginate capsules (>1 mm) containing probiotic bacteria on the sensorial characteristics and acceptability of a smooth-textured product such as yogurt. To fill this gap, we manufactured probiotic yogurt with *Lactobacillus acidophilus* ATCC 4356 encapsulated in large Ca-alginate capsules to provide gastric protection and then evaluated sensory properties and acceptability of the yogurt. Survivability was measured periodically during 4 wk of refrigerated storage of yogurt and after incubation in artificial gastric and bile juices. Following this, sensory evaluations, using trained panelists, were performed on yogurt, which was evaluated for appearance and color, body and texture, flavor, odor, and overall liking.

MATERIALS AND METHODS

Materials

Lactobacillus acidophilus ATCC 4356 probiotic strain was obtained from Microbiologics Inc. (St. Cloud, MN), and thermophilic lyophilized yogurt cultures were obtained from Chr. Hansen (Hørsholm, Denmark). Sodium alginate and bile bovine were purchased from Sigma-Aldrich Co. (St. Louis, MO); de Man, Rogosa, and Sharpe (**MRS**) broth and agar were from Oxoid Inc. (Basingstoke, UK); peptone was from EMD Chemicals Inc. (Gibbstown, NJ); and $CaCl_2 \cdot H_2O$, HCl, NaCl, NaH_2PO_4, and Na_2HPO_4 were of analytical reagent grade. Pasteurized and homogenized milk was obtained from Ataturk University's Dairy Processing Plant (Erzurum, Turkey), and skim milk powder (Pinar Dairy Products Inc., Izmir, Turkey) was purchased from a local store.

Bacterial Growth Conditions

Lyophilized cultures of ATCC 4356 were prepared by sequential transfer twice into MRS broth cultures were then incubated anaerobically at 37°C for 18 h. Following overnight incubation, the media-containing cells were centrifuged at 3,900 × *g* for 10 min at 4°C, after which the supernatant was removed and the cells further washed twice (3,900 × *g* for 10 min at 4°C) in sterile 0.1% (wt/vol) peptone water. The washed cells were suspended to approximately 10^{10} cfu/mL in peptone water by comparing optical density to a previously prepared standard curve ($R^2 \geq 0.9$; data not shown).

Encapsulation

Bacterial cells were encapsulated in alginate by the modified extrusion method of Muthukumarasamy et al. (2006). Briefly, 10 g of cell suspension (~10^{10} cfu/mL) was mixed with 50 g of a 4% sterile sodium alginate solution with continuous gentle stirring to immobilize the bacteria. The alginate–culture mixture was then added using a sterile glass syringe with regular-bevel 21-gauge needle, into 250 mL of 0.2 *M* of $CaCl_2$ with stirring at 300 rpm. The syringe needle was held 10 cm above the calcium solution and the alginate–culture mixture added dropwise to maintain a constant droplet size, as described by Krasaekoopt et al. (2003). The sodium alginate within the droplets was cross-linked to form calcium-alginate beads as they entered the calcium solution and were retained in the calcium solution for 30 min to gain rigidity. The solidified beads were then collected by filtration through Whatman #4 filter paper and washed with sterile water (Ellenton, 1998).

Capsule Attributes

Fifty capsules were randomly collected and their diameter measured by visual observation against a millimeter-scale ruler. The shape of the capsules was

observed visually, photographed using a Sony DSC-W370 14 MP digital camera (Sony Electronics Inc., San Diego, CA), and the image enhanced using Adobe Photoshop Elements 7 (Adobe Inc., San Jose, CA) to improve capsule appearance.

Yogurt Manufacture

Pasteurized and homogenized bovine milk containing 2% fat was fortified with skim milk powder to 15% TS content and heated to 43°C. Warmed milk was inoculated with thermophilic yogurt cultures according to the yogurt manufacturer's directions, and 9 g of inoculated milk was poured into each of 20 (50-mL) sterile plastic tubes. All samples were incubated at 43°C until the pH reached 4.6 (~3.5 h). Yogurt samples were kept at 4°C for 12 h; free bacteria were added to 10 of the samples and alginate-encapsulated bacteria were added to the remaining 10 samples. All yogurt samples were mixed thoroughly and stored at 4°C for 28 d. Then, ATCC 4356 were enumerated at 0, 7, 14, 21, and 28 d to observe the relative survival rates of the free versus encapsulated bacteria.

Bacterial Enumeration

Bacteria were enumerated from yogurt containing free ATCC 4356 after mixing 10 g of yogurt with 90 mL of 0.1% peptone water in a stomacher for 10 min. For yogurt containing encapsulated ATCC 4356, 0.2 *M* phosphate buffer (pH 7) was used instead of peptone water to disrupt the alginate gel and release the encapsulated bacteria. Strain ATCC 4356 was selectively enumerated on MRS agar containing 0.2% bile (Lima et al., 2009). Bacterial enumerations were performed after 0, 7, 14, 21, and 28 d of refrigerated storage of yogurt samples.

pH Analysis

The pH of all yogurt samples was measured by using a digital pH meter (pH meter 211, Hanna Inc., Ann Arbor, MI) by directly submerging the probe into the yogurt samples. The pH meter was calibrated using reference pH 4.0 and pH 7.0 buffer solutions.

Artificial Gastric Digestive System

To investigate the influence of pH on survival of probiotic bacteria, sterile-filtered AGJ based on Sun and Griffiths (2000) containing 0.2% NaCl was prepared using 0.08 *N* HCl at a final pH of 1.5. Before adding 1 g of Ca-alginate capsules or free ATCC 4356, 9 g of AGJ was tempered to 37°C, and the mixture was held at 37°C for up to 2 h with periodic shaking. The samples were taken at 0, 30, 60, and 120 min during incubation in AGJ and spread-plated on MRS agar followed by anaerobic incubation at 37°C for 48 h. The experiments were performed in duplicate and the results were presented in mean number of surviving bacteria ± standard deviation.

Artificial Bile Juice

Artificial bile juice (**ABJ**; Klaenhammer and Kleeman, 1981; Song et al., 2003) was prepared by adding 1.2% bile into MRS broth prewarmed to the 37°C. One gram of capsule was added to 9 g of ABJ and then incubated for 6 h at 37°C with periodic shaking. The samples were taken at 0 and 360 min of incubation in ABJs and spread-plated on MRS agar in which anaerobic incubation at 37°C for 48 h was performed. The experiments were performed in duplicate and the results were presented in mean number of surviving bacteria ± standard deviation.

Sensorial Evaluation

Yogurt was manufactured as described above in 1-kg batches with free ATCC 4356 added to one batch, and encapsulated ATCC 4356 added to the second batch; the control batch contained only the yogurt starter cultures. The yogurts were mixed thoroughly and ten 100-g yogurt samples were dispensed from each batch into 10 plastic cups. Three types of yogurt samples were evaluated by 10 well-trained panelists using a hedonic-type scale from 1 to 9, where 1 = least desirable and 9 = most desirable. Panelists evaluated the 3 types of yogurts in terms of appearance and color, body and texture, flavor, odor, and overall liking. A score of 5 was considered as the limit of acceptability or unacceptability. The experiments were performed in duplicate and the results were presented as mean scores ± standard deviation.

Statistical Analysis

For comparisons, the percentage survival in each treatment, instead of real numbers, was used to determine the change in the survival of *Lb. acidophilus* ATCC 4356 over time (Adhikari et al., 2003). Percentage survival of bacteria during storage and as a consequence of AGJ or ABJ incubation were analyzed using ANOVA, and differences between means were evaluated by the Duncan multiple comparison test using SPSS 13.0.0.246 for Windows (SPSS Inc., Chicago, IL) as a 2-way factorial, with encapsulation as the treatment effect with 2 replicates. The sensorial evaluation

Table 1. Effect of encapsulation on the viability of *Lactobacillus acidophilus* ATCC 4356 and pH in stirred-type yogurts over a period of 28 d at 4°C (means ± SD)

		Encapsulated			Free		
Day	No.	Actual count[1]	pH	Survival (%)[2]	Actual count	pH	Survival (%)
0	4	8.26 ± 0.014	4.54 ± 0.014	100^{a,x}	9.47 ± 0.021	4.54 ± 0.007	100^{a,x}
7	4	7.83 ± 0.127	4.51 ± 0.049	94.79^{b,x}	8.68 ± 0.014	4.59 ± 0.098	91.66^{b,x}
14	4	7.53 ± 0.091	4.37 ± 0.028	91.16^{c,x}	8.48 ± 0.148	4.43 ± 0.007	89.55^{b,x}
21	4	7.42 ± 0.176	4.38 ± 0.014	89.83^{c,x}	8.71 ± 0.120	4.30 ± 0.070	91.97^{b,x}
28	4	7.16 ± 0.071	4.38 ± 0.007	86.68^{c,x}	8.56 ± 0.042	4.37 ± 0.007	90.39^{b,x}
Significance		**	**	**	**	**	**

$^{a–c}$Means within a same column with the same letters are not significantly different at $^{**}P < 0.01$ by Duncan multiple comparisons.

^{x}Means within same row with same letters are not significantly different ($P > 0.05$).

[1]Log_{10} cfu/g of yogurt.

[2]Calculated by dividing the final viable population (cfu/g) by initial viable population (cfu/g) of the test organism.

data and pH changes in yogurts were analyzed using ANOVA and differences between means were evaluated by the Duncan multiple comparison test using SPSS 13.0.0.246 for Windows. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

pH Alterations of Yogurts During Refrigerated Storage

The pH changes in the yogurts containing free and encapsulated probiotic bacteria during storage at 4°C for 4 wk are shown in Table 1. Both yogurt samples had similar pH fluctuations through the 28 d of refrigerated storage. The pH of yogurts was 4.54 and 4.54 during the first day of storage and decreased to 4.37 and 4.38 at the end of the storage period for yogurts with free and encapsulated bacteria, respectively. These results were similar to those found by Krasaekoopt et al. (2006). They reported that the initial percentage acidity of yogurts containing free and encapsulated *Lb. acidophilus* was equal (at d 0), and the percentage acidity of 2 types of yogurts was the same at the end of 4 wk of storage. Kailasapathy (2006) reported less post-acidification when encapsulated probiotic bacteria were added to yogurt.

Survival of ATCC 4356 During 28 Days of Refrigerated Storage

The reductions in the numbers of free and encapsulated ATCC 4356 in yogurts after 28 d of refrigerated storage are shown in Table 1. No differences were observed in the numbers of probiotic bacteria incorporated in yogurts before or after yogurt fermentation (Krasaekoopt et al., 2006). Therefore, we preferred to add the free and encapsulated ATCC 4356 cells into the yogurts after fermentation to ensure homogeneous mixing of the capsules in yogurt and to avoid sedimentation of capsules during fermentation.

Initial numbers of probiotic ATCC 4356 in yogurts were 9.47 and 8.26 log cfu/g and decreased to 8.56 and 7.16 log cfu/g at the end of the refrigerated storage for yogurts containing free and encapsulated bacteria, respectively. The reduction (~1 log cfu/g) in the numbers of free and encapsulated ATCC 4356 over 4 wk was similar and statistically significant ($P < 0.01$). This 1-log reduction was probably due to the bactericidal activity of lactic and acetic acids produced by the yogurt starters and β-galactosidase activity during storage of yogurts (post-acidification). Similar results were reported by Sultana et al. (2000), who observed reductions of 0.75 and 0.57 log cfu/g in the numbers of free and encapsulated *Lb. acidophilus* 2401 during 4 wk of refrigerated storage, respectively. In contrast, Krasaekoopt et al. (2006) and Kailasapathy (2006) reported that encapsulation increased the viability of *Lb. acidophilus* during 4 wk of refrigerated storage of stirred-type yogurts. In our experiment, however, the reductions in the numbers of free and encapsulated probiotic *Lb. acidophilus* ATCC 4356 did not exceed 1 log over 28 d refrigerated storage of yogurts and, as mentioned above, we obtained relatively better survival of both free and encapsulated probiotic ATCC 4356 than did Krasaekoopt et al. (2006) and Kailasapathy (2006). The greater survival in the present study was probably due to the use of larger capsules, use of different strains, or occurrence of different post-acidifications in yogurts during refrigerated storage.

Survival of ATCC 4356 in Artificial Gastric and Bile Juices

When ATCC 4356 was incubated in 0.08 *N* HCl at a pH of 1.5 (AGJ), the numbers of free ATCC 4356

decreased from 10^9 to 6×10^2 cfu/g after a 30-min exposure of the bacteria to the AGJ ($P < 0.01$). We observed only a 0.25-log reduction (from 1.8×10^8 to 8×10^7 cfu/g; $P > 0.05$) in the numbers of encapsulated ATCC 4356 after a 30-min exposure to AGJ (Table 2). Free ATCC 4356 did not survive (detectable level was 10^2 cfu/g) after a 60-min incubation in AGJ. Nonetheless, encapsulated ATCC 4356 survived at a significantly higher rate (77% after a 60-min incubation in AGJ; $P < 0.05$). The viability rate of encapsulated ATCC 4356 after a 2-h incubation in AGJ was 61.54% ($\sim 10^5$), which is close to the therapeutic minimum (10^6) for probiotics.

The environment inside the capsules most likely protected the Ca-alginate encapsulated cells from high acidic stress factors via restriction of acid diffusion into the beads. Encapsulation clearly exerts a protective effect on *Lb. acidophilus* ATCC 4356 cells in AGJ. According to Sun and Griffiths (2000), this protection is due to the formation of a pH gradient in beads, which depends on bead size and the exposure time of the beads in AGJ. Therefore, when placed into AGJ, free cells were exposed to the extremely low pH immediately; whereas beads were subjected to a different pH, which provides protection to the bacteria against the hyper-acidic conditions in the capsules. In this study, about 10^5 cfu/g of ATCC 4356 cells would be alive when 2×10^8 cfu/g of cells in beads were incubated in AGJ (pH 1.5) for 120 min.

It generally takes about 90 min to empty half the human stomach, which has a pH of 1 to 3 (Giannella et al., 1972). Therefore, it is beneficial to bacteria if they have enough protection to survive the 90-min transit through the harsh acidic environment of the stomach. Encapsulation limits the exposure of bacteria to gastric juice by slowing the rate of diffusion of gastric juice into the cells. The rate of diffusion is determined by capsule size (Sun and Griffiths, 2000; Muthukumarasamy et al., 2006) and the concentration of alginate used to encapsulate the bacteria (Mandal et al., 2006). Larger capsules (Figure 1) obtained by the extrusion method (1.5 to 2.5 mm) provide greater protection because the distance between the gastric acid and the cell is increased, diffusion time is therefore increased, and consequently, bacteria survival rate is increased.

In addition to above-mentioned deceleration of diffusion mechanisms, the other reason for the higher survival rates in the encapsulated cells in our study may be that in larger (~2-mm) capsules the bacteria in the outer regions (which are more exposed to the acid) die off, while the bacteria in the inner regions are protected. This would also explain having only a ~3-log reduction in encapsulated ATCC 4356 compared with no survival (>7-log loss) of free bacteria.

The results of present study overlap with those reported by Krasaekoopt et al. (2004), who encapsulated *Lb. acidophilus* in large alginate capsules at an average diameter of 1.62 mm. They also observed greater survival of encapsulated bacteria with only a 3.2-log reduction (1.6×10^9 to 8.5×10^5) compared with a 5.9-log reduction in free bacteria (2×10^9 to 2.3×10^3). Muthukumarasamy et al. (2006) also observed a good protective effect of large alginate capsules with an average diameter of 2.37 mm. They reported that larger (2 to 4 mm) extruded capsules protected the *Lb. casei* cells better than the smaller (20 μm to 1 mm) emulsified capsules simply by increasing the distance between encapsulated cells and the surrounding acid. Lee and

Table 2. Mean counts (means ± SD) and percentage (%) survival (n = 2) of encapsulated and free *Lactobacillus acidophilus* ATCC 4356 during incubation in artificial gastric juice (AGJ) or artificial bile juice (ABJ)

Incubation time (min)	No.	Encapsulated		Free	
		Actual count[1]	Survival (%)[2]	Actual count	Survival (%)
AGJ					
0	4	8.255 ± 0.021	100[a,x]	9.015 ± 0.007	100[a,x]
30	4	8.04 ± 0.042	97.4[a,x]	2.775 ± 0.007	30.78[b,y]
60	4	6.385 ± 0.12	77.35[b,x]	ND[3]	ND
120	4	5.08 ± 0.552	61.54[c,x]	ND	ND
Significance		**	**	**	**
ABJ					
0	4	8.255 ± 0.021	100[a,x]	9.08 ± 0.057	100[a,x]
360	4	8.24 ± 0.014	99.82[a,x]	8.99 ± 0.042	99[a,x]
Significance		NS	NS	NS	NS

[a–c]Means within a same column with same letters are not significantly different at **($P < 0.05$).

[x,y]Means within same row with same letters are not significantly different ($P < 0.05$).

[1]Log_{10} cfu/g of capsule.

[2]Calculated by dividing the final viable population (cfu/g) by initial viable population (cfu/g) of the test organism inoculated in AGJ and ABJ.

[3]Not detected (detectable level was 10^2 cfu/g).

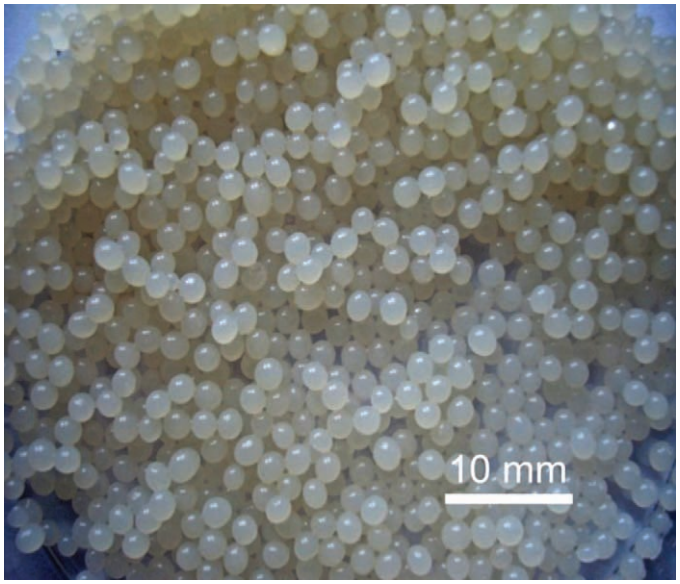

Figure 1. Calcium alginate capsules containing probiotic *Lactobacillus acidophilus* ATCC 4356 encapsulated by the extrusion method. Color version available in the online PDF.

Heo (2000) reported that when bifidobacteria encapsulated in calcium alginate capsules were subjected to AGJ (pH 1.55) and a bile salt solution, the death rate of cells in the beads decreased proportionally with an increase in alginate gel concentration and in bead size.

When the encapsulated and free ATCC 4356 were incubated in ABJ for 360 min, no reductions occurred in the numbers of both encapsulated and free ATCC 4356 cells ($P > 0.05$; Table 2). It is known that probiotic ATCC 4356 has bile tolerance. Similar results were reported by Kim et al. (2008), who observed no loss of free and encapsulated *Lb. acidophilus* ATCC 43121 after a 6-h incubation in artificial intestinal juice (**AIJ**). Nonetheless, they reported a significant protective effect of encapsulation on the viability of *Lb. acidophilus* ATCC 43121 after 24 h. Free *Lb. acidophilus* ATCC 43121 had a 1-log loss but encapsulated bacteria did not have a significant loss after a 24-h incubation in AIJ at 37°C. Brinques and Ayub (2011) also reported that viability of free and encapsulated *Lb. plantarum* BL011 was not affected by AIJ, and noted that BL011 has a natural resistance to bile, similar to that of ATCC 4356.

As mentioned above, most of the probiotic bacteria survive or even recover in the intestinal tract because once the encapsulated bacteria leave the stomach and enter the neutral pH environment of the intestines, the capsules are neutralized by intestinal juice, the capsules are softened, and might be broken by the peristalsis of the small intestine, resulting in release of the probiotic bacteria (Sun and Griffiths, 2000). As Antoine (2011) observed, this neutralization and release allow the probiotics to metabolize normally and resume growth. Although the luminal detergent bile salts still interfere with the cell membrane of dividing bacteria, the glucose-rich digesta in the gut lumen will provide a more readily available source of energy than lactose. Therefore, it will not be a challenge for probiotic bacteria to shift from an acid dairy matrix to a warm nutrient-rich gut lumen.

When probiotic bacteria are consumed with foods, the food matrix itself provides some protection. However, Sharp et al. (2008) have shown that when yogurts containing probiotic bacteria were added into an AGJ in which the pH was constant (no buffering), the survivability of bacteria was $<10^1$ cfu/g after a 30-min exposure to this acidic environment. Therefore, yogurt itself may provide little protection to the viability of ingested probiotic bacteria. As a result, consumption of yogurts containing larger capsules might increase the survival rate of the probiotic bacteria in the gastrointestinal tract compared with consumption as a pure liquid culture. Thus, consumers would more benefit from the therapeutic effects of these health-promoting microbes in encapsulated forms.

Sensorial Evaluation

Statistical analysis showed that the sensorial scores of 3 types of yogurts differed only for body and texture ($P < 0.05$); none of the other sensorial characteristics were statistically significant ($P > 0.05$). Yogurts that contained encapsulated ATCC 4356 had the lowest overall liking score (5.65 out of 9), and the control yogurts (no additional probiotic bacteria) had the highest overall liking score (6.9 out of 9); a score of 5 was considered the limit of acceptability (Table 3).

The body/texture of yogurts containing encapsulated ATCC 4356 was below the acceptable level (4.7 out of 9) and body/texture was considered a defect in the encapsulated type of yogurts by the panelists. Control yogurts and those containing free ATCC 4356 had acceptable scores of 6.6 and 5.6, respectively, in terms of body/texture. Panelists complained about the coarser texture of the yogurts that contained encapsulated ATCC 4356. Similar results were reported by Kailasapathy (2006), who observed a significant change in the textural properties, particularly smoothness, of yogurts containing microencapsulated probiotic bacteria. In our study, the capsules were approximately 2 mm in diameter and were thus visible to the panelists, which is an undesirable quality parameter for yogurt. Utilization of exopolysaccharide-producing probiotic cultures

Table 3. Sensory evaluation scores (mean ± SD) of yogurts[1]

Treatment[2]	No.	Appearance and color	Body and texture	Flavor	Odor	Overall liking
Yogurt + EnLa	10	6.0 ± 1.33[a]	4.7 ± 1.57[b]	5.8 ± 1.47[a]	6.8 ± 0.92[a]	5.65 ± 1.56[a]
Yogurt + FLa	10	6.5 ± 1.43[a]	5.6 ± 1.51[ab]	6.1 ± 2.08[a]	6.5 ± 0.85[a]	6.2 ± 1.47[a]
Control	10	7.2 ± 0.79[a]	6.6 ± 0.97[a]	7 ± 1.15[a]	6.4 ± 1.65[a]	6.9 ± 0.99[a]
Significance		NS	*	NS	NS	NS

[a,b]Mean values within a column with the same letters are not significantly different from each other at $P < 0.05$ (*) by Duncan multiple comparison test.

[1]Average of 2 replications, with each replicate analyzed in duplicate. Sensory attributes were scored on a 1 to 9 scale, where 9 = most desirable, 5 = acceptable, 1 = least desirable.

[2]EnLa = encapsulated *Lactobacillus acidophilus* ATCC 4356; FLa = free (nonencapsulated) *Lb. acidophilus* ATCC 4356; Control = yogurts that contained no additional probiotic bacteria (starter cultures only).

or addition of polysaccharides to tighten the yogurt gel might improve the body and textural attributes of yogurts containing larger capsules.

CONCLUSIONS

During 4-wk refrigerated storage of yogurts containing *Lb. acidophilus* ATCC 4356, we observed a 1-log reduction in free and encapsulated ATCC 4356. When free ATCC 4356 was incubated for 2 h in HCl-NaCl solution (pH 1.5) to simulate gastric digestion, we observed no survival (>7-log reduction) of the probiotic strain. Encapsulating the probiotic strain in 2-mm alginate capsules increased its survivability by >4 logs, with only a 3-log reduction occurring. Incubation of the probiotic in ABJ resulted in no decrease in ATCC 4356 counts. Overall liking scores did not differ for the control yogurt (no additional probiotic) and yogurts containing free ATCC 4356 or encapsulated ATCC 4356, but the yogurt with encapsulated bacteria had the lowest score for texture and had a noticeably coarser texture. Further research is needed to determine an optimal capsule size that will provide adequate protection during gastric digestion without adversely affecting textural attributes (smoothness) of yogurt.

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