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Preparation of *Bifidobacterium breve* encapsulated in low methoxyl pectin beads and its effects on yogurt quality

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ABSTRACT

Yogurt is a popular product worldwide partly because of the health-promoting effects of the probiotics that it contains. Probiotics with high survivability constitute a promising direction for fortified yogurt products. This study aimed to prepare *Bifidobacterium breve*–loaded yogurt with the bacteria surviving transit to the lower part of small intestine or colon. *Bifidobacterium breve* beads were prepared through an ion-crosslinking method using low methoxyl pectin as the encapsulating material. Features such as encapsulation efficiency and stability during storage and passage through the simulated gastrointestinal tract were studied in vitro. A commercial starter was used for yogurt fermentation, and *B. breve* with or without encapsulation was added as a probiotic supplement with the starter or 3 to 4 h after fermentation. The effects of *B. breve* beads on yogurt characteristics were evaluated after different fermentation processes: BC, milk fermented with marketed yogurt starter; UBFF, unencapsulated *B. breve* added to fresh milk and then fermented; EBFF, encapsulated *B. breve* added to fresh milk and then fermented; UBAF, unencapsulated *B. breve* added after fermentation with the starter; and EBAF, encapsulated *B. breve* beads added 3 to 4 h after fermentation with the starter. Evaluation was based on texture, electronic nose, and electronic tongue analyses. The particle size analysis of *B. breve* beads showed that they were uniform, mostly spherical, 1 to 1.5 mm in diameter with encapsulating efficiency higher than 99%. Following treatment with the simulated gastrointestinal tract conditions, the number of *B. breve* decreased by 1.76 and 4.82 log cfu/g for *B. breve* beads and unencapsulated *B. breve*, respectively. The EBAF group showed the lowest viscosity (2,235.67 cP) at d 0, and the lower postfermentation degree was reflected by the slow increase in yogurt viscosity. All groups kept a relatively stable pH during storage. The cohesiveness values of the EBAF and UBAF groups were significantly higher than those of the other groups. The trends in texture changes within the BC, UBFF, and EBFF groups were similar, and the UBAF and EBAF groups showed similar trends. In conclusion, *B. breve* beads showed good stability in vitro and improved yogurt characteristics by increasing the survival rate of the encapsulated cells. Good compatibility of low methoxyl pectin beads with yogurt was also observed.

Key words: *Bifidobacterium breve*, low methoxyl pectin, beads, yogurt fermentation

INTRODUCTION

Functional foods containing probiotics have undergone rapid growth in the field of fortified foods (Champagne et al., 2018). The term probiotic can only be used for products that deliver a suitable amount of well-defined, living microorganisms that benefit the well-being of the host. Live cultures include probiotic and non-probiotic organisms. The former includes probiotic drugs, probiotic medical foods, probiotic foods, and non-oral probiotics, among others, whereas nonprobiotic cultures include fermented foods with an undefined microbial content (Hill et al., 2014). These active microorganisms are present in the host gut and have beneficial effects, such as maintaining the intestinal microbial balance, inhibiting pathogen invasion, and reducing intestinal permeability. Based on the important physiological functions of probiotics, probiotics-based products are becoming increasing common (Ranadheera et al., 2010; Aryana and Olson, 2017; Sarao and Arora, 2017; Champagne et al., 2018). Probiotics that reach the gastrointestinal (**GI**) tract may be helpful for the production of short-chain fatty acids (Sarao and Arora, 2017),

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but to have health-promoting effects, the concentration of viable cells should be at least 10^7 cfu/mL (Chavarri et al., 2010). Attaining this number can be difficult due to conditions during production, transportation, and preservation processes and exposure to gastric juice and bile during transit through the GI tract. Therefore, effective measures are needed to increase the vitality of probiotics, and encapsulation of the bacteria shows great promise in this regard. Probiotics can be encapsulated in materials such as polysaccharides, proteins, and lipids, among others, for protection against the above-mentioned harsh conditions. Pectin, a member of the polysaccharide family, exists widely in natural foods and is commonly used as a food additive. Low methoxyl pectin (**LMP**) can be cross-linked with Ca^{2+} to form microparticles or nanoparticles, and it is widely used as a carrier to deliver drugs to the colon (Bourgeois et al., 2006; Nguyen et al., 2014; Ghibaudo et al., 2018). In the current study, we prepared LMP beads to encapsulate *B. breve*. We then evaluated the performance of the beads in terms of encapsulation efficiency and performance in simulated GI and storage stability tests.

Yogurt is a good vehicle for carrying probiotics to the human intestinal tract, not only because it is a popular food, but also it typically has a high concentration of probiotics (Yang and Sheu, 2012; Champagne et al., 2018). Over the past 100 years, yogurt has undergone improvements and new developments, and it is the leading cultured dairy product in the United States (Aryana and Olson, 2017). Several strategies have been used to improve the functions of yogurt with probiotics (Champagne et al., 2018); for example, yogurt supplemented with *Lactobacillus paracasei* N1115 can be used to protect elderly people against respiratory infections (Pu et al., 2017).

During fermentation, the yogurt pH decreases, which is detrimental to many microorganisms. In addition, when consumed, the yogurt passes through the GI tract, where extreme pH and bile salt may destroy probiotics (Champagne et al., 2018). Therefore, in this study, *B. breve* beads were first prepared using LMP to protect the bacteria. We then evaluated the effects of *B. breve* beads on yogurt features, including pH, acidity, and viscosity during storage. We also used electronic nose and tongue equipment to analyze odor and flavor characteristics of the yogurt.

MATERIALS AND METHODS

Materials

Bifidobacterium breve (strain no. CICC 6182) was purchased from the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China); LMP was purchased from Anhui Yu Ning Biotechnology Co. Ltd. (Suzhou, China); anhydrous calcium chloride was purchased from Zhejiang Juhua Xinlian Chemical Co. Ltd. (Quzhou, China); and anaerobic tank AG035 and gas production anaerobic bag C3500 were purchased from Mitsubishi (Tokyo, Japan).

Strain Activation and Culture

Bifidobacterium breve powder was dispensed into 0.5 mL of sterilized TPY liquid medium. The bacterial dispersion was transferred onto TPY solid culture medium and cultured anaerobically for 48 h at 37°C. A single colony was then transferred to de Man, Rogosa, and Sharpe (MRS) liquid culture medium supplemented with L-cysteine monohydrochloride (5 mg/100 mL) and incubated at 37°C for 24 h anaerobically.

Preparation of B. breve Beads

The 100-mL bulk cultures of *B. breve* were centrifuged at 1,684 × *g* for 15 min, and the pellets were then resuspended in 5 mL of sterile saline. The LMP beads were prepared as previously reported (Aydin and Akbuga, 1996; Sandoval-Castilla et al., 2010; Oehme et al., 2011; Sandolo et al., 2011; Nguyen et al., 2014; Ghibaudo et al., 2018). In brief, 5 mL of 2% LMP solution (wt/vol) was prepared by dissolving 0.1 g of LMP into 4.9 mL of distilled H_2O. The solution was then mixed with the concentrated bacterial suspension, using a vortex mixer. The mixture was transferred into a beaker containing 300 mL of a $CaCl_2$ solution (300 mmol/L) using a syringe with a no. 7 needle, with stirring on a magnetic stirrer. The prepared beads were filtered through gauze and thoroughly washed with sterile water. Finally, the cleaned beads were mixed well with cryoprotectant and lyophilized.

Encapsulation Efficiency Determination

The encapsulation efficiency was determined as previously described (Sandoval-Castilla et al., 2010; Vaziri et al., 2018), with some modifications. Briefly, the collected bulk cultures of *B. breve* were subjected to serial dilution and enumerated by the plate colony count, which was recorded as the primary number of bacteria (A). Wet beads (0.1 g) as prepared above were treated with 30 mL of EDTA (50 mmol/L, pH 8.0) under agitation for complete disintegration, and the cell pellets were collected by centrifugation at 1,684 × *g* for 15 min and washed twice with sterilized saline. The pellets were then diluted for plate counting, and the number of cells (B) was calculated. The encapsulation efficiency

(EE) was calculated according to Equation [1]: EE (%) = $(B/A) \times 100\%$.

Simulation of GI Conditions

The in vitro simulated GI tests were conducted as previously reported (Tomaro-Duchesneau et al., 2012), with some modifications. In brief, 1 mL of *B. breve* suspension (containing 10.45 log cfu *B. breve*) or 0.1 g of *B. breve* beads (containing 10.38 log cfu *B. breve*) was subjected to treatment with 30 mL of simulated gastric fluid (**SGF**, 0.03 *M* NaCl solution containing 1.6 g of pepsin in 500 mL of water, pH 2.0), with shaking at 180 rpm for 2 h. Afterward, the pellets were collected by centrifugation at 1,684 × *g* for 15 min, and the collected cells were divided. One half was used for plate counting as above, while the other half was placed in 30 mL of simulated bile juice (**SBF**), which was prepared by adding 1 g of porcine bile extract to 100 mL of 0.2 *M* phosphate buffer solution (pH 7.4), with shaking at 180 rpm for 20 min. The mixture was centrifuged for 15 min at 1,684 × *g* after SBJ treatment, and viable cells were counted. The pellets were then placed in 30 mL of simulated intestinal fluid (**SIF**), which was prepared by dissolving 2 g of pancreatin in 400 mL of 0.2 *M* PBS (pH 7.4). After 180 rpm shaking for 2 h, the viable cells were counted.

Stability Test

Vacuum freeze-dried *B. breve* beads were placed at −20°C, 4°C, and ambient temperature (20 ± 2°C) conditions, respectively, for 13 wk. Each week, 0.1 g of beads under each temperature condition were removed and placed in 30 mL of EDTA solution (50 mmol/L, pH 8.0) and stirred at 180 rpm at 37°C until completely disintegrated. The resultant solution was then serially diluted and plated for counting. The freeze-dried unencapsulated *B. breve* powder treated under the same conditions was used as control. The cell survival rate was calculated as the percentage of colonies after a certain period of storage based on the number before storage.

Yogurt Fermentation

Fresh milk (Yili, Hohhot, Inner Mongolia, China) was purchased from a local supermarket. The yogurt was produced as previously described (Adhikari et al., 2000; Yang and Sheu, 2012; Caleja et al., 2016). Briefly, the fresh milk was heated at 85°C for 15 min. Food-grade $CaCl_2$ was added to the milk at 0.04% (wt/wt), and 0.5% corn starch (wt/wt) was dissolved in hot water and added to the milk. After being mixed, the solution was cooled to 37 to 45°C. Next, a commercial yogurt starter was activated and added to the milk at a ratio of 1 g of starter to 500 mL of milk. Then, the milk was poured into autoclaved bottles and fermented at 42 to 45°C for 6 to 8 h.

Five types of yogurt were produced: blank control with milk fermented with commercial yogurt starter (**BC**); unencapsulated *B. breve* added along with the yogurt starter to fresh milk and then fermented (**UBFF**); encapsulated *B. breve* added together with the yogurt starter to fresh milk and then fermented (**EBFF**); unencapsulated *B. breve* added 3 to 4 h after yogurt fermentation with the commercial starter (**UBAF**); and encapsulated *B. breve* beads added 3 to 4 h after yogurt fermentation with the commercial starter (**EBAF**).

The *B. breve* with or without encapsulation was added to the milk at a concentration of 7.5 log cfu/g milk at the same time as the starter or 3 to 4 h after fermentation. Figure 1 shows the flowchart for preparation of yogurt containing the *B. breve* beads. All yogurts were stored at 4°C, and characteristics were tested at 0, 1, 3, 5, and 7 d after fermentation. The pH of the samples was measured using a pH meter, and pictures were taken of all products.

Yogurt Characterization

Acidity. The acidity of the yogurt was determined by acid-base titration. Ten milliliters of yogurt was transferred into 150-mL Erlenmeyer flasks, and then mixed with 20 mL of distilled water containing 2 drops of phenolphthalein indicator. The mixture was then titrated with sodium hydroxide standard solution, and the volume of the titration solution consumed (V) was recorded. The acidity values of the sample are expressed in degrees Theurer (°T) as follows: °T = $V \times 10$.

Viscosity. The viscosity of the samples was determined at 20°C using a Brookfield DV-II Viscometer (Ametek Brookfield, Middleborough, MA) with no. 64 rotor at 1.0 rpm and 20 to 80% torque.

Texture Determination. Yogurts were analyzed for hardness and cohesiveness using a TA-XT Express physical property tester with P/36R probe (Stable Micro Systems, Surrey, UK) as previously reported (Curti et al., 2017). Textural properties were analyzed by performing 2 sequential compression tests with a cylindrical-shaped probe (25-mm diameter) separated by a rest phase of 30 s. Each sample was pressed up to 70% of its original length. The test distance was 15.0 mm. The probe speeds before, during, and after test were 5.0, 2.5, and 2.0 mm/s, respectively. The measure-

ment time was 5.00 s, and the induction force was 5.0 × *g*.

Bifidobacterium breve Activity. The number of original bacteria in yogurt was determined by plate counting as done previously. The simulated GI test for all the samples during 7 d storage was conducted as above.

Sample Discrimination. After fermentation, the yogurt samples were placed at 4°C for 3 d and then underwent electronic nose and electronic tongue analysis.

Electronic Nose Analysis. The electronic nose analysis followed a previously reported procedure (Zhao et al., 2018). Before the experiment, the prepared yogurt samples were pipetted into 50-mL clean glass bottles with disposable 10-mL syringes and left to equilibrate for 30 min at 25°C to develop headspace for detection. Conditions for electronic nose (iNose system, Isenso, New York, NY) use were as follows: sample preparation time for 5 s, detection time for 60 s, measurement count for 1 s, automatic zero adjustment time for 10 s, cleaning time for 240 s, internal flow at 400 mL/min, and injection flow rate at 400 mL/min. Each sample was measured once, and the average value of triplicate samples was used in principal component analysis and discriminant function analysis.

Electronic Tongue Analysis. Thirty-milliliter samples from each group were mixed with 60 mL of sterile water and centrifuged at 3,790 × *g* for 10 min at 20°C. The supernatant was vacuum filtered, and the filtrate was directly poured into a special container for the electronic tongue analysis (25 mL per sample). The SmarTongue system (Isenso) was used in this experiment, and the primary parts of the system included a sensor array, signal excitation acquisition system, and multivariate mathematical statistics system. The detection probe was composed of a specific multichannel cross-sensitive sensor array. The working electrodes included a gold electrode, palladium electrode, silver electrode, platinum electrode, titanium electrode, and tungsten electrode. The auxiliary electrode was a platinum electrode with a diameter of 2 mm, and a silver/silver chloride electrode was used as the reference electrode. Distilled water was used as the cleaning solvent. The sampling time was 180 s, once per second (n = 5). Finally, 3 stable test data were selected for principal component analysis and discriminant function analysis.

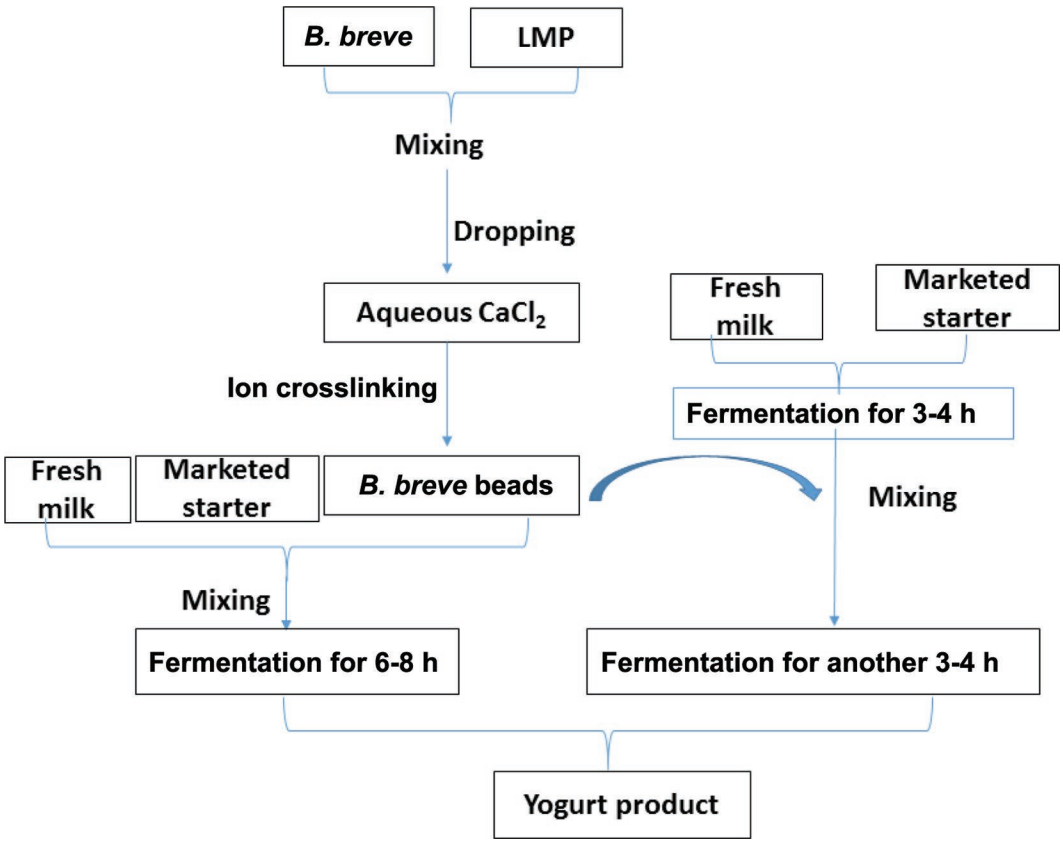

Figure 1. Schematic diagram of the preparation of yogurt containing *Bifidobacterium breve* beads. LMP = low methoxyl pectin.

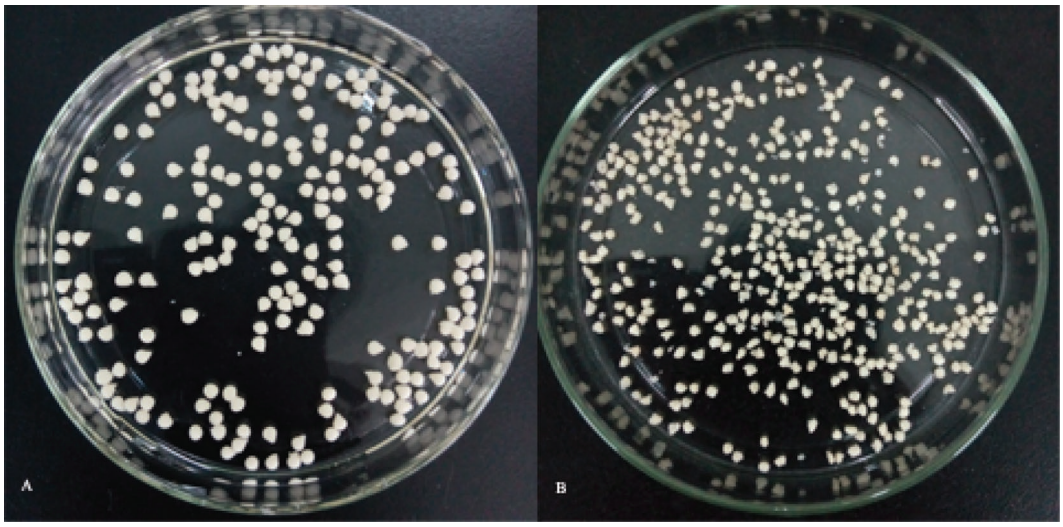

Figure 2. Morphology of *Bifidobacterium breve* beads before (A) and after (B) freeze drying.

Statistical Analysis

At least 3 samples were used for each analysis. The Origin 8.0 software(OriginLab, Northampton, MA) was used for data analysis, and the data are expressed as mean ± standard deviation (SD). The statistical significance was analyzed by 1-way ANOVA at $P < 0.05$ level.

RESULTS AND DISCUSSION

Probiotics and their derived products show great promise in improving human health (Saldanha, 2008; Champagne, 2012; Vieira da Silva et al., 2016; Champagne et al., 2018). A pivotal question is how to ensure the survival of probiotics under different environmental conditions, such as temperature, water content, and transport in vitro and exposure to gastric acid and bile juice in vivo (Champagne, 2012; Cook et al., 2012; Khosravi Zanjani et al., 2014; Sarao and Arora, 2017). Another challenge involves how to protect the activity of probiotics in popular foods, such as yogurt and other fermented products (Ranadheera et al., 2012; Yang and Sheu, 2012; Pu et al., 2017). To address these issues, we developed a process for loading *B. breve* into pectin beads and then characterized the in vitro stability. We also studied the use of these beads in yogurt fermentation.

Characterization of the Pectin Beads Loaded with B. breve

Figure 2 shows that the beads prepared in this study were white, uniform in particle size distribution (1–1.5 mm in diameter), spherical, and smooth, with good fluidity. No cross-linking among particles was present after lyophilization, and the encapsulation efficiency was higher than 99%. This excellent encapsulation efficiency was significantly higher than reported previously (Rodklongtan et al., 2014).

The rationale for using LMP to encapsulate *B. breve* in this study was based on several points. First, it is plant derived, with no toxicity (Vincent and Williams, 2009), and it has been widely used as a delivery vector for colon-targeted medications (Liu et al., 2006; Bigucci et al., 2008; Das et al., 2011; Jung et al., 2013). In addition, the beads can be easily prepared by a simple ion-crosslinking process (Liu et al., 2006; de Souza et al., 2009; Jung et al., 2013; Ribeiro et al., 2014; Belscak-Cvitanovic et al., 2015). These points collectively indicated that LMP could also be used as carrier for probiotics delivery.

Simulated Gastrointestinal Test

As shown in Table 1, unencapsulated *B. breve* showed a decrease of 4.82 log cfu/g after treatment with simulated gastric juice, bile, and intestinal juice, while the *B. breve* in beads decreased by only 1.76 log cfu/g after the same treatment. The gastric juice showed the largest destructive effects for unencapsulated *B. breve*, with more than 4 log cfu loss after treatment in SGF for 2 h, while less than 1 log cfu was lost when the bacteria were encapsulated in LMP beads. This experiment revealed that *B. breve* counts declined rapidly in the gastric environment, similar to other probiotics (Rodklongtan et al., 2014), and LMP beads could protect *B. breve* effectively.

The stability of probiotics during storage and in vivo digestion is very important because the number of viable cells reaching the colon determines whether these probiotics are able to exert a healthful effect. The effect arises not only because of the enrichment of various flora in the colon, but also because of the interactive effects of intestinal flora and health also occur here. For most probiotics, such as *Lactobacillus* and especially *Bifidobacteria*, several factors may restrict their use, including susceptibility to oxygen, gastric juice, and bile salt. Therefore, protective measures must be taken, of which microencapsulation has shown great promise (Pedroso et al., 2013; Rosas-Flores et al., 2013; Solanki et al., 2013; Rodklongtan et al., 2014; Li et al., 2016). In this study, 8.62 log cfu/g *B. breve* survived after SGF, SBF, and SIF treatments, which is sufficient to enable health-promoting effects.

Storage Stability

As shown in Figure 3, the number of viable cells in the beads decreased by 0.94, 1.42, and 3.29 log cfu/g after storage for 13 wk at −20 and 4°C and ambient temperature (20 ± 2°C), respectively. Meanwhile, the unencapsulated cells decreased by 1.46, 2.10, and 4.11 log cfu/g, respectively at −20 and 4°C and ambient temperature (20 ± 2°C) for 13 wk. The results showed that encapsulation of probiotics could significantly increase the survival rate of the cells, and the storage effect of the probiotics was the best at −20°C. Under different temperatures, a high number of cells were alive in beads than among those unencapsulated cells ($P < 0.05$).

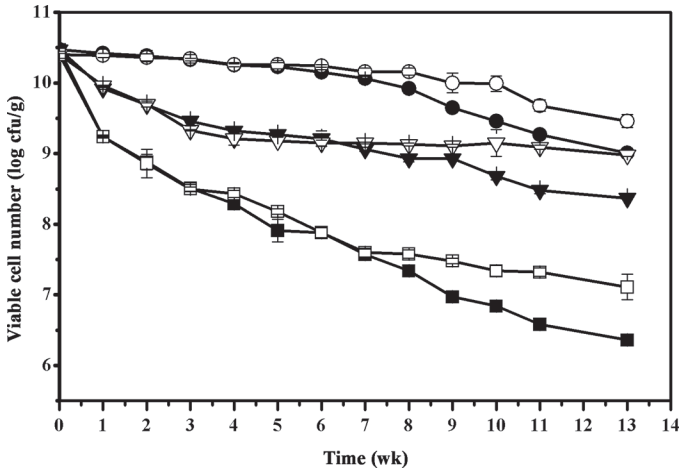

Figure 3. Storage stability of *Bifidobacterium* breve beads (○ −20°C; △ 4°C; □ ambient temperature) versus unencapsulated *B. breve* (● −20°C; ▲ 4°C; ■ ambient temperature) over 3 mo of storage at different temperatures. The data are expressed as mean ± SD (n = 6).

Table 1. Results of simulated gastrointestinal tract test (log cfu/g)[1]

Group	Unencapsulated *Bifidobacterium breve*	*B. breve* beads
Primary colonies	10.45 ± 0.01	10.38 ± 0.02
Colonies after SGF treatment	6.17 ± 0.07	9.45 ± 0.06
Colonies after SBF treatment	5.74 ± 0.03	8.62 ± 0.07
Colonies after SIF treatment	5.63 ± 0.07	8.62 ± 0.06

[1]Three batches of beads were prepared independently, and the results are expressed as mean ± SD. SBF = simulated bile juice; SGF = simulated gastric fluid; SIF = simulated intestinal fluid.

Yogurt Evaluation

Viscosity. The viscosity of yogurt influences both the taste and stability of the product (Rivera and Matheus, 2009; Janiaski et al., 2016). Figure 4 shows the viscosity changes of each group during 7-d storage. At d 0, the EBAF group showed the lowest viscosity (2,235.67 cP), while the viscosity of both the UBFF and EBFF groups was higher than that of the BC group, suggesting that *B. breve* was involved in the yogurt fermentation when added together with the commercial starter. The higher viscosity of the EBFF group could be attributable to the agglomeration effects of pectin with milk protein during fermentation. At the end of the experiment, the viscosity of the UBAF group was lowest, followed by that of the EBAF group. The slower increase of the yogurt viscosity in the EBAF group revealed the lower degree post fermentation.

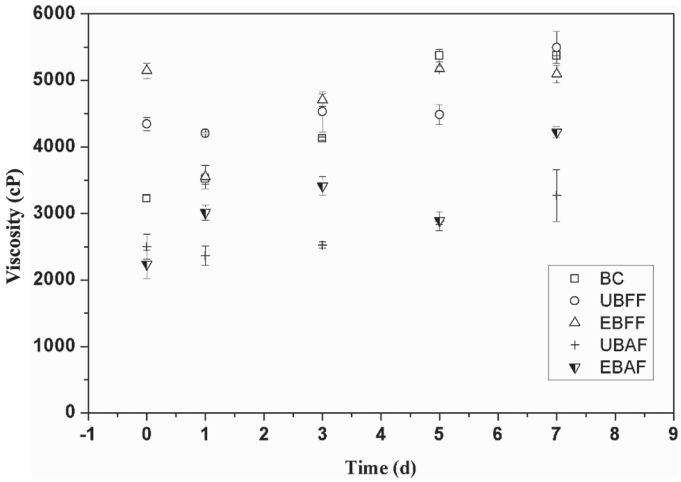

Figure 4. The yogurt viscosity of each group was measured in triplicate, and the values are expressed as mean ± SD. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter.

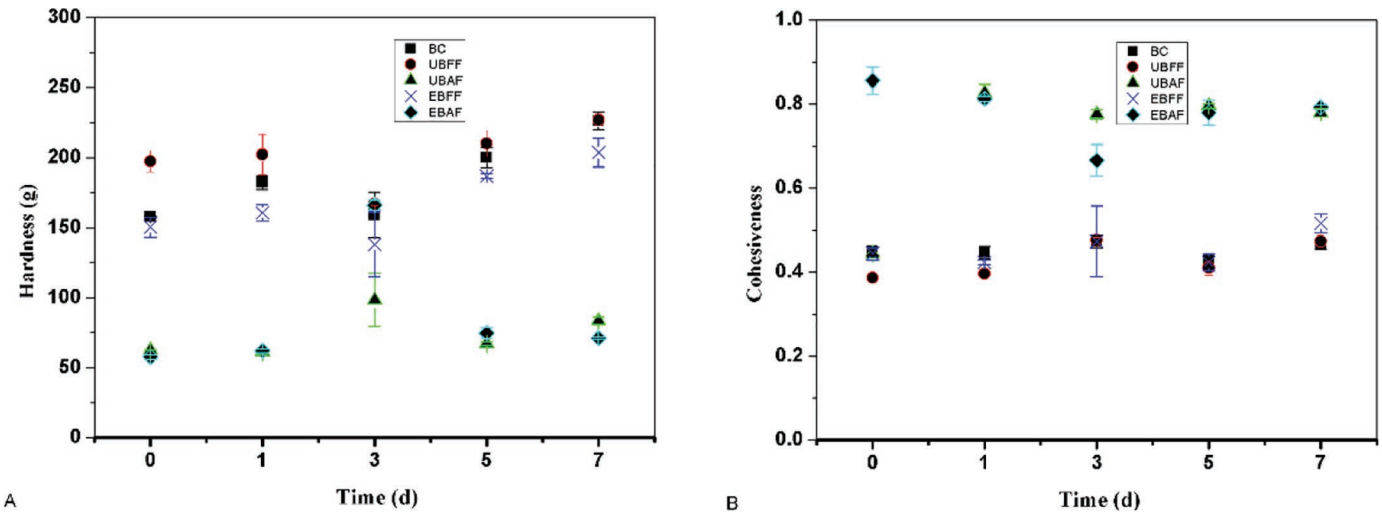

Figure 5. Hardness and cohesiveness of yogurts. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter. Yogurt samples from each group were measured in triplicate, and the values are expressed as mean ± SD.

Hardness and cohesiveness are important in evaluating the yogurt texture (Mudgil et al., 2017), and they were determined by texture analysis for the different treatment groups after storage for 0, 1, 3, 5, and 7 d (Figure 5). Hardness is regarded as the force required to attain a certain deformation, and it serves as a measure of yogurt firmness (Mudgil et al., 2017). The hardness of the BC, UBFF, and EBFF groups were significantly higher than those of the UBAF and EBAF groups ($P < 0.01$). The hardness of the UBAF and EBAF groups was most stable within 7 d (Figure 5A). Our results show that the trends of texture changes from BC, UBFF, and EBFF groups were similar, while the UBAF and EBAF groups showed similar trends. The values of the UBFF group were closest to those of the BC group. The texture index values of the UBAF group and the EBAF group were significantly different from those of the BC group. The unencapsulated bacteria and the *B. breve* beads added after fermentation had a significant effect on the texture of the yogurt, while the changes of the different indexes during the storage period were relatively stable within a certain range. Figure 5B shows that the cohesiveness values of the EBAF and UBAF groups were significantly higher than those of the other groups ($P < 0.01$). The cohesiveness of the EBAF group showed a downward trend in the first 3 d and gradually increased thereafter. The overall changes in other groups were not obvious. Yogurt cohesiveness is regarded as the level to which the tested yogurt can be deformed before it ruptures, and it thus serves as a measure of the strength of internal bonds. It is related to consumer acceptability of yogurt and is an important aspect of the yogurt texture (Mudgil et al., 2017). Therefore, both the EBAF and UBAF groups showed better eating quality compared with the other groups. The other 3 groups had no significant differences in cohesiveness.

pH. The pH value is an important indicator for evaluating the yogurt quality and represents the amount of free H^+ in the yogurt. As shown in Figure 6, during storage of yogurt for 7 d at 4°C, the pH values of all groups remained steady.

Acidity. Yogurt pH determines the amount of ionized H^+ in the yogurt, while the acidity measures the total acidity of the yogurt, including the number of ionized H^+ and non-ionized H^+. The degree of non-ionized H^+ in the yogurt may cause the acidity and the pH measurement results to differ. As shown in Figure 7, the acidity of the BC group did not change much during the 7-d storage and stayed the lowest among all groups, while the acidity of EBAF remained relatively higher but the difference was not significant. The acidity of yogurt may affect the yogurt viscosity because under acidic conditions, casein molecules form tiny subcolloidal molecular groups. When the acidity of yogurt reaches a certain value, the subcolloidal molecules are connected as colloidal molecules by the action of calcium phosphate, and the colloidal molecules aggregate again to form a homogeneous network of hydrated proteins, reaching a peak viscosity.

***Bifidobacterium breve* Viability.** The *B. breve* viability was determined after the bacteria were subjected to the simulated GI test. At d 0, the survival rate of the *B. breve* was 32.17, 35.85, 35.01, 76.37, and 77.63%

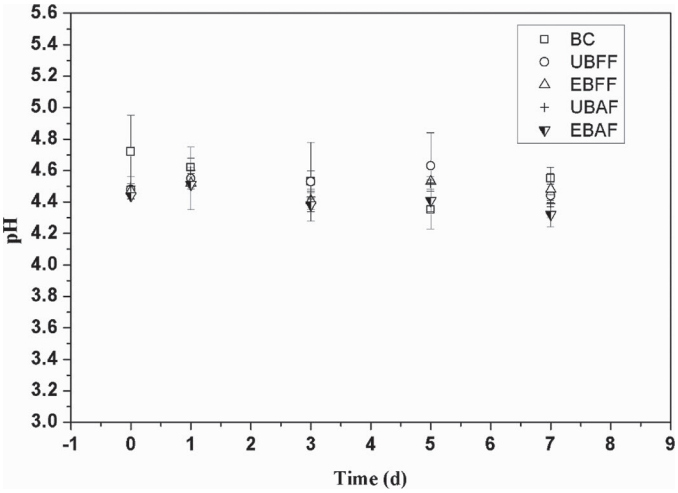

Figure 6. pH values of yogurt. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter. Yogurt samples from each group were measured in triplicate, and the values are expressed as mean ± SD.

for BC, UBFF, UBAF, EBFF, and EBAF groups, respectively. After storage for 1 d, the survival rates of *B. breve* were 33.96, 39.34, 37.28, 79.94, and 78.07% for BC, UBFF, UBAF, EBFF, and EBAF groups,

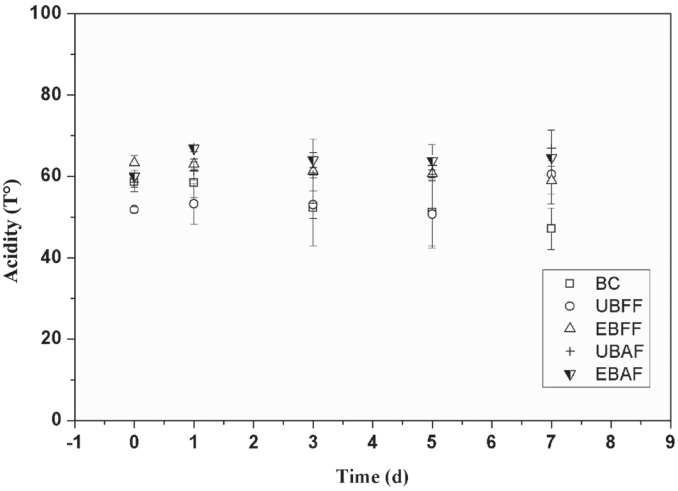

Figure 7. Determination of yogurt acidity (degrees Theurer, °T). BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter. Yogurt samples from each group were measured in triplicate, and the values are expressed as mean ± SD.

respectively (Table 2). At the end of the d 7, the *B. breve* survival rates were 29.72, 32.03, 32.39, 67.04, and 74.75% for BC, UBFF, UBAF, EBFF, and EBAF groups, respectively. The encapsulation of *B. breve* into LMP beads protected the cells from damage during storage and during passage through the simulated GI tract. The microencapsulation process increased the *B. breve* number, which was in agreement with other studies (Adhikari et al., 2000; Allgeyer et al., 2010; Jones et al., 2012).

Figure 8 shows the images of different yogurt product. The products did not differ in appearance and seemed to be homogeneous (see also Supplemental Video; https://doi.org/10.3168/jds.2018-15597). The homogeneous appearance of the products suggests that the LMP beads are compatible with yogurt.

Discrimination of Different Yogurt by Electronic Nose and Electronic Tongue. Linear discriminant analysis (**LDA**) is a statistical method for discriminating the sample type, in which the new components or items of unknown category are discriminated and classified into known categories by establishing a certain function (a linear combination of independent variables versus a discriminant function) based on the nature of the things of the known category (independent variables). Linear discriminant analysis has the advantages of a good classification effect and ease of use, and consequently, it is one of the commonly used pattern recognition methods for electronic nose and electronic tongue analysis. Figure 9A shows the LDA component graph from discriminant function analysis by iNose evaluation, in which the discrimination index (**DI**) was 91.8%. The clustering trend of samples from each group on the classification map is obvious, and the samples from each group were well separated, indicating that the discriminant function could effectively classify the yogurt samples from different fermentation treatments. The yogurt sample difference between the EBAF and the BC group was the smallest, indicating that the *B. breve* beads added during the late stages of yogurt fermentation had little effect on the flavor of the yogurt itself and did not substantially change its odor. Figure 10A is the LDA component graph from discriminant function analysis after SmarTongue evaluation, in which the DI reaches 95.1%. The clustering trend of samples from each group on the classification map is obvious, and the distance among samples from each group is relatively great, indicating that the discriminant function can effectively classify the yogurt samples that underwent different fermentation conditions.

The purpose of PCA in electronic nose or electronic tongue detection is to use a few new variables instead of the original variables to represent the original informa-

Table 2. Results of simulated gastrointestinal release test (log cfu/g)[1]

Time (d)	Group	BC	UBFF	EBFF	UBAF	EBAF
0	PC	7.43 ± 0.01	7.61 ± 0.06	7.57 ± 0.02	7.67 ± 0.04	7.54 ± 0.03
	SGF	3.32 ± 0.06	3.58 ± 0.01	6.81 ± 0.05	3.61 ± 0.02	6.97 ± 0.02
	SBF	2.88 ± 0.04	3.07 ± 0.03	6.11 ± 0.05	3.17 ± 0.03	6.04 ± 0.06
	SIF	2.56 ± 0.07	2.81 ± 0.04	5.95 ± 0.12	3.01 ± 0.01	5.93 ± 0.09
	Survival rate (%)	32.17	35.85	76.37	35.01	77.63
1	PC	7.36 ± 0.04	7.55 ± 0.23	7.39 ± 0.03	7.51 ± 0.02	7.48 ± 0.01
	SGF	3.20 ± 0.05	3.62 ± 0.05	6.53 ± 0.11	3.21 ± 0.07	6.89 ± 0.04
	SBF	2.79 ± 0.03	3.11 ± 0.03	5.91 ± 0.02	2.87 ± 0.14	5.97 ± 0.03
	SIF	2.50 ± 0.12	2.97 ± 0.13	5.76 ± 0.03	2.80 ± 0.09	5.84 ± 0.12
	Survival rate (%)	33.96	39.34	77.94	37.28	78.07
3	PC	7.18 ± 0.03	7.42 ± 0.05	7.11 ± 0.11	7.37 ± 0.05	7.33 ± 0.03
	SGF	2.98 ± 0.03	3.27 ± 0.03	6.34 ± 0.08	3.15 ± 0.04	6.32 ± 0.02
	SBF	2.58 ± 0.11	2.70 ± 0.21	5.58 ± 0.05	2.64 ± 0.06	5.78 ± 0.10
	SIF	2.31 ± 0.13	2.66 ± 0.15	5.43 ± 0.11	2.58 ± 0.12	5.69 ± 0.09
	Survival rate (%)	32.17	35.85	76.37	35.01	77.63
5	PC	7.11 ± 0.05	7.28 ± 0.07	7.27 ± 0.04	7.21 ± 0.02	7.16 ± 0.02
	SGF	2.68 ± 0.10	3.10 ± 0.03	5.74 ± 0.02	3.08 ± 0.01	6.35 ± 0.01
	SBF	2.31 ± 0.06	2.57 ± 0.02	5.11 ± 0.06	2.44 ± 0.05	5.43 ± 0.08
	SIF	2.18 ± 0.11	2.43 ± 0.13	4.95 ± 0.09	2.39 ± 0.25	5.39 ± 0.11
	Survival rate (%)	30.60	33.38	68.09	33.15	75.28
7	PC	6.83 ± 0.07	7.15 ± 0.02	7.13 ± 0.02	7.10 ± 0.05	7.09 ± 0.04
	SGF	2.66 ± 0.06	3.01 ± 0.01	6.01 ± 0.04	3.03 ± 0.04	6.21 ± 0.02
	SBF	2.25 ± 0.05	2.41 ± 0.05	4.99 ± 0.03	2.38 ± 0.03	5.47 ± 0.09
	SIF	2.03 ± 0.09	2.29 ± 0.09	4.78 ± 0.17	2.30 ± 0.05	5.30 ± 0.07
	Survival rate (%)	29.72	32.03	67.04	32.39	74.75

[1]The yogurt samples from different groups were taken at different times after fermentation and then treated with simulated gastric fluid, bile, or intestinal juice in turn. After this treatment, the number of *Bifidobacterium breve* was enumerated. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *B. breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter; PC = primary colonies; SGF = simulated gastric fluid; SBF = simulated bile fluid; SIF = simulated intestinal fluid.

tion as much as possible after the dimensionality reduction processing (Ciosek et al., 2005). The scatter plots for the main component were obtained after the PCA of the original data obtained by iNose evaluation, in which each point represents 1 sample, and the distance between points represents the characteristic difference among different detection points (Figure 9B). PC1 and PC2 have variance contribution rates of 98.0 and 1.0%, respectively, with a cumulative contribution rate of 99.0%, which can reflect a large amount of information of the sample as a whole. The PCA DI value is 91.8%, indicating an excellent discriminant result, and yogurt samples from the different groups can be distinguished well. The yogurt samples in BC, UBFF, and EBFF groups were clustered in different regions of the PCA diagram, indicating that the electronic nose could distinguish these yogurt samples. The partial overlap of the EBAF and UBAF groups reflected the similarity of the yogurt volatilization from these 2 groups. The distance between the BC and EBFF groups was the largest in the PCA chart, indicating significant different volatile components between them. Meanwhile, the distance between the BC and EBAF group in the PCA chart was the smallest, indicating that the flavor difference between the BC and EBAF groups was not significant. Flavor difference also existed between the yogurt samples from the UBFF and UBAF groups, suggesting that the unencapsulated *B. breve* contributed to the flavor to yogurt and the encapsulating process caused the *B. breve* to be unavailable for fermentation process.

The PCA score plot for the electronic tongue analysis after pretreatment of the yogurt samples is shown in Figure 10B. PC1 and PC2 have variance contribution rates of 37.5% and 26.2%, respectively, with a cumulative contribution rate of 63.7%, which represents the major information of the samples as a whole. The PCA DI value is 95.1%, indicating an excellent discriminant result; yogurt samples from the different groups could be distinguished well. Clustering in different regions of the PCA diagram indicated that the electronic tongue could distinguish among these yogurt samples. The distance between the BC and EBFF groups was shortest in the PCA chart, with the longest distance found between the BC and UBAF groups. The distance between the BC and EBAF yogurts was shorter than that between the BC and UBAF yogurts. These results indicated that the *B. breve* was fully isolated from fermentation process when encapsulated.

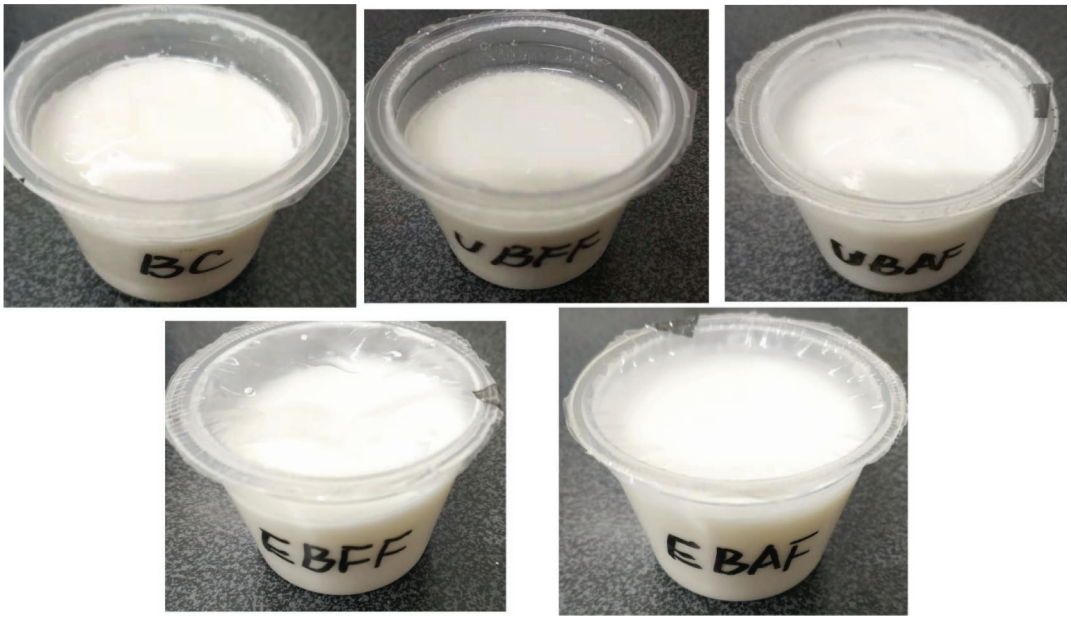

Figure 8. Images of yogurt products. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter.

CONCLUSIONS

In this study, we prepared *B. breve* beads using LMP as the encapsulating material. The beads were white and the particle size distribution was uniform and spherical, with encapsulation efficiency higher than 99%. The number of viable *B. breve* in LMP beads decreased by 1.76 log cfu/g after simulated GI tests, while unencapsulated *B. breve* decreased by 4.82 log cfu/g after the same treatment. The results of the storage stability test showed that microencapsulation could significantly improve the viability of the encapsulated

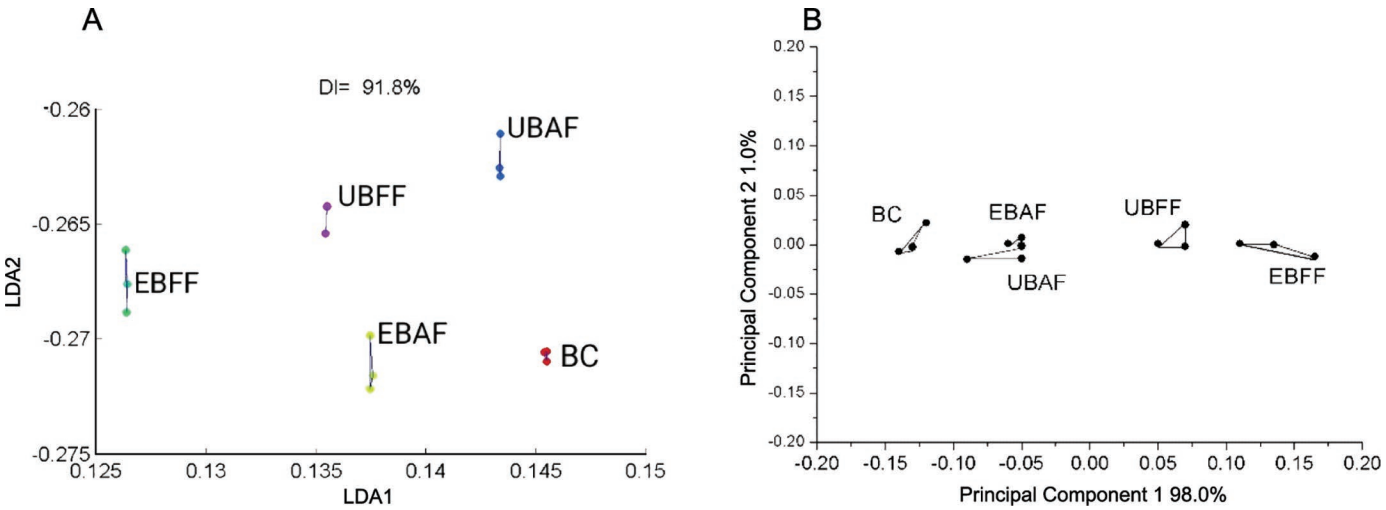

Figure 9. iNose (iNose system, Isenso, New York, NY) evaluation of the different yogurt samples with (A) linear discriminant analysis (LDA) and (B) principal component analysis. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter. DI = discrimination index.

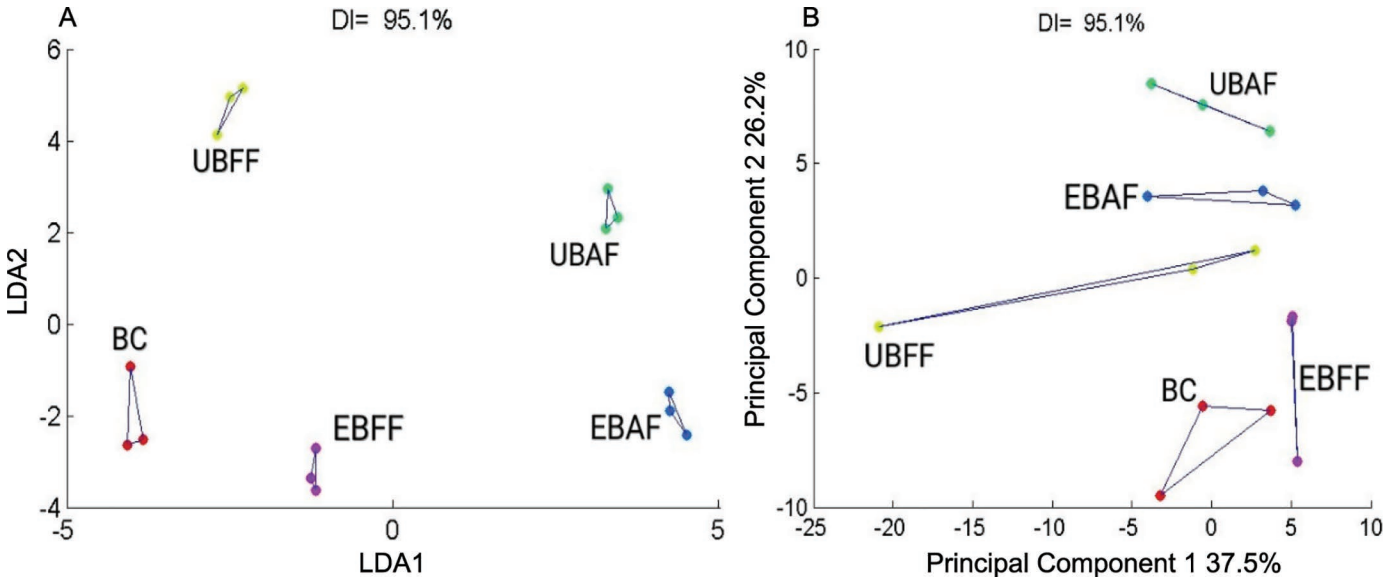

Figure 10. SmarTongue (SmarTongue system, Isenso, New York, NY) evaluation of the different yogurt samples with (A) linear discriminant analysis (LDA) and (B) principal component analysis. BC = milk fermented with commercial yogurt starter; UBFF = unencapsulated *Bifidobacterium breve* added to fresh milk and then fermented; EBFF = encapsulated *B. breve* added to fresh milk and then fermented; UBAF = unencapsulated *B. breve* added after yogurt fermentation with commercial starter; EBAF = encapsulated *B. breve* beads added after yogurt fermentation with commercial starter. DI = discrimination index.

cells, and the storage effect was best at −20°C. The encapsulation of *B. breve* into LMP also increased the stability of the *B. breve* in yogurt during storage at 4°C. The difference in yogurt samples between the EBAF and BC groups was the smallest, indicating that *B. breve* beads added during the middle and late stages of yogurt fermentation had little effect on the flavor of yogurt itself and did not cause large differences in yogurt odor. In conclusion, LMP appears to be ideal for encapsulating *B. breve* and shows potential use in yogurt fermentation.

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