Effect of the incorporation of *Bifidobacterium* BB-12 microencapsulated with sweet whey and inulin on the properties of Greek-style yogurt

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Abstract The effect of the addition of *Bifidobacterium* BB-12 microencapsulated by spray drying with sweet whey and inulin on the microbiological, physicochemical and texture properties of Greek-style yogurt was evaluated during 28 days of storage. The survival of this probiotic under simulated gastrointestinal conditions was assessed after this storage time. Three formulations of Greek-style yogurt were produced: Control (with free cells); SW (with microcapsules produced only with sweet whey); and SWI (with microcapsules produced with sweet whey and inulin). The counts of *Bifidobacterium* BB-12 remained stable during storage in the Control yogurt and in the SW yogurt, while a slight decrease occurred in the SWI yogurt. Nevertheless, the addition of microcapsules improved the survival of *S. thermophilus* and *L. bulgaricus* in the SW yogurt after 14 days of storage. In general, incorporation of microcapsules into Greek-style yogurt affected specific physicochemical and textural properties of the samples. A decrease in the probiotic survival rate during gastrointestinal simulation was observed for all samples; however, it was more pronounced in the SWI yogurt. Even though the protective effect of the microcapsules was not evident, the results showed that Greek-style yogurt is a promising carrier for incorporation of *Bifidobacterium* BB-12.

Keywords Probiotic yogurt · Microencapsulation · Whey · Prebiotic · In vitro simulated gastrointestinal

Introduction

Probiotics are reported to provide a number of potential health benefits since they help to maintain a good balance and composition of the intestinal tract, and thus protect the human body from invasion of pathogens (Ranadheera et al. 2010). Nevertheless, to provide the claimed health benefits, it has been recommended that probiotic bacteria be present at a minimum level of 6 log CFU per gram of food product until the end of its shelf life. Besides the survival of probiotic bacteria during storage of the product, there is also the concern regarding the ability of the probiotics to resist the passage through the gastrointestinal tract in order to be effective in the host (Madhu et al. 2012; Tripathi and Giri 2014).

Yogurt and fermented milks are considered as the most popular carriers for the delivery of probiotics because of their great acceptance by consumers and their excellent nutritional value (Madhu et al. 2012; Yüksel and Bakırcı 2015). Greek-style yogurt or concentrated yogurt, and also known as strained yogurt in Europe, is a semi-solid product traditionally produced by straining fermented yogurt in cloth bags to reach the desired amount of solids by removing acid whey (Desai et al. 2013). Because of its high amount of total solids, Greek-style yogurt may be a suitable matrix for probiotic bacteria (Abd El-Salam et al. 2011). However, several factors, such as strain type, interaction with starter cultures, pH, concentration of metabolites, oxygen and hydrogen peroxide contents, and storage conditions, have been claimed to influence on the viability of probiotic cultures in yogurts and other dairy

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products (Paseephol and Sherkat 2009; Tripathi and Giri 2014; da Silveira et al. 2015).

Microencapsulation has emerged as an alternative for protection of probiotics and several researches have applied this technique to improve the survival of probiotics in yogurt and during passage through the gastrointestinal tract (Ribeiro et al. 2014; Iravani et al. 2015; De Prisco and Mauriello 2016). Among the existing microencapsulation techniques, spray drying is acknowledged as one of the most convenient in terms of energy requirements, cost and process yield. However, thermal and osmotic damage to the probiotic cells has to be minimized by careful selection of the operating conditions and the carrier media composition (Yonekura et al. 2014; Avila-Reyes et al. 2014). Concerning biopolymers applied as carrier agents, sweet whey has been found effective in improving the survival rate of probiotic bacteria during the drying process and during exposure to acidic and bile conditions (Jantzen et al. 2013; Maciel et al. 2014). Pinto et al. (2015) found that *Bifidobacterium* BB-12 microencapsulated by spray drying using liquid sweet whey was more resistant to simulated gastrointestinal conditions than the free cells. Moreover, prebiotics such as inulin have been successfully applied as co-components for microencapsulation in order to improve the effectiveness of the probiotic (Fritzen-Freire et al. 2013; Krasaekoopt and Watcharapoka 2014).

To the best of our knowledge, there are no previous studies that prepared a Greek-style yogurt with the addition of microencapsulated probiotic bacteria. Therefore, the aim of this present study was to develop a Greek-style yogurt with addition of *Bifidobacterium* BB-12 microencapsulated with sweet whey and inulin by using the spray drying technique, and also to evaluate the effect of the addition of microcapsules regarding the microbiological, physicochemical, and texture properties of the product during 28 days of storage at 4 ± 1 °C. The survival of the free and of the microencapsulated probiotic bacteria during exposure to simulated gastrointestinal conditions was also assessed.

Materials and methods

Materials

A probiotic culture composed of *Bifidobacterium* BB-12 (BB-12[®], Chr. Hansen, Hørsholm, Denmark) was used as the active material for the microcapsules, while commercial skim milk powder (Molico[®], Nestlé, São Paulo, Brazil) was employed for preparation of bacterial suspension. The carrier agents used were liquid sweet whey (6.51% total solids and 0.77% protein) and the prebiotic inulin (Orafti[®] HPX, Beneo, Tienen, Belgium), with degree of

polymerization (DP) ≥ 23 . MRS Agar (Difco, Sparks, MD, USA), M17 Agar (Fluka, Buchs, Switzerland), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Fluka, Buchs, Switzerland) and AnaeroGen[®] (Oxoid, Basingstoke, Hampshire, UK) were used for the microbiological analysis. Pepsin from porcine gastric mucosa (EC 3.4.23.1) (≥ 400 U/mg protein), α -amylase from *Aspergillus oryzae* (EC 3.2.1.1) (28.75 U/mg protein), pancreatin from porcine pancreas (8 $\times$ USP specification) and bile salts used for the simulated gastrointestinal conditions were purchased from Sigma-Aldrich (St. Louis, MO, USA). The commercial ingredients used in the manufacture of Greek-style yogurt were: commercial pasteurized milk (3% fat, Tirol[®], Treze Tílias, SC, Brazil), sucrose (Caramelas[®], Ariranha, SP, Brazil), skim milk powder (Molico[®], Nestlé, São Paulo, Brazil) and culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* YF-L812 (Chr. Hansen, Valinhos, SP, Brazil). All other reagents used were of analytical grade.

Preparation of the probiotic bacterial suspension

The probiotic bacterial suspension was prepared following the procedures described by Fritzen-Freire et al. (2012). Twenty-five grams of freeze dried probiotic cells of *Bifidobacterium* BB-12 were rehydrated in 1 L of a solution of reconstituted skim milk (120 g/L) and frozen as stock solution at -20 ± 1 °C in sterile glass bottles. Before spray drying, this probiotic stock solution was incubated at 37 °C for 2 h. This suspension was inoculated into the feed solutions for microencapsulation by spray drying or directly used as free cells (Control yogurt).

Preparation of the drying media and microencapsulation

The feed solutions used for the production of spray dried *Bifidobacterium* BB-12 microcapsules consisted only of sweet whey or sweet whey and 100 g/L of inulin. The media were homogenised and heat treated at 80 ± 1 °C for 30 min. Before addition of the bacterial suspension, the feed solutions were left to cool down to room temperature. Then, the bacterial suspension (10.40 log CFU/g) was added to two feed solutions at a concentration of 50 mL/L.

Microencapsulation of probiotic was performed in a laboratory scale spray dryer (B-290 mini spray dryer, Buchi, Flawil, Switzerland), operating at constant air inlet temperature of 150 °C and outlet temperature of 50 ± 3 °C. The feed solutions were kept under stirring at room temperature and fed into the main chamber through a peristaltic pump, with a feed flow of 6 mL/min, drying air flow rate of 35 m³/h and compressor air pressure of 0.7 MPa. After spray drying, the microcapsules were

collected from the base of the cyclone, placed in sterile plastic bottles and stored at -20 ± 1 °C.

Manufacture of the Greek-style yogurts

Commercial pasteurized milk was added of sucrose (50 g/L) and skim milk powder (10 g/L), submitted to a heat treatment at 90 ± 1 °C for 5 min and subsequently cooled to 42 ± 1 °C in an ice bath. The yogurt was produced by inoculating the above mixture with YF-L812 culture according to the producer's recommendation followed by incubation at 42 ± 1 °C. The fermentation was stopped when pH reached 4.6, and it was cooled down to 4 ± 1 °C and kept so for 12 h. After overnight refrigeration, the yogurt was drained in a cloth bag for about 18–20 h, as suggested by Şanlıdere Aloğlu and Öner (2013). The strained yogurt was stirred and divided into three portions to which the free or microencapsulated bifidobacteria were added. The first portion, denoted as Control, was added with 4 mL/kg of bacterial suspension of bifidobacteria (free cells); while the other two were added with 10 g/kg of different formulation of microcapsules, as follows: SW was added with microcapsules produced only with sweet whey; and SWI was added with microcapsules produced with sweet whey and inulin. The products obtained were packaged in plastic cups of 100 mL capacity, sealed with aluminum foil lids, and stored at 4 ± 1 °C. Three complete replications of the experiment were performed.

Evaluation of the Greek-style yogurt samples in relation to their physicochemical composition was performed on day 1 of storage, whereas the analyses for microbiological properties, total solids content, pH, titratable acidity, and texture were performed on days 1, 14, and 28 of storage at 4 ± 1 °C. The survival of *Bifidobacterium* BB-12 under simulated gastrointestinal conditions was carried out after 28 days of storage (4 ± 1 °C).

Microbiological analysis of the yogurts

For microbiology analysis, 25 g of the yogurts were diluted in 225 mL of phosphate buffer (0.1 mol/L, pH 7.0) followed by homogenization using a magnetic stirrer for 10 min to release the microorganisms from the microcapsules. The same procedure was performed for the yogurt containing bifidobacteria in their free form. Serial dilutions of yogurt samples were made using peptone water (1 g/L) and bacteria were enumerated by the pour plate technique. The enumeration of *Bifidobacterium* BB-12 was carried out according to Vinderola and Reinheimer (1999). *L. delbrueckii* subsp. *bulgaricus* colonies were enumerated in MRS agar and the plates were incubated under aerobic conditions at 37 ± 1 °C for 72 h (Dave and Shah 1996). The enumeration of *S. thermophilus* colonies were

performed using M17 agar with sterile lactose solution (100 g/L) and the plates were incubated under aerobic conditions at 37 ± 1 °C for 48 h (IDF 1997). After the respective incubation periods, the count of viable probiotic and starter cultures was carried out and expressed as log colony-forming units per gram (log CFU/g). All the analyses were carried out in triplicate.

Physicochemical characterization

The yogurts were analyzed for total solids content and for fat content, as described by the analytical norms of the Adolfo Lutz Institute (IAL 2005). The protein content was estimated through the determination of the total nitrogen level by Kjeldahl method ($N \times 6.38$), and the ash content by subjecting the samples at 550 °C in a muffle furnace (AOAC 2005). The pH values were measured using a pH meter (model W3B, BEL Engineering, Piracicaba, SP, Brazil), while the titratable acidity (% lactic acid) was determined according to AOAC (2005).

Texture analysis

Texture parameters were determined using a texturometer model TA-XT plus (Stable Micro Systems, Texture Exponent software, Surrey, UK) equipped with a 50 kg load cell. A double compression test was employed on the yogurt samples (50 mm diameter and 20 mm height) contained in individual plastic cups by a 25 mm diameter aluminum cylinder probe. The measurements were made at 4 ± 1 °C and the probe penetrated to a depth of 10 mm with a test speed of 1.0 mm/s, according to the methodology described by Fritzen-Freire et al. (2013). The data for force (N) as a function of time (s) were obtained using the Texture Exponent 32 software version 4.0.13.0 (Stable Micro Systems). The following parameters were obtained: firmness (N), cohesiveness, adhesiveness (N s) and gumminess (N). For each yogurt sample the results were expressed as the average of six measurements.

Survival of *Bifidobacterium* BB-12 under simulated gastrointestinal conditions

The survival of *Bifidobacterium* BB-12 in the yogurts during exposure to simulated gastrointestinal conditions was determined according to Verruck et al. (2015). The typical conditions prevailing in the human mouth, esophagus-stomach, duodenum and ileum were sequentially simulated. All the enzyme solutions were freshly prepared and filter-sterilized using a 0.22 µm-membrane filter (MF-Millipore, Billerica MA, USA) prior to use; after sterilization, all the solutions were kept in an ice bath during the entire period of simulation. The yogurt samples (25 g)

were placed into sterile flasks and a water bath shaker (Dist DI950 M, Florianópolis SC, Brazil) at 37 ± 1 °C was used to simulate the temperature and peristaltic movements that prevail during human gastrointestinal transit. For the mastication step (mouth) simulation the pH was adjusted to 6.9 with 1 mol/L NaHCO_3 and a saliva solution prepared with 100 U/mL of the α -amylase in 1 mmol/L CaCl_2 was added to the samples at a rate of 0.6 mL/min for 2 min, with stirring at 200 rpm. In the esophagus–stomach step, the pH of the samples was decreased until it reached 2.0 using 1 mol/L HCl. After 0.05 mL of pepsin solution (25 mg/mL in 0.1 mol/L HCl) was added in equal-sized aliquots during the whole gastric phase, totaling 90 min at 130 rpm of stirring. To simulate the duodenum step the acidity was raised to pH 5.0 by addition of 1 mol/L NaHCO_3 . Then, at the beginning of this step, 0.25 mL of pancreatin-bovine bile salts solution (2 g/L of pancreatin and 12 g/L of bovine bile salts in 0.1 mol/L NaHCO_3) was added per gram of sample. In this step, stirring at 45 rpm for 20 min was employed. Finally, for the ileum step, in which the stirring was also maintained at 45 rpm for 90 min, the pH was increased to 6.5 by addition of 1 mol/L NaHCO_3 . At the end of each step, samples were analyzed to evaluate the count of viable bifidobacteria cells, as previously described. The simulated gastrointestinal conditions were continuous, thus the overall working volume increased, i.e., exactly as happens during actual digestion. Therefore, the dilution of each sample was corrected with peptone water (1 g/L) until reaching 225 mL per 25 of yogurt.

Statistical analysis

The data analysis was carried out with the STATISTICA 7.0 software (StatSoft Inc., Tulsa, USA). Analysis of variance (ANOVA) was applied to determine significant differences ($P < 0.05$) between results and Tukey's test was used to compare means values. The data were expressed as mean $\pm$ standard deviation.

Results and discussion

Microbiological analysis

The variation in viable cell count of *Bifidobacterium* BB-12, *L. bulgaricus*, and *S. thermophilus* in the yogurt samples throughout the storage period of 28 days at 4 ± 1 °C is shown in Fig. 1. The viable cell counts of *Bifidobacterium* BB-12 was above 9 log CFU/g in all the yogurt formulations and the minimum therapeutic level was maintained until the end of the storage period, suggesting that Greek-style yogurt provided a suitable environment for

this probiotic bacteria. During the whole storage period, the counts of *Bifidobacterium* BB-12 in the Control yogurt and in the SW yogurt remained stable ($P > 0.05$). However, a mild but significant decrease ($P < 0.05$) in *Bifidobacterium* BB-12 counts occurred in the SWI yogurt. This behavior

Fig. 1 *Bifidobacterium* BB-12, *S. thermophilus* and *L. bulgaricus* counts in the Greek-style yogurt samples with free or microencapsulated *Bifidobacterium* BB-12 during 28 days of storage at 4 ± 1 °C. *Black square* Control yogurt added with bifidobacteria in the free form, *open square* SW yogurt added bifidobacteria microencapsulated with sweet whey; *gray square* SWI yogurt added bifidobacteria microencapsulated with sweet whey and inulin. Values are expressed as mean $\pm$ standard deviation ($n = 3$). ^{a–c} Different lowercase letters denote significant differences between the different samples for the same storage period ($P < 0.05$). ^{A–D} Different uppercase letters denote significant differences between the different storage periods for the same sample ($P < 0.05$)

could be related to the chemical characteristics of the spray drying media that were used. Several studies have shown that different encapsulating agents provided different levels of protection to spray dried probiotic microorganisms during storage of the microcapsules and when incorporated in a food product (Fritzen-Freire et al. 2013; Yonekura et al. 2014; Avila-Reyes et al. 2014).

The high survival rate of bifidobacteria found in the present study may be attributed to the probiotic strain-specific response and also to the particular properties of the product. Ranadheera et al. (2010) reported that the survival of probiotic microorganisms in food products is closely related to the properties of the food product (concentration and type of proteins, fat content, dissolved oxygen, pH, among others) in addition to the storage conditions. According to Iravani et al. (2015) the main factors that restrict the stability of probiotic bacteria in fermented products are the low pH and titrable acidity of the products. Nevertheless, the pH and titratable acidity values obtained in the present study (Table 2) do not seem to have affected the viability of the probiotic bacteria. A similar behavior was observed by Abd El-Salam et al. (2011), who noted that the counts of *L. acidophilus* and *L. casei* added in labneh remained high (above 8 log CFU/g) throughout the storage period. In this case, the authors suggested that the high viability of the added probiotics may be related to the high total solids content in labneh.

The counts of *S. thermophilus* and *L. bulgaricus* (Fig. 1) were significantly affected by the addition of the microcapsules in the Greek-style yogurt. The viability of *S. thermophilus* was higher ($P < 0.05$) in the yogurts added with the microcapsules (SW and SWI) compared with the Control yogurt on day 1 of storage. The same behavior was noted by Ribeiro et al. (2014) in stirred yogurts added with microencapsulated *L. acidophilus* LA-5. Moreover, the SW yogurt showed a slight increase ($P < 0.05$) in the *S. thermophilus* and *L. bulgaricus* counts after 14 days of storage. Since the major fraction of whey solids is lactose along with soluble proteins (Walstra et al. 2006), this improvement in the viability of starter cultures may have been because of the availability of nutrients in sweet whey. Pescuma et al. (2008) reported that *S. thermophilus* and *L.*

delbrueckii subsp. bulgaricus strains were able to lower the concentration of lactose in reconstituted whey and also to degrade the major whey protein (β -Lactoglobulin). In contrast, a decline in the viable cell counts of *S. thermophilus* and *L. bulgaricus* ($P < 0.05$) was observed until the end of the storage period for the SWI yogurt. This behavior is in accordance with the findings of Paseephol and Sherkat (2009), who noted that supplementation of probiotic yogurts with different inulin powders did not improve the viability of *S. thermophilus* and *L. bulgaricus* in comparison to the Control yogurt.

Physicochemical characterization

The addition of the microcapsules did not affect ($P > 0.05$) the protein and fat content in the samples (Table 1). On the other hand, the yogurts added with the microcapsules (SW and SWI) showed higher total solids and ash content ($P < 0.05$) than the Control yogurt. An increase in the total solids content (Table 2) was also noted by Fritzen-Freire et al. (2013) in samples of ricotta cream. The highest ash content ($P < 0.05$) was noted in the SW yogurt, followed by the SWI yogurt. This result may be attributed to the composition of the microcapsules that were added in the SW yogurt since they were produced only with sweet whey.

An overall decrease in the pH values accompanied by an increase in the acidity levels was observed during storage for all the yogurts. These alterations were more accentuated after 14 days of storage (Table 2). A similar behavior was noted by Glibowski and Rybak (2016) in stirred yogurts with partial replacement of skim milk powder by different inulins. According to Beal et al. (1999), this behavior could be because of the continuous production of lactic acid and other organic acids resulting from lactose consumption by the starter and probiotic cultures.

Table 1 Physicochemical properties of the Greek-style yogurt samples with free or microencapsulated *Bifidobacterium* BB-12 on day 1 of storage (4 ± 1 °C)

Analysis (%)	Yogurt samples		
	Control	SW	SWI
Total solids	20.98 ± 0.36^b	21.82 ± 0.09^a	21.89 ± 0.07^a
Protein	6.75 ± 0.08^a	6.80 ± 0.02^a	6.83 ± 0.11^a
Fat	6.50 ± 0.01^a	6.46 ± 0.11^a	6.50 ± 0.04^a
Ash	0.71 ± 0.02^c	0.88 ± 0.01^a	0.82 ± 0.01^b

Values are expressed as mean $\pm$ standard deviation ($n = 3$)

Control yogurt added with bifidobacteria in the free form, SW yogurt added bifidobacteria microencapsulated with sweet whey, SWI yogurt added bifidobacteria microencapsulated with sweet whey and inulin

^{a–c} Within a row, different lowercase letters denote significant differences between samples ($P < 0.05$)

Table 2 pH, titratable acidity and total solids content of the Greek-style yogurt samples with free or microencapsulated *Bifidobacterium* BB-12 during 28 days of storage (4 ± 1 °C)

Analysis	Time (days)	Yogurt samples		
		Control	SW	SWI
pH	1	4.21 ± 0.01 ^{cA}	4.30 ± 0.02 ^{aA}	4.27 ± 0.01 ^{bA}
	14	4.05 ± 0.01 ^{cC}	4.10 ± 0.01 ^{bC}	4.14 ± 0.01 ^{aC}
	28	4.08 ± 0.01 ^{cB}	4.13 ± 0.01 ^{bB}	4.16 ± 0.01 ^{aB}
Titratable acidity (% lactic acid)	1	1.08 ± 0.01 ^{cB}	1.11 ± 0.02 ^{bB}	1.14 ± 0.01 ^{aB}
	14	1.26 ± 0.02 ^{aA}	1.25 ± 0.02 ^{aA}	1.19 ± 0.01 ^{bA}
	28	1.24 ± 0.01 ^{aA}	1.24 ± 0.01 ^{aA}	1.18 ± 0.02 ^{bA}
Total solids (%)	1	20.98 ± 0.36 ^{bB}	21.82 ± 0.09 ^{aB}	21.89 ± 0.07 ^{aB}
	14	21.77 ± 0.03 ^{bAB}	22.69 ± 0.05 ^{aA}	22.83 ± 0.01 ^{aA}
	28	22.05 ± 0.02 ^{bA}	22.84 ± 0.23 ^{aA}	22.97 ± 0.02 ^{aA}

Values are expressed as mean ± standard deviation (n = 3)

Control yogurt added with bifidobacteria in the free form, SW yogurt added bifidobacteria microencapsulated with sweet whey, SWI yogurt added bifidobacteria microencapsulated with sweet whey and inulin

^{a–c} Within a row, different lowercase letters denote significant differences between samples ($P < 0.05$)

^{A–B} Within a column, different uppercase letters denote significant differences between storage periods ($P < 0.05$)

After the first day of storage, the yogurts added with the microcapsules (SW and SWI) showed higher ($P < 0.05$) pH values than the Control yogurt. Conversely, the acidity of the SW and SWI yogurts was higher ($P < 0.05$) than that of the Control yogurt at day 1 of storage. However, the decrease in pH and the increase in titratable acidity throughout storage were less prominent in the SWI yogurt. Thus, it can be inferred that the addition of the microcapsules containing sweet whey and inulin in the yogurt led to lower post-acidification, independent of the pH and titratable acidity values at the beginning of storage. Shoji et al. (2013) and Ribeiro et al. (2014) noted a lower post-acidification in yogurts containing microencapsulated probiotic bacteria when compared to their free form. However, in the present study the same behavior was not observed in the yogurt added with microcapsules produced only with sweet whey (SW). This result may be associated with the higher viability of the *S. thermophilus* and *L. bulgaricus* cultures in this sample (Fig. 1). Shah et al. (1995) reported that yogurt starter cultures are active even at refrigeration temperatures and can still lower the level of lactose through its conversion to lactic acid, which may explain the higher post-acidification of the SW yogurt. In addition, Kavaz and Bakirci (2014) noted that the levels of organic acids (lactic, citric, orotic, acetic and pyruvic acids) in probiotic yogurt samples were generally increased when the proportion of demineralised whey powder added was higher.

Concerning the total solids content, all the three yogurts showed an increase during the 28 days of storage ($P < 0.05$). This behavior probably occurred due to the formation of liquid droplets on the inside of the aluminum foil lid during storage, indicating a slight syneresis.

Texture analysis

It was possible to note a similar increase ($P < 0.05$) in the firmness, adhesiveness, and gumminess in all the samples throughout the storage time (Table 3). This increase may be related to the increase in the total solids content of the yogurts throughout the storage (Table 2). Conversely, it was possible to note a similar decrease ($P < 0.05$) in the cohesiveness of the yogurt samples during storage. Cohesiveness is a measure of how well a product withstands a second deformation relative to its resistance under the first deformation. It can be interpreted as how tight the binding inside the gel needs to be in order to resist deformation (Liu et al. 2013). Therefore, a decrease in cohesiveness values could indicate a lower association between protein–protein linkages after 14 days of storage. The same changes in these texture parameters during storage were reported by Bedani et al. (2014) for probiotic soy yogurts added with inulin and/or okara flour.

Regardless of the storage time, the addition of microcapsules produced only with sweet whey contributed to a decrease ($P < 0.05$) in the firmness and the gumminess of the SW yogurt. Oliveira et al. (2001) and Amatayakul et al. (2006b) correlated the decrease in firmness of yogurts to their lower contents of total solids and protein as well as to the type of protein. Nevertheless, in the present study, the total solids content of the SW yogurt was slightly higher than that of the Control yogurt (Table 1) and such behavior cannot have resulted in lower firmness. Moreover, the protein content did not significantly vary between the yogurts (Table 1). Therefore, the lower firmness noted in the SW yogurt could be related to the decrease in the

Table 3 Texture parameters of the Greek-style yogurt samples with free or microencapsulated *Bifidobacterium* BB-12 during 28 days of storage (4 ± 1 °C)

Texture parameters (days)	Time	Yogurt samples		
		Control	SW	SWI
Firmness (N)	1	1.13 $\pm$ 0.04 ^{aC}	1.03 $\pm$ 0.02 ^{bC}	1.16 $\pm$ 0.03 ^{aC}
	14	1.46 $\pm$ 0.03 ^{aB}	1.26 $\pm$ 0.03 ^{bB}	1.47 $\pm$ 0.03 ^{aB}
	28	1.57 $\pm$ 0.08 ^{aA}	1.43 $\pm$ 0.09 ^{bA}	1.67 $\pm$ 0.08 ^{aA}
Cohesiveness (–)	1	0.54 $\pm$ 0.01 ^{bA}	0.54 $\pm$ 0.01 ^{bA}	0.55 $\pm$ 0.01 ^{aA}
	14	0.50 $\pm$ 0.01 ^{aB}	0.50 $\pm$ 0.01 ^{aB}	0.51 $\pm$ 0.01 ^{aB}
	28	0.51 $\pm$ 0.01 ^{aB}	0.49 $\pm$ 0.01 ^{aB}	0.50 $\pm$ 0.03 ^{aB}
Adhesiveness (N s)	1	4.62 $\pm$ 0.32 ^{aB}	4.25 $\pm$ 0.11 ^{bC}	4.57 $\pm$ 0.24 ^{abB}
	14	5.56 $\pm$ 0.34 ^{abA}	5.29 $\pm$ 0.28 ^{bB}	5.92 $\pm$ 0.38 ^{aA}
	28	5.86 $\pm$ 0.63 ^{aA}	5.97 $\pm$ 0.42 ^{aA}	6.51 $\pm$ 0.75 ^{aA}
Gumminess (N)	1	60.43 $\pm$ 2.24 ^{bC}	55.10 $\pm$ 0.75 ^{cC}	63.96 $\pm$ 1.40 ^{aC}
	14	72.61 $\pm$ 2.46 ^{aB}	62.86 $\pm$ 1.28 ^{bB}	73.74 $\pm$ 1.58 ^{aB}
	28	79.66 $\pm$ 4.79 ^{aA}	70.04 $\pm$ 4.25 ^{bA}	80.99 $\pm$ 0.47 ^{aA}

Values are expressed as mean $\pm$ standard deviation (n = 6)

Control yogurt added with bifidobacteria in the free form, SW yogurt added bifidobacteria microencapsulated with sweet whey, SWI yogurt added bifidobacteria microencapsulated with sweet whey and inulin

^{a–c} Within a row, different lowercase letters denote significant differences between samples ($P < 0.05$)

^{A–B} Within a column, different uppercase letters denote significant differences between storage periods ($P < 0.05$)

casein: whey protein ratio caused by the presence of sweet whey in the microcapsules. Amatayakul et al. (2006a) reported that the decrease in the casein: whey protein ratio in set yogurt manufactured with different levels of whey protein concentrated promoted a reduction in the firmness of the product. On the other hand, the addition of microcapsules produced with sweet whey and inulin did not affect the firmness of the samples throughout the entire storage period. A similar behavior was noted by Costa et al. (2015) in low-fat cupuassu goat milk yogurts added prepared with inulin. According to Meyer et al. (2011), the effect of inulin on texture depends on the amount added to the product and is strongly affected by the composition and structure of the dairy matrix.

Survival of *Bifidobacterium* BB-12 under simulated gastrointestinal conditions

The survival rate of *Bifidobacterium* BB-12 in the Greek-style yogurts on day 28 of storage exposed to the simulated gastrointestinal conditions is shown in Table 4. Although the viable cell counts decreased during the gastrointestinal simulation, they were different for each yogurt formulation.

No significant decrease ($P > 0.05$) in viable cell counts was detected after exposure to simulated mouth conditions. As reported by Verruck et al. (2015), this behavior may be attributed to the short time of contact with the α -amylase enzyme and also to the buffering capacity of the sodium bicarbonate present in the enzyme solution. On the other

hand, the survival rate of *Bifidobacterium* BB-12 decreased ($P < 0.05$) after exposure to the simulated esophagus-stomach conditions for all the yogurt samples, thus suggesting their sensitivity towards simulated gastric juice containing HCl and pepsin.

The exposure to the simulated esophagus-stomach conditions had a more drastic effect on the yogurts containing microcapsules. The SW and SWI yogurts suffered a decrease of 3.58 and 3.06 log cycles in viable cell numbers relative to the starting situation, respectively. As for the Control yogurt, the viable cell count dropped 2.73 log cycles compared with the initial count. Yonekura et al. (2014) reported that the lower tolerance of microencapsulated bacteria may be associated with sub lethal heat injuries suffered by the cells during the spray drying process, which may have caused variations in the acid tolerance of the probiotic cells. Moreover, it can be hypothesized that the yogurt storage period of 28 days may have contributed to an ever greater increase in the susceptibility of the microencapsulated probiotic cells to acid conditions due to detrimental changes on the shape and structure of the microcapsules during cold storage.

Interestingly, when the SW yogurt was submitted to simulated duodenum and ileum conditions no decrease ($P > 0.05$) in the viable cell counts was noted. Conversely, the bifidobacteria cells in the Control and in the SWI yogurts were more susceptible to simulated duodenum and ileum conditions since the viable cell counts showed a decrease of 1.21 and 1.16 log cycles relative to the esophagus–stomach step, respectively. These results are in

Table 4 Survival of *Bifidobacterium* BB-12 (log CFU/g) in the Greek-style yogurt samples stored (4 ± 1 °C) for 28 days during sequential exposure to simulated gastrointestinal conditions

Gastrointestinal compartment	Sampling (min)	Yogurt samples		
		Control	SW	SWI
Initial	0	9.44 ± 0.09^{aA}	9.39 ± 0.03^{abA}	9.25 ± 0.10^{bA}
Mouth (pH 6.9, α -amylase, 200 rpm)	2	9.33 ± 0.11^{aA}	9.40 ± 0.18^{aA}	9.22 ± 0.02^{aA}
Esophagus-Stomach (Δ pH 5.5–2.0, pepsin, 130 rpm)	90	6.71 ± 0.19^{aB}	5.81 ± 0.04^{cB}	6.19 ± 0.04^{bB}
Duodenum (Δ pH 2.0–5.0, pancreatin + bile salts, 45 rpm)	110	5.81 ± 0.07^{aC}	5.79 ± 0.07^{aB}	5.36 ± 0.03^{bC}
Ileum (Δ pH 5.0–6.5, 45 rpm)	200	5.50 ± 0.05^{bC}	5.83 ± 0.04^{aB}	5.03 ± 0.11^{cD}

Control yogurt added with bifidobacteria in the free form, SW yogurt added bifidobacteria microencapsulated with sweet whey, SWI yogurt added bifidobacteria microencapsulated with sweet whey and inulin

^{a-c} Within a row, different lowercase letters denote significant differences between different samples for the same sampling period ($P < 0.05$)

^{A-D} Within a column, different uppercase letters denote significant differences between different sampling periods for the same sample ($P < 0.05$)

agreement with those of a study conducted by Pinto et al. (2015), where the survival of *Bifidobacterium* BB-12 microencapsulated with sweet whey did not decrease when submitted to the same simulated duodenum and ileum conditions.

After all the steps of the simulated gastrointestinal conditions, the survival of the microencapsulated bacteria in the SW yogurt was slightly higher ($P < 0.05$) than that of the free bacteria in the Control yogurt. On the other hand, no protection was provided by the microencapsulation of the bacteria with sweet whey and inulin in the SWI yogurt. These less favorable results that are reported herein are important to note since they show that microencapsulation does not necessarily mean cell protection. A vast number of publications (Shoji et al. 2013; Maciel et al. 2014; Krasaekoopt and Watcharapoka 2014) have shown the potential effect of encapsulation to protect probiotic bacteria from gastrointestinal conditions. However, very few studies (Ribeiro et al. 2014; Rodríguez-Huez et al. 2014) have been carried out with encapsulated bacteria incorporated into food matrices, as was the case in the present study. Therefore, the resistance of the probiotic bacteria to simulated gastrointestinal conditions, besides being dependent on the bacterial strain, it appears to also depend on the microencapsulation technique and the type of encapsulating agents used, as well as the food matrix in which the probiotics are incorporated.

Conclusion

A high level of *Bifidobacterium* BB-12 viability was maintained in the Greek-style yogurts throughout the 28 days of storage; however, the protective effect of microencapsulation on the probiotic cells was not evident. Meanwhile, there was an improvement in the survival of *S.*

thermophilus and *L. bulgaricus* cultures in the yogurt added with microcapsules produced only with sweet whey. The addition of either of the microcapsule formulations increased the total solids and ash content of the yogurts. The incorporation of microcapsules produced only with sweet whey decreased the firmness and the gumminess of the yogurt in comparison to the sample with free cells. Moreover, the yogurt containing microcapsules with sweet whey and inulin resulted in lower values of post-acidification. Besides that, the microcapsules produced with sweet whey and inulin were not effective in protecting *Bifidobacterium* BB-12 incorporated into Greek-style yogurts during exposure to simulated gastrointestinal conditions. Even though this present study showed that sometimes microencapsulation does not render cell protection, its results showed the feasibility of the incorporation of microencapsulated *Bifidobacterium* BB-12 in Greek-style yogurt since the general characteristics of the product were barely affected. Moreover, the microbiological results suggested that Greek-style yogurt is a good matrix for maintaining the viability of *Bifidobacterium* BB-12 during 28 days of storage at 4 ± 1 °C.

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