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The Use of Probiotic-Loaded Single- and Double-Layered Microcapsules in Cake Production

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Abstract

To date, the probiotic product development studies have mostly focused on dairy-based foods. However, endowing bakery products with probiotic properties not only provides a variety in food selection but would also potentially improve public health when the consumption rates are taken into consideration. This study aimed to incorporate single- and double-layered microcapsules containing *Saccharomyces boulardii*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*, produced by spray drying and chilling, in cake production. Microcapsules were added after baking to the three different types of cakes (cream-filled, marmalade-filled, and chocolate-coated). Additionally, the microcapsules were injected into the center of the cake mix and baked at 200 °C for 20 min, for plain cake only. After baking of plain cakes, the count of *S. boulardii* and *L. acidophilus* as determined in the double-layered microcapsules produced by spray chilling was 2.9 log cfu/g. The survivability rates of *S. boulardii* and *L. acidophilus* were also determined as 67.4 and 70.7% in this microcapsule, respectively. However, there were no viable *B. bifidum* detected after baking. The free forms of these probiotics did not survive in any plain cake experiments. Single-layered microcapsules produced by spray chilling provided a better protective effect on the probiotics in cream-filled and marmalade-filled cake samples during storage, particularly the cream-filled cakes. This study showed that combined spray chilling and spray drying microencapsulation techniques (double-layered microcapsules) could increase the survivability of probiotic microorganisms during the cake baking process. During storage, the cake samples had a near neutral pH value, and the textural properties deteriorated due to staling. However, cake staling had a limited effect on the sensorial attributes of the cakes and the samples could be readily consumed after storage for 90 days.

Keywords Probiotic · Microencapsulation · Spray chilling · Spray drying · Bakery product

Introduction

Nowadays the demand for functional food is increasing in direct response to health consciousness and health-care costs. Functional foods are substances intended not only to satisfy hunger and provide nutrients to humans in a daily diet but also to prevent nutrition-related diseases and increase the physical and mental well-being of consumers [1]. Probiotics are beneficial microorganisms with immunostimulatory activities that contribute to protecting against gastrointestinal infections manifested as anticarcinogenic, antibacterial, and antimutagenic effects [2]. Consequently, the global probiotic world market is predicted to reach about 45 billion USD in 2018 [3].

The main problem in the production of probiotic foods is ensuring an adequate number of bacteria in the product, and their protection during storage [4] as probiotics are sensitive microorganisms and can be readily inactivated under extreme conditions. For this purpose, various microencapsulation techniques have been developed such as extrusion, emulsion, spray drying, freeze drying, and spray chilling [5–8].

To date, milk-based products are the main (48%) food applications of probiotic microcapsules, with only 10% based on bakery products [9]. Although the improvement of non-dairy products containing microencapsulated probiotic microorganisms is of major interest in the food industry [10–12], new microencapsulation techniques allowing improved preservation of microorganism viability during the production of

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probiotic products are required. The development of new probiotic functional foods not only provides a variety of food selection but would also potentially improve public health [13]. The production of new and high temperature-resistant probiotic microcapsules would provide an opportunity for enrichment of cereal-based probiotic bakery products [14–16].

A plethora of literature studies relates to the microencapsulation of probiotics by extrusion and emulsion techniques, and the addition of these microcapsules to non-heat treated and non-dairy foods, such as mayonnaise, fruit juices, sausage, and chocolate [14–16]. However, there are very limited studies about the enrichment of bakery products with probiotics because these products are baked at high temperatures, at which the viability of probiotics is completely lost.

The probiotics are incorporated into bakery products by adding them directly to the dough and cream filling or spreading on the product surface after baking [9]. Acceptable bacteria counts can be achieved in non-heat treatment, but when probiotic microorganisms are added to the dough, inactivation occurs during baking. A possible solution is to develop heat-resistant probiotic microcapsules. Therefore, this study was aimed to produce single- and double-layered microcapsules containing *Saccharomyces boulardii*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* by spray drying and chilling techniques to make them heat-resistant for suitability in cake production. In addition, the viability of the probiotic microorganisms and the textural and sensorial properties of the cake samples were also investigated during storage.

Material and Methods

Materials

The probiotic yeast *S. boulardii* was supplied by Reflor (Biocodex, Beauvais, France), and the probiotic bacteria, *L. acidophilus* LA-5 and *B. bifidum* BB-12, were purchased from Chr. Hansen (Horsholm, Denmark).

Results of the preliminary experiments showed that the use of gum Arabic as the wall material ensured the highest protection to the probiotic microorganisms. Also, the addition of β-cyclodextrin into the wall material solution, containing gum Arabic, increased the microencapsulation performance. Accordingly, these two wall materials, gum Arabic (Sigma, Taufkirchen, Germany) and β-cyclodextrin (Sigma, Taufkirchen, Germany), were used in the spray drying process. For producing encapsulated probiotic microorganisms by the spray chilling technique, hydrogenated palm oil (melting point of 38–40 °C, HYSOC-40, Cargıll, MN, USA) was used as the wall material.

Cake ingredients were purchased from the market, and the chemical reagents were obtained from either Merck (Darmstadt, Germany) or Sigma (Taufkirchen, Germany).

Encapsulation

Preparation of Probiotic Microorganisms

Saccharomyces boulardii was cultured in sterile 50 mL yeast malt broth (YM Broth) (Sigma, Taufkirchen, Germany) to late log phase (48 h) in a 37 °C shaking incubator (CERTOMAT IS, Goettingen, Germany). *L. acidophilus* was cultured in sterile and degassed 50 mL De Man, Rogosa, and Sharp broth (MRS Broth) (Merck, Darmstadt, Germany) (pH adjusted to 6.2) to late log phase (18 h) in a 37 °C CO_2 incubator (INC153MED, Memmert, Carla, Germany). *B. bifidum* was cultured in sterile and degassed 50 mL MRS broth enriched with 0.05% L-cysteine (pH adjusted to 6.5), for 12 h at 37 °C [17]. After the first incubation, 5 mL of *B. bifidum* culture suspension was added to 50 mL MRS broth and incubated under the same conditions. At the end of the incubations, probiotic suspensions were centrifuged at 7500 rpm for 10 min and washed twice with a quarter strength Ringer solution (Merck, Darmstadt, Germany). The probiotic culture concentrations of *S. boulardii*, *L. acidophilus*, and *B. bifidum* were 10^{10}, 10^{10}, and 10^{11} cfu/mL, respectively. Probiotic microorganism cultures were suspended in 20% glycerol and stored at −18 °C until further use.

Production of Single-Layered Probiotic Microcapsules by Spray Drying and Chilling

For the spray drying experiment, 20 g of the wall material (gum Arabic and β-cyclodextrin at 9:1) (w/w) was suspended in 200 mL water and autoclaved at 121 °C. After cooling the solution, *S. boulardii*, *L. acidophilus*, and *B. bifidum* cultures were separately added, at a ratio of 5% (v/w) dry matter of wall materials (1 mL, each of probiotic culture). The probiotic containing hydrophilic carrier solution was then microencapsulated, using a laboratory scale spray dryer (Buchi-290, Flawil, Switzerland). The spray dryer was operated at 120 °C inlet air temperature and 50 °C outlet air temperature which were determined after a preliminary experiment with the two-fluid nozzle operated by compressed air at 0.3 bar. The aspiration rate was about 38 m^3/h while the feeding rate of the solution was approximately 16.5 mL/min.

For the spray chilling experiment, 20 g hydrogenated palm oil was melted on a hot plate and 2% Tween 80 was added. The molten carrier was cooled to 45 °C, and the probiotic cultures were added at the 5% (v/w) ratio of the wall materials amount (1 mL). The probiotic containing hydrophobic carrier was homogenized using a disperser (Ultraturrax-IKA, Staufen, Germany) at 8200 rpm for 1 min, and the emulsion was microencapsulated using a laboratory scale spray chiller (B-290-spray chilling unit, Buchi, Flawil, Switzerland) at room temperature. The feeding solution bath was set at 45 °C, and the nozzle was fixed at 38 °C. The nozzle was

operated by compressed air at 0.3 bar, and the aspiration rate was about 20 m³/h.

The probiotic microcapsules were separated by the cyclone, gathered in the collection vessel, and stored at − 18 °C, for further analysis. The produced microcapsules were named *GS* (spray-dried single-layered microcapsules) and *P* (spray-chilled single-layered microcapsules).

After the microencapsulation process, the *S. boulardii*, *L. acidophilus*, and *B. bifidum* counts of *P* and *GS* microcapsules were determined [18].

Production of Double-Layered Probiotic Microcapsules—Core Wall Spray-Chilled and Outer Wall Spray-Dried GS/P

Spray-chilled capsules were used for the production of microcapsules with spray-dried outer wall. Twenty grams of the wall material (gum Arabic and β-cyclodextrin at 9:1, (w/w)) was dissolved in 200 mL water and sterilized by autoclaving at 121 °C for 15 min. After cooling, 1% (v/v) lecithin was added to the solution. *P* microcapsules (5% w/w) were dispersed in the above solutions using a disperser at 8200 rpm for 1 min. The spray dryer was operated at 80 °C inlet air temperature and 35 °C outlet air temperature, under a constant feeding rate of 12 mL/min.

Production of Double-Layered Probiotic Microcapsules—Core Wall Spray-Dried and Outer Wall Spray-Chilled P/GS

Spray-dried capsules were used for the production of microcapsules with spray-chilled outer wall. Twenty grams of hydrogenated palm oil was melted on a hot plate and 2% Tween 80 was added, as per the previous section. In this instance, however, the *GS* microcapsules (5% w/w) were dispersed in the above solution using a disperser at 8200 rpm for 1 min. The other conditions were similar to those used for the single-layered spray chilling experiments.

The collected microcapsules were stored at − 18 °C for further analysis, and the viability of cells was assessed [18].

Cake Production

Standard pound cake formulation was prepared with the addition of flour, egg, vegetable oil, and sugar (1:1:1:1, (w/w/w/w)). Vanilla essence and baking powder were added at 0.6% of the cake mix, and the ingredients were mixed using a blender [19].

In plain cake production, 25 g of the cake mix was spread into the tin, and 0.5 g of microcapsules containing the probiotic microorganism (*GS*, *P*, *GS/P*, and *P/GS*) was added individually in the center of the cake mix, followed by a further 25 g of cake mix spread over the top and was baked at 200 °C for 20 min. This procedure ensured that the microcapsules were located in the center of the cake mix, where they would

be most protected from the baking heat [20]. The temperature in the cake center was measured as 102 ± 2 °C during baking. In another set of cakes, non-encapsulated probiotic microorganisms were introduced into the center of the cake mix before baking, as controls.

To determine the protection of probiotic microcapsules in various types of cakes, non-plain cakes were also prepared; by filling the plain cakes with strawberry marmalade, chocolate cream, and chocolate covering, respectively. Probiotic microcapsules were added to the marmalade-filled, cream-filled, and chocolate-coated cakes after they were baked. Thus, the probiotic microcapsules in non-plain cakes were not exposed to the oven temperature. To produce the three non-plain cakes, 50 g of cake mix without probiotic microcapsules was baked under the same conditions as the plain cake. After cooling, the baked cakes were filled with either 5 g strawberry marmalade or chocolate cream, each containing 0.5 g of the probiotic microcapsules, using an injector. For the production of the chocolate-coated cakes, solid chocolate was first melted and cooled to 40 °C before the addition of 0.5 g of microcapsules; the probiotic added chocolate mix was then spread over the baked cake. The four different cakes (plain, marmalade-filled, cream-filled, and chocolate-coated), each containing 1% probiotic microcapsules (*GS*, *P*, *GS/P*, and *P/GS*), were stored in plastic bags at 4 °C for 3 months.

The bottom and top diameters, height, and specific volume were measured for determining the dimensions of the produced cakes, and these values were 52.47 ± 0.74 mm, 64.95 ± 0.33 mm, 49.72 ± 0.67 mm, and 1.75 ± 0.07 mL/g, respectively.

Probiotic Microorganism Enumerations

Sample Preparation

The probiotic microorganism counts in plain, cream-filled, and marmalade-filled cakes were determined in samples (1 g) taken from the core of the cakes since microcapsules were placed in the center of the cakes (3 × 3 cm) and from the chocolate layer of the chocolate-coated cakes. The samples were homogenized in 9 mL sterile 2% sodium citrate solution and incubated in a water bath at 45 °C for 10 min to allow the fat matrix to melt.

Saccharomyces boulardii Counts

S. boulardii was enumerated after spreading on yeast extract glucose chloramphenicol agar (YGC) (Merck, Darmstadt, Germany) plates and incubation at 37 °C for 72 h [2].

Lactobacillus acidophilus Counts

L. acidophilus was enumerated by pour plates of MRS-BC (bromcresol green and clindamycin) (Merck, Darmstadt,

Germany) agar, containing 1% cycloheximide, with the pH adjusted to 6.2 (1 N NaOH). The MRS agar and bromcresol green (0.2%) (Sigma, Taufkirchen, Germany) were sterilized separately at 121 °C for 15 min. Bromcresol green was added into the agar medium at a final concentration of 2% (v/v). Filter sterilized clindamycin (50 mg/L) (Sigma, Taufkirchen, Germany) was also added. The plates were inoculated with *L. acidophilus* and incubated under anaerobic conditions at 37 °C for 72 h before counting [2].

Bifidobacterium bifidum Counts

B. bifidum count was enumerated by pour plate method in MRS-NNLP agar (pH 6.5) containing 1% (w/v) cyclohexi-mide. Filter sterilized antibiotic mixture (NNLP; neomycin sulfate, nalidixic acid, lithium chlorite, and paromomycin sulfate) was added to the medium at 20% (v/v) concentration. The plates were inoculated and incubated under anaerobic conditions at 37 °C for 72 h before counting [2].

pH and Total Titratable Acidity Analysis

The pH value was determined using a pH meter (3410, WTW, Wellheim, Germany). For this purpose, 5-g sample was taken from the homogenized whole cake and diluted in 20 mL of distilled water, and a few drops of the phenolphthalein indicator were added. The total titratable acidity was determined via titration of the sample with 0.1 N NaOH, and the result was expressed as the percentage of lactic acid.

Texture Analysis

The textural properties of cake samples were measured using a texture analyzer (TA-XT plus, Stable Micro Systems, Surrey, UK) equipped with a 100-mm diameter aluminum cylinder probe (SMSP/100). The cake samples were compressed to 30% of their original height by a double compression at a test speed of 3 mm/s. Hardness (the maximum force of the first compression), cohesiveness (the area of work during the second compression divided by the area of work during the first compression), springiness (the distance of the detected height during the second compression divided by the original compression distance), and chewiness (the product of gumminess and springiness) were obtained from the texture profile analysis at 0th, 30th, 60th, and 90th days of storage.

Sensory Analysis

The sensory test was carried out by a panel consisting of eight trained panelist who were aware of the characteristics of cake and studied as research assistants in the Department of Food Engineering. The panelist evaluated the color, appearance, structure, texture, flavor, smell, and overall properties of the stored cake samples according to a 5-point hedonic scale, where 1 = "dislike extremely", 5 = "like extremely", and the mid-point of the range 3 = neither like nor dislike [21].

Statistical Analysis

During the research, cakes were produced in duplicate, and analyses were performed in parallel. The factorial randomized block design (RBD) was used as an experimental design, and one-way ANOVA was used for data evaluation. The data were subject to analysis of variance, and appropriate mean separation was conducted using Duncan's Multiple Range Test ($p < 0.05$). All statistical calculations were performed by SAS Statistical Software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The Viability of *Saccharomyces boulardii* in Cake

After baking, the *S. boulardii* count in plain cake with the addition of the *P/GS* microcapsules was 2.9 log cfu/g (Table 1), but no viable *S. boulardii* was detected in cake produced with other microcapsules (*GS*, *P*, and *GS/P*) and free (non-encapsulated) yeast cells. Double-layered microencapsulation (*P/GS*) is highly promotive to produce probiotic bakery products via mixing the probiotics in the dough. The double-layered microencapsulation, whereby the outer layer was fabricated by spray chilling with a hydrophobic material and the inner layer was produced by spray drying with a hydrophilic material, increased the survivability of *S. boulardii* cells. The hydrophobic and hydrophilic combination of materials may have protected the cells from the heat. The outer layer of P/GS microcapsule was hydrophobic, and the core layer was hydrophilic. When this microcapsule was exposed to heat, the outer hydrophobic layer melted, and it absorbed heat during melting. As a result, the temperature of the microcapsule center, which contains the probiotics, could remain cooler than its surroundings. In addition, heat may have transferred from the outer layer to the center across the hydrophobic/hydrophilic layer interface, due to the limit of conventional heat transfer by restriction of water movement. Consequently, the microcapsule centers were remained comparatively cooler, protecting the probiotic microorganisms against the detrimental effects of heat.

The change in the *S. boulardii* counts during storage of the cake samples (Table 2) indicated that single-layered microcapsules ensured higher counts than double-layered microcapsules in non-heated cake samples during storage.

In general, S. *boulardii* counts were high in cake produced with the addition of *P* microcapsule except for chocolate-coated cake. It is possible that the hydrophobic wall material may have remained insoluble in the filling and coating cake

Table 1 Probiotic microorganism counts (log cfu/g) of plain cake before and after baking (mean ± standard error)

Microcapsule	S. boulardii		L. acidophilus		B. bifidum	
	N_0	N_t	N_0	N_t	N_0	N_t
P	6.2 ± 0.1	< 1.0	6.1 ± 0.2	2.4[ba] ± 0.5	6.2 ± 0.1	< 1.0
GS	5.9 ± 0.1	< 1.0	5.9 ± 0.1	2.5[ba] ± 0.3	5.6 ± 0.1	< 1.0
P/GS	4.3 ± 0.2	2.9 ± 0.1	4.1 ± 0.1	2.9[a] ± 0.3	4.0 ± 0.1	< 1.0
GS/P	4.2 ± 0.1	< 1.0	4.1 ± 0.1	1.9[b] ± 0.3	4.2 ± 0.1	< 1.0
Control	6.0 ± 0.2	< 1.0	5.8 ± 0.1	< 1.0	6.4 ± 0.1	< 1.0

Superscript letters beside the mean values denote in the same column that are significantly different by Duncan's multiple range test ($P < 0.05$)

N_0 microorganism counts of plain cakes samples before baking, N_t microorganism counts of plain cakes samples after baking

P spray-chilled single-layered microcapsule, GS spray-dried single-layered microcapsule, P/GS outer layer was spray-chilled and inner layer was spray-dried double-layered microcapsule, GS/P outer layer was spray-dried and inner layer was spray-chilled double-layered microcapsule

materials, thereby better protecting the yeast cells. It was reported that hydrophobic wall materials limited the permeability of oxygen and water and decreased oxidative stress so that probiotics remained in a latent state [22, 23]. However, free *S. boulardii* cells (non-encapsulated) were protected to the same extent as the microencapsulated forms. Probably *S. boulardii* may be resistant to harsh conditions and can easily adapt to limiting environments. Furthermore, the yeast cell wall material consists of chitin, mannose, and glucan whereas the bacterial cell wall is composed of peptidoglycan [24].

There was no significant difference between cake types on *S. boulardii* survivability except for the plain cake sample. Cream-filled, marmalade-filled, and chocolate-coated cakes ensured an average of 3.5 log cfu/g counts of yeast cells during storage. The *S. boulardii* viability decreased during cake storage, possibly due to the adverse effect of prolonged storage time and factors, such as oxidative stress, differences in osmotic pressure, and redistribution of moisture.

There are limited studies regarding the microencapsulation of *S. boulardii* by various techniques [25–29] and deficient in research on the use of microencapsulated and free *S. boulardii*

Table 2 *S. boulardii* counts (log cfu/g) of cakes during storage (mean ± standard error)

Cake type	Layer		Storage day				Averages (log cfu/g)		
			0	30	60	90			
Cream-filled	S	P	5.5[a] ± 0.1	5.2[a] ± 0.1	5.1[a] ± 0.1	4.4[a] ± 0.2	5.1[a]	4.9[a]	3.6[a]
		GS	5.3[ba] ± 0.2	5.0[ba] ± 0.1	4.9[b] ± 0.1	3.8[a] ± 0.4	4.8[b]		
	D	P/GS	3.6[c] ± 0.1	< 1.0[b]	< 1.0[b]	< 1.0[b]	1.2[c]	1.2[c]	
		GS/P	2.7[d] ± 0.1	2.5[c] ± 0.1	< 1.0[b]	< 1.0[b]	1.3[c]		
	Control		4.9[b] ± 0.1	4.9[b] ± 0.1	4.7[c] ± 0.1	4.1[a] ± 0.1	4.6[b]	4.6[b]	
Marmalade-filled	S	P	5.2[b] ± 0.1	5.1[a] ± 0.1	4.7[a] ± 0.3	4.5[a] ± 0.1	4.9[a]	4.5[b]	3.5[a]
		GS	6.1[a] ± 0.1	4.1[b] ± 0.2	3.7[b] ± 0.2	2.6[c] ± 0.1	4.1[c]		
	D	P/GS	3.6[c] ± 0.1	2.3[c] ± 0.3	< 1.0[c]	< 1.0[d]	1.5[d]	1.4[c]	
		GS/P	2.7[d] ± 0.1	2.5[c] ± 0.1	< 1.0[c]	< 1.0[d]	1.3[d]		
	Control		5.1[b] ± 0.2	4.8[a] ± 0.1	4.6[a] ± 0.1	4.1[b] ± 0.2	4.6[b]	4.6[a]	
Chocolate-coated	S	P	5.6[a] ± 0.1	5.0[a] ± 0.1	3.9[c] ± 0.1	3.6[a] ± 0.1	4.5[b]	4.6[a]	3.5[a]
		GS	5.9[a] ± 0.0	5.1[a] ± 0.5	5.0[a] ± 0.1	3.2[b] ± 0.1	4.8[a]		
	D	P/GS	3.6[b] ± 0.1	3.1[c] ± 0.1	< 1.0[d]	< 1.0[c]	1.7[c]	1.5[c]	
		GS/P	2.8[b] ± 0.5	2.3[d] ± 0.1	< 1.0[d]	< 1.0[c]	1.3[d]		
	Control		5.1[a] ± 0.1	4.3[b] ± 0.1	4.2[b] ± 0.1	3.5[a] ± 0.0	4.3[b]	4.3[b]	

Superscript letters beside the mean values denote in the same column that are significantly different by Duncan's multiple range test ($P < 0.05$)

S single-layered microcapsules and D double-layered microcapsules. P spray-chilled single-layered microcapsule, GS spray-dried single-layered microcapsule, P/GS outer layer was spray-chilled and inner layer was spray-dried double-layered microcapsule, GS/P outer layer was spray-dried and inner layer was spray-chilled double-layered microcapsule

in cake production. To date, the only relevant study was that aimed to coat apple slices with alginate gel containing *S. boulardii* [26].

The Viability of *Lactobacillus acidophilus* in Cake

L. acidophilus viability was detected in all plain cake samples produced by the four different microcapsules (*GS*, *P*, *GS/P*, and *PG/S*) but whereas free (non-encapsulated) *L. acidophilus* cells could not survive. The highest cell survival of 2.9 log cfu/g was observed in *P/GS* microcapsules (Table 1) after baking. Each type of microcapsule material protected *L. acidophilus* cells by preventing heat transfer, with the double-layered spray-chilled microcapsules (*P/GS*) being the most protective. The reason could be due to the protective mechanism as speculated above for higher survivability of *S. boulardii* after baking.

Reid et al. [12] enriched biscuit dough with microencapsulated *L. rhamnosus* cultures and determined nearly 79% cell survivability after baking, with a center temperature during baking of around 92 °C. In comparison, however, lower survivability rates have been reported in the literature at lower baking temperatures. When microencapsulated *L. reuteri* cells were added to souffle before baking at 180 °C for 10 min (center temperature of 80 °C), the survivability rate of *L. reuteri* was between 7 and 10% [30]. Seyedain-Ardabili et al. [31] microencapsulated *L. acidophilus* by an emulsion technique and inoculated them into hamburger bun dough and white pan bread dough and baked. For *L. acidophilus*, the cell count decreased from 11 log cfu/g to 5.45 and 3.2 log cfu/g in the white pan bread and hamburger buns, less than 24 h after baking at 180 °C, respectively. In another study, microencapsulated *L. plantarum* cells were added into noodle formulations, but no viable cells were observed after boiling the dried noodles for 3.5 min [32].

The variability in of *L. acidophilus* counts during storage of the cake samples (Table 3) verified that in single- or double-layered encapsulation, the type of encapsulation material and cake type significantly (*P* < 0.05) influenced the cell survivability rates.

The single-layered microcapsules (*P* and *GS*) presented a higher survival rate than the double-layered microcapsules (*P/GS* and *GS/P*), among the various types of cakes, with a mean count of 4.7 log cfu/g. However, it averaged 3.3 log cfu/g for the control cake. This result indicates that microencapsulated *L. acidophilus* cells could be mixed in the filling and used to cover the cake.

At the beginning of the storage, an adequate probiotic microorganism number (> 10^6) was achieved in cream-filled, marmalade-filled, and chocolate-covered cake samples with added *P* and *GS* microcapsules, but this was not maintained for 30 days.

Regarding the cake types, *L. acidophilus* exhibited high viability in cream-filled cake samples (*P* < 0.05), potentially because the cream filling was injected into the cake center, so the microencapsulated *L. acidophilus* was better protected than in the chocolate coating. The marmalade filling did not provide the same protection in the cake center due to its low

Table 3 *L. acidophilus* counts (log cfu/g) of cakes during storage (mean ± standard error)

Cake type	Layer	Microcapsule	Storage day				Average (log cfu/g)		
			0	30	60	90			
Cream-filled	S	P	5.9^a ± 0.1	5.2^b ± 0.1	4.4^a ± 0.1	3.7^a ± 0.2	4.8^a	4.9^a	3.7^a
		GS	6.5^a ± 0.2	5.8^a ± 0.1	4.1^b ± 0.2	3.6^a ± 0.4	5.0^a		
	D	P/GS	4.7^b ± 0.2	2.8^c ± 0.1	2.0^c ± 0.1	< 1.0^c	2.4^c	2.3^c	
		GS/P	4.4^b ± 0.5	2.9^c ± 0.2	1.9^c ± 0.1	< 1.0^c	2.3^c		
	Control		5.7^a ± 0.1	4.9^b ± 0.1	2.7^b ± 0.1	2.4^b ± 0.1	3.9^b	3.9^b	
Marmalade-filled	S	P	6.0^a ± 0.1	4.8^a ± 0.1	4.4^a ± 0.2	3.0^b ± 0.1	4.5^a	4.5^a	3.1^b
		GS	6.0^a ± 0.2	4.6^a ± 0.4	3.7^b ± 0.2	3.4^a ± 0.1	4.4^a		
	D	P/GS	4.7^b ± 0.3	2.6^c ± 0.1	1.8^c ± 0.1	< 1.0^c	2.3^c	2.2^c	
		GS/P	4.4^b ± 0.4	2.8^c ± 0.1	1.6^c ± 0.2	< 1.0^c	2.2^c		
	Control		5.0^b ± 0.2	3.5^b ± 0.1	2.5^c ± 0.2	< 1.0^c	2.7^b	2.7^b	
Chocolate-coated	S	P	6.1^b ± 0.1	5.4^a ± 0.1	4.0^a ± 0.1	3.6^a ± 0.1	4.8^a	4.6^a	3.2^b
		GS	6.9^a ± 0.1	3.9^b ± 0.2	4.0^a ± 0.1	3.1^a ± 0.3	4.5^b		
	D	P/GS	4.9^c ± 0.2	2.9^c ± 0.1	< 1.0^c	< 1.0^c	1.9^d	1.9^c	
		GS/P	4.4^c ± 0.4	3.2^{cb} ± 0.1	< 1.0^c	< 1.0^c	1.9^d		
	Control		4.8^c ± 0.1	3.6^b ± 0.3	2.2^b ± 0.1	2.2^b ± 0.1	3.2^c	3.2^b	

Superscript letters beside the mean values denote in the same column that are significantly different by Duncan's multiple range test (*P* < 0.05)

S single-layered microcapsules and D double-layered microcapsules. P spray-chilled single-layered microcapsule, GS spray-dried single microcapsule, P/GS outer layer was spray-chilled and inner layer was spray-dried double-layered microcapsule, GS/P outer layer was spray-dried and inner layer was spray-chilled double-layered microcapsule

acidity. Moreover, *L. acidophilus* counts decreased continuously during 90 days of storage.

There are currently limited reports describing the formulation of probiotic bakery products with spray-chilled probiotic microcapsules. In cereal bars consisting of individually baked ingredients, spray-chilled *L. acidophilus* microcapsules were added in the molding step of the cereal bars, and the cell counts decreased from 10.5 to 3.10 log cfu/g after 120 days [22].

The Viability of *Bifidobacterium bifidum* in Cake

In contrast to the *S. boulardii* and *L. acidophilus* results, single- or double-layered microencapsulation did not ensure additional protection for *B. bifidum* during baking (Table 1) and no viable free (non-encapsulated) *B. bifidum* cells were detected in cakes after baking. The heat tolerance of *B. bifidum* cells could be lower than the other probiotic cultures used in this study. An earlier investigation reported a sharp decrease in *B. lactis* cell counts in the 3 min of bread baking, and no viability was detected after 12 min [20].

The number of layers of encapsulated material, type of encapsulation material, and cake type had a significant effect ($P < 0.01$, $P < 0.05$) on the survival of *B. bifidum* cells during storage (Table 4).

The mean *B. bifidum* counts of single- and double-layered microcapsules of all types of cakes were 3.2 and 2.0 log cfu/g respectively. The presence of oxygen may have a detrimental effect on *B. bifidum* during storage of cake samples since it is an obligate anaerobic bacterium. It has been reported that

spray-dried microcapsules had poor storage stability [33], and the survival of free (non-encapsulated) *B. bifidum* cells was only 12%.

B. bifidum survivability was high in *P* microcapsules since the hydrophobic wall material may have remained undissolved in the filling and covering cake material, thereby better protecting the cells. Furthermore, similar to *L. acidophilus* survivability results, cream-filled cake samples had better *B. bifidum* survivability (2.7 log cfu/g) than other cake samples.

During storage, *B. bifidum* counts decreased (Table 4) in all cake types. The *B. animalis* cell count declined from 10.3 to 5.2 log cfu/g after 120 days in cereal bars produced with spray-chilled microcapsules containing *B. animalis* cells [22].

pH and Total Titrable Acidity

The pH of the cakes varied between 6.76 and 7.11 during storage, with a slight decrease in the average pH value of the sample to 6.90 after 90 days. The lowest pH value measured in the marmalade-filled cake sample (6.76) was probably due to the strawberry organic acids. The pH value of used marmalade was measured to be 3.18 ± 0.05. Similar results were reported by Zanjani [34]; they could not detect any measurable change in pH values of cream-filled cakes at the end of 4-week storage.

The addition of cream or marmalade had no significant effect on the titratable acidity content of cakes (on average 0.42% as lactic acid), which decreased from 0.59 to 0.36% during 90 days of storage.

Table 4   *B. bifidum* counts (log cfu/g) of cakes during storage (mean ± standard error)

Cake type	Layer	Microcapsule	Storage day				Average (log cfu/g)		
			0	30	60	90			
Cream-filled	S	P	6.1ᵃ±0.1	4.5ᵃ±0.1	4.4ᵃ±0.1	2.4ᵃ±0.1	4.7ᵃ	3.0ᵃ	2.7ᵃ
		GS	5.7ᵃ±0.2	<1.0ᵈ	<1.0ᵈ	<1.0ᶜ	1.4ᶜ		
	D	P/GS	3.6ᵇ±0.1	2.6ᶜ±0.1	2.4ᶜ±0.1	2.3ᵇ±0.2	2.7ᵇ	2.4ᶜ	
		GS/P	3.5ᵇ±0.2	2.5ᶜ±0.1	2.3ᶜ±0.1	<1.0ᶜ	2.1ᵇ		
	Control		5.6ᵃ±0.2	3.0ᵇ±0.1	2.8ᵇ±0.1	<1.0ᶜ	2.8ᵇ	2.8ᵇ	
Marmalade-filled	S	P	6.1ᵃ±0.2	3.4ᵃ±0.1	3.1ᵃ±0.1	2.2ᵃ±0.1	3.7ᵃ	2.9ᵃ	1.9ᶜ
		GS	6.0ᵃ±0.2	2.7ᵇ±0.4	<1.0ᶜ	<1.0ᵇ	2.2ᵇ		
	D	P/GS	3.2ᶜ±0.1	2.4ᵇ±0.1	2.3ᵇ±0.1	<1.0ᵇ	2.0ᵇ	2.0ᵇ	
		GS/P	3.5ᶜᵇ±0.1	2.3ᵇ±0.1	2.2ᵇ±0.4	<1.0ᵇ	2.0ᵇ		
	Control		3.7ᵇ±0.1	<1.0ᶜ	<1.0ᶜ	<1.0ᵇ	0.9ᵇ	0.9ᶜ	
Chocolate-coated	S	P	6.4ᵃ±0.1	4.8ᵃ±0.1	4.5ᵃ±0.1	4.0ᵃ±0.1	4.9ᵃ	3.7ᵃ	2.4ᵇ
		GS	6.2ᵃ±0.1	3.7ᵇ±0.2	<1.0ᵇ	<1.0ᵇ	2.5ᵇ		
	D	P/GS	4.3ᶜ±0.2	3.4ᵇ±0.1	<1.0ᵇ	<1.0ᵇ	1.9ᶜ	1.7ᶜ	
		GS/P	3.5ᵈ±0.3	2.4ᶜ±0.1	<1.0ᵇ	<1.0ᵇ	1.5ᵈ		
		Control	5.4ᵇ±0.2	2.2ᶜ±0.1	<1.0ᵇ	<1.0ᵇ	1.9ᶜ	1.9ᵇ	

Superscript letters beside the mean values denote in the same column that are significantly different by Duncan's multiple range test ($P < 0.05$)

S single-layered microcapsules and D double-layered microcapsules. P spray-chilled single-layered microcapsule, GS spray-dried single microcapsule, P/GS outer layer was spray-chilled and inner layer was spray-dried double-layered microcapsule, GS/P outer layer was spray-dried and inner layer was spray-chilled double-layered microcapsule

Table 5 Textural properties of cakes samples during storage (mean ± standard error)

Type of cake	Textural properties					
	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Plain	2865.5[a] ± 288	0.84[a] ± 0.02	0.67[a] ± 0.04	1887.2[a] ± 112	1637.5[a] ± 74	0.29[a] ± 0.02
Cream-filled	2737.5[a] ± 188	0.83[a] ± 0.02	0.64[ba] ± 0.03	1769.4[a] ± 103	1425.7[ba] ± 99	0.27[ba] ± 0.02
Marmalade-filled	2404.5[b] ± 212	0.85[a] ± 0.02	0.64[ba] ± 0.03	1606.7[a] ± 133	1278.9[b] ± 97	0.26[ba] ± 0.02
Chocolate-coated	2633.8[ba] ± 243	0.86[a] ± 0.05	0.59[b] ± 0.06	1524.3[a] ± 81	1243.5[b] ± 69	0.25[b] ± 0.03
Storage days						
0	1784.1[c] ± 107	0.92[a] ± 0.02	0.79[a] ± 0.03	1499.8[b] ± 149	1239.6[b] ± 100	0.36[a] ± 0.02
30	2650.4[b] ± 110	0.82[b] ± 0.03	0.60[b] ± 0.01	1660.9[ba] ± 74	1329.9[b] ± 77	0.25[b] ± 0.01
60	2971.3[a] ± 98	0.81[b] ± 0.01	0.57[b] ± 0.02	1712.8[ba] ± 74	1438.9[ba] ± 99	0.24[b] ± 0.01
90	3235.5[a] ± 187	0.83[b] ± 0.02	0.58[b] ± 0.02	1914.1[a] ± 112	1577.4[a] ± 91	0.22[b] ± 0.01

Mean ± standard error, $n = 8$ for cake type and storage. Superscript letters beside the mean values denote in the same column that are significantly different by Duncan's multiple range test ($P < 0.05$)
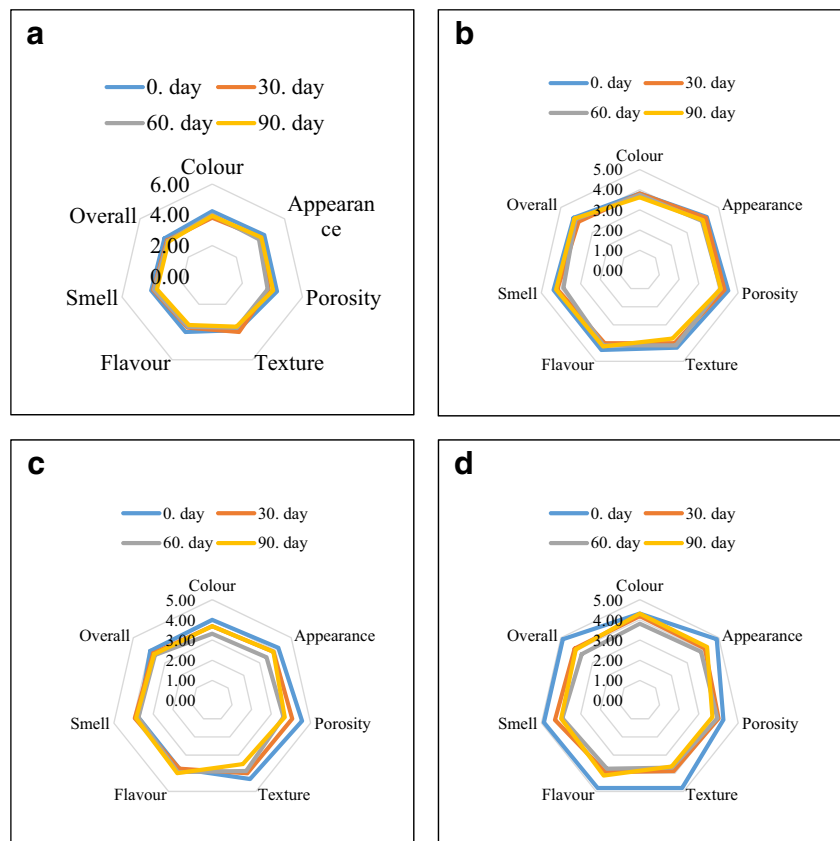
Textural Properties of Cakes

Based on the textural properties of the cakes (Table 5), the cake type had a significant ($P < 0.01$, $P < 0.05$) effect on the hardness, cohesiveness, chewiness, and resilience properties while it had no significant impact ($P > 0.05$) on the springiness and gumminess. Also, the storage time significantly affected ($P < 0.01$, $P < 0.05$) all these characteristics. The highest

hardness, cohesiveness, chewiness, and resilience values were obtained in the plain cake sample, and the addition of filling or covering material decreased these parameters.

At the end of storage, the hardness value had nearly doubled due to cake staling, resulting in physical changes in the cake formulations as a result of moisture during storage. The high value of hardness means that the more force is required to break down the cake structure, so it increases gumminess and

Fig. 1 Sensory analysis of cakes containing probiotic microcapsules. **a** Plain cake sample, **b** chocolate-filled cake sample, **c** marmalade-filled cake sample, and **d** chocolate-covered cake sample

chewiness. Consequently, the increased hardness was associated with a decrease in springiness and cohesiveness in the cake samples.

Sensorial Properties of Cakes

According to the sensorial scores (Fig. 1), the type of cake had a significant ($P < 0.05$) effect on smell and overall properties of the cakes while storage time significantly ($P < 0.01$, $P < 0.05$) impacted on the texture, appearance, smell, and overall attributes of the cakes.

The sensorial qualities of the cake samples decreased during storage but remained acceptable because the sensorial score was higher than 3 on the 5-point hedonic scale. The panel preferred the chocolate-coated cake sample, with receiving the highest rating of 4.2 out of 5-point hedonic scale. Additionally, the overall features of the cake samples decreased on average from 4.3 at the beginning of storage to 3.9 at the end of storage. These results revealed that cake staling had a limited effect on the sensorial characteristics of the cakes, and the samples could be readily consumed after storage for 90 days. This finding may have been associated with the low quantity of water in the cake formulations.

Conclusion

The increased production of probiotic bakery foods can be achieved using new and high temperature-resistant probiotic microcapsules. Double-layered microcapsules (*P/GS*) comprising an inner layer produced by spray drying with gum Arabic and β-cyclodextrin, and an outer layer produced by spray chilling with hydrogenated palm oil, promoted the survival of *S. boulardii* and *L. acidophilus* during baking. In contrast, no viable *B. bifidum cells* were detected after baking, suggesting *L. acidophilus* and *S. boulardii* are more heat-tolerant than *B. bifidum*. Therefore, *L. acidophilus* and *S. boulardii* are preferred for microencapsulation in the formulation of probiotic bakery products. The single-layered microcapsules produced by spray chilling (*P*) were more protective than other microcapsules to *S. boulardii* and *B. bifidum* during storage in cream-filled and marmalade-filled cakes. Among the cake types, cream-filled probiotic cake samples exhibited better cell survivability during storage, but it is recommended that the shelf life of these products should not extend beyond 30 days, under refrigerated conditions.

Compliance with Ethical Standards

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