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Encapsulated *Bifidobacterium* BB-12 addition in a concentrated lactose-free yogurt: its survival during storage and effects on the product's properties

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Abstract

This work aims to manufacture a new concentrated lactose-free probiotic yogurt. For this purpose, the probiotic *Bifidocaterium* BB-12 was incorporated in a concentrated lactose-free yogurt, both in its free form and previously encapsulated. Previous cell encapsulation was performed using the spray-drying technique with the following wall materials: lactose-free milk, lactose-free milk and inulin, and lactose-free milk and oligofructose. Thus, three different probiotic powders were obtained and added separately to three fractions of concentrated lactose-free yogurt. The probiotic survival of both powders and yogurts was evaluated during refrigerated storage. Likewise, the viability of starter cultures in yogurt (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was controlled. In addition, the physicochemical properties of the four yogurts were also measured (color, pH and acidity, and texture properties). All three powders showed good probiotic viability (>8 log CFU g^{-1}) throughout 120 days of storage at 4 ºC. In turn, yogurt formulations (with the addition of powders or free bifidobacteria) presented probiotic viability above 7 log CFU g^{-1} after storage; as well as the starter cultures (> 8 log UFC g^{-1}). Yogurt with probiotic powder from lactose-free milk showed a more yellowish color; however, these differences would not be detected by the human eye ($\Delta E < 3.00$). The yogurt with bifidobacteria free cells showed a greater post-acidification process (pH 4.18 to 4.02 and titratable acidity 1.52 to 1.89). It was not observed differences for firmness values of yogurt with free cells addition and yogurt with lactose-free milk and oligofructose powder addition. A slight significant decrease in the cohesiveness was observed in the yogurt elaborated with bifidobacteria free cells. The gumminess showed fluctuating values between all

concentrated lactose-free yogurts. At the end of this study, we conclude that these probiotic powders can be incorporated into innovative lactose-free yogurts.

Keywords Bifidobacteria, Lactose-free, Skyr-style yogurt, Probiotic food, Inulin, Texture.

1. Introduction

Probiotic microorganisms are known to provide several consumer health benefits when administered in adequate amounts. Strain characteristics and mechanisms of *Bifidobacterium* BB-12 against pathogen inhibition, barrier function enhancement, and immune interactions are mechanisms demonstrated for this type of bacteria. *Bifidobacterium* BB-12 has proven its beneficial health effect in numerous clinical studies within gastrointestinal health and immune function. Furthermore, bifidobacteria have been shown to improve bowel function, have a protective effect against diarrhea, and reduce side effects of antibiotic treatment, such as antibiotic-associated diarrhea. In terms of immune function, clinical studies have shown that bifidobacteria increases the body's resistance to common respiratory infections as well as reduces the incidence of acute respiratory tract infections microbiota (Jungersen et al., 2014). Therefore, it is recommended that the minimum daily intake of probiotic viable cells should be between 10^6 and 10^7 CFU per g of food product (Wang, Lin, & Zhong, 2020).

Hansen, Allan-Wojtas, Jin, and Paulson (2002) highlighted that although bifidobacteria are being increasingly recognized as probiotics that have advantageous properties, they are also fastidious, obligate anaerobes, and, therefore, pose a technological challenge for the food industry. Therefore, the encapsulation of probiotic cells shows advantages and possibilities of incorporating produced particles into food matrices. Microencapsulation techniques, as spray drying, were used to achieve better probiotic survival during food processing, storage, and passage through the gastrointestinal tract (Dos Santos et al., 2019; Pinto et al., 2015; Verruck et al., 2017). The spray drying technique enables the use of different types of encapsulating materials, among them, prebiotics and milk products have been employed for many types of

research. Prebiotic like inulin or oligosaccharide is not metabolized by humans. The prebiotic property allows for unique applications such as determination of colonic targeting, making use of metabolization by microbiota present in the colon, or by probiotic bacterium present in the microparticles from the encapsulation process. Moreover, the presence of prebiotics in the composition of microparticles can result in a longer dissolution time in the water. According to Pinto *et al*. (2015), this long dissolution time ensures good control of the probiotic release when in contact with the dairy product water, such as of a concentrated yogurt.

Since lactose intolerance affects approximately 75% of the adult world population (Suri et al., 2019), products free of this disaccharide are essential to make up the diet of intolerant people. To avoid traces of lactose contamination in dairy products, lactose-free milk and prebiotics were used by Dantas, Verruck, de Liz, Hernandez, and Prudencio (2021a), aiming the future application of probiotic spray-dried powders in lactose-free dairy products. In this study, lactose-free skim milk was used as an innovative wall material for *Bifidobacterium* BB-12 encapsulation. Dantas et al. (2021a) observed better survival rates in spray-dried powders with encapsulated bifidobacteria (80 to 88%) than bifidobacteria-free cells (~75%), after in vitro gastrointestinal condition stages. These results encourage us to obtain an innovative dairy product.

A variety of dairy products has been elaborated with the addition of different probiotic microorganisms (Angelopoulou et al., 2017; Aspri, Leni, Galaverna, & Papademas, 2018; Machado et al., 2017; Meira et al., 2015; Nadelman et al., 2017; Ranadheera, Evans, Adams, & Baines, 2016; Silva, Costa, Vieira, & Conte-Junior 2019; Verruck et al., 2020). Particularly, yogurt and other fermented milk products are the most common food carriers for probiotic bacteria (Pinto et al., 2019). Besides, concentrated fermented milks, for example, the skyr, has become popular in the past

few years (Körzendörfer, Schäfer, Hinrichs, & Nöbel, 2019). Skyr is a traditional product of Iceland made from skimmed sheep and cow milk, and fermented including a traditional starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) (Macori & Cotter, 2018). The skyr has even gained popularity as a low-fat and high-protein food, being a very important dairy product of the inhabitants of Iceland, providing essential nutrients (Steingrímsdóttir, Thorkelsson, & Eythórsdóttir, 2018). As above mentioned, lactose intolerance, a gastrointestinal disorder, can limit consumption of these types of food products. This gastrointestinal disorder is characterized by symptoms such as flatulence, diarrhea, abdominal pain, and bloating (Roškar et al., 2017). Milk fermentation is a process that naturally reduces the lactose content (Harju, Kallioinen, & Tossavainen, 2012); nevertheless, *skyr*-style yogurt can contain significant intact amounts of this disaccharide. It is noteworthy that for a food product to be claimed as lactose-free, the concentration of lactose must be lower than 0.1 g 100 g^{-1}, according to the advice of the European Food Safety Agency (Trani et al., 2017) and a new resolution in Brazilian legislation (Brasil, 2017). Pereira et al. (2021) elaborated and evaluated a skyr yogurt with mango pulp, fructooligosaccharide, and natural sweeteners. The potential of lactose-free greek-style yogurt as a new matrix for incorporation of spray-dried microparticles containing the probiotic *Bifidobacterium* BB-12 was evaluated by Pinto, Fritzen-Freire, Dias, and Amboni (2019). However, to our knowledge, the present study is the first report about lactose-free skyr-style yogurt.

Considering that different combinations of wall materials can result in spray-dried powders with different physical characteristics and structures, and based on the previous results obtained by Dantas *et al*. (2021a) and Dantas *et al*. (2021b), we noted the importance to evaluate the impact of the application of these spray-dried powders on the lactose-free skyr-style yogurt. In this context, this study aimed to investigate the

potential of spray drying encapsulation of *Bifidobacterium* BB-12 with lactose-free milk, lactose-free milk and inulin, and lactose-free milk and oligofructose in the production of a concentrated lactose-free yogurt. Therefore, the texture, physicochemical, and microbiological properties of the dairy product were assessed throughout 30 days of storage at 5 ºC. It was also evaluated the survival of the encapsulated probiotic bacteria during 120 days of storage under refrigeration temperature.

2. Material and methods

2.1 Materials

The probiotic *Bifidobacterium animalis* ssp. *lactis* BB-12 (NU-TRISH® BB-12®, Chr. Hansen, Hørsholm, Denmark) was employed as the active material for the microparticle. Lactose-free skim milk powder (Aurora®, Cooperativa Central Aurora Alimentos, Santa Catarina, Brazil) (85.51 g total solids 100 g^{-1}, 32.50 g protein 100 g^{-1}, 0.00 g fat 100 g^{-1}, 3.01 g ash 100 g^{-1} and 50.00 g carbohydrates 100 g^{-1}), inulin (DP ≥ 10, Orafti® Gr, Orafti, Tienen, Belgium) and oligofructose (2 ≤ DP ≤ 8, Orafti® P95, Orafti, Tienen, Belgium) were used as wall materials. Sodium propionate (Fluka, Neu-Ulm, Germany), lithium chloride (Vetec, Rio de Janeiro, Brazil), M17 agar (Sigma-Aldrich, St. Louis, MO, USA), AnaeroGen® (Oxoid, Hampshire, UK), MRS Agar (Merck, Darmstadt, Germany) and MRS Broth (Difco, Sparks, USA) were used for the microbiological assays. For the preparation of the concentrated lactose-free yogurt was used lactose-free skim milk (Aurora®, Cooperativa Central Aurora Alimentos, Santa

Catarina, Brazil) (8.80 g total solids 100 mL^{-1}, 3.30 g protein 100 mL^{-1}, 0.32 g fat 100 mL^{-1}, 0.18 g ash 100 mL^{-1} and 5.00 g carbohydrates 100 mL^{-1}) and thermophilic starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Yo-Flex® L812, Chr. Hansen, Horrsholm, Denmark).

2.2 Obtaining and characterization of probiotic spray-dried powders

2.2.1 Encapsulation of Bifidobacterium BB-12

Three feed solutions were prepared for the production of spray-dried powders containing *Bifidobacterium* BB-12. The feed solutions prepared with sterile distilled water were obtained from lactose-free skim milk powder (200 g L^{-1}); lactose-free skim milk powder (100 g L^{-1}) and inulin (100 g L^{-1}); and lactose-free skim milk powder (100 g L^{-1}) and oligofructose (100 g L^{-1}); and were denoted as 1, 2, and 3, respectively. The spray-dried powders derived from feed solutions 1, 2, and 3 were also denoted as 1, 2, and 3. A precipitate of probiotic cells was added to each feed solution. A laboratory-scale spray dryer (B 290 mini spray dryer, Buchi, Flawil, Switzerland) equipped with a cyclone was used to obtain the three spray-dried powders. The spray dried under optimum conditions used was 150 °C inlet temperature and 44 °C outlet temperature to obtain probiotic three spray-dried powders. The mini spray dryer, with an integrated standard two-fluid nozzle (0.7 mm liquid orifice diameter, a 1.1 mm liquid outer diameter, and a 1.5 mm gas orifice diameter), using compressed air, was used to disperse each one feed solutions into fine droplets. The compressor air pressure, drying airflow rate, and feed rate were set at 0.7 MPa, 35 m3 h^{-1}, and 12 mL min^{-1},

respectively. The powder and wet air were separated and collected by the cyclone. As observed by Dantas et al. (2021b), the three spray-dried powders formulation and operating conditions result in clusters formation, and therefore, the spray-dried powders contain microparticles, which can be called microspheres. However, it was chosen the denomination of microparticle in the present study.

2.2.2 Effect of storage on microparticle probiotic viability

Spray-dried powders (1, 2, and 3) with bifidobacteria microparticles were stored for 120 days at refrigeration temperature (4 °C). Viable cells counts were performed every 30 days; for this, we used the following methodological steps: first, the entrapped bacteria were released from the microparticle according to Sheu, Marshall, and Heymann (1993) with some modifications. One gram of spray-dried powder was resuspended in 9 mL of sterile phosphate buffer (0.1 mol L^{-1}, pH = 7) followed by homogenization in a vortex (VTX-F-100; Biomixer, São Paulo, Brazil) for 10 min. Second, the mixtures were serially diluted in peptone water (Oxoid; 0.1 g 100 mL^{-1}), and inoculated on MRS agar modified by the addition of lithium chloride (0.2 g 100 g^{-1}) and sodium propionate (0.3 g 100 g^{-1}), as described by Vinderola and Reinheimer (1999). And finally, the plates were incubated at 37 °C for 72 h in anaerobic jars using AnaeroGen®. After the incubation period, the number of colonies formed on the agar was counted and expressed in log colony-forming units per gram (log CFU g^{-1}).

2.3 Elaboration and characterization of probiotic concentrated lactose-free yogurts

2.3.1 Manufacture of probiotic concentrated lactose-free yogurts

Free *Bifidobacterium* BB-12 and the different spray-dried powders were incorporated into the concentrated lactose-free yogurt to produce novel dairy products. At the end of the fermentation process of the lactose-free skyr-style yogurt, each one of the spray-dried powders was incorporated individually for each formulation, in the amount of 10 g 100 g^{-1}. In turn, the suspension of free bifidobacteria (10 log CFU g^{-1}) was added in the amount of 10 mL 100 g^{-1}. The yogurt manufacture process began with the heating of the lactose-free skim milk until 42 °C. At this point, the milk was mixed with starter culture according to the producer's recommendation, fermented until 6 h, and cooled to 18 ºC to stop the fermentation. The fermented milk was centrifuged (8000*g*) (Nova Técnica NT825, São Paulo, Brazil) at this same temperature for 15 min, as suggested by Moineau-Jean, Champagne, Roy, Raymond, and LaPointe (2019). The whey was discarded and the concentrated curds were pooled. Then, the incorporation of the additives was performed, giving rise to the distinct probiotic concentrated lactose-free yogurts, which are presented in Table 1. Finally, the yogurts were transferred to a refrigerator (5 °C ± 1) and stored for thirty days. The microbiology of the product and its chemical and physical properties were studied at storage days 0, 15, and 30.

2.3.2 Microbiological analysis

Twenty-five grams of each yogurt were diluted in 225 mL of phosphate buffer (pH 7.0, 0.1 mol L^{-1}) followed by homogenization using a magnetic stirrer for 10 min. Serial dilutions were made according to the methodology by Vinderola and Reinheimer (1999) already described (section 2.2.2). So, plates incubation and enumeration of *Bifidobacterium* BB-12 also were performed according to section 2.2.2.

The *S. thermophilus* count was carried out by the pour plate technique using M17 agar with the addition of lactose solution (10 g 100 mL^{-1}), incubated aerobically at 37 ± 1°C for 48 h (IDF, 1997). Enumeration of *L. delbrueckii* subsp. *bulgaricus* was realized using MRS agar under aerobic conditions at 37 ± 1 °C for 72 h (Dave & Shah, 1996). The total viable count (determined in triplicate) was expressed as log of colony-forming units per gram of yogurt (log CFU g^{-1}).

2.3.3 Physical and chemical properties

The textural properties of the yogurts were measured using a texturometer model TA-HD plus (Stable Micro System, Texture Analyser, Surrey, UK). The double compression analysis was carried out using a 25 mm-diameter aluminum probe (P25/L). The analysis was performed in a 50 mL glass capsule with the samples at a temperature of 6 ± 1 ºC. The test velocity, the time, and the distance were set at 1.0 mm s^{-1}, 5.0 s, and 10.0 mm, respectively. The parameters firmness, gumminess, and cohesiveness were obtained by the software Exponent version 6.1.1.0. (Stable Micro Systems, Surrey, United Kingdom).

A pH meter (model PHS3BW, BEL Engineering, Monza, Italy) was employed to measure the pH values, while the titratable acidity (% lactic acid) was determined following AOAC (2005).

Color measurements were carried out using a Chromameter CR-400 (Konica Minolta, Osaka, Japan) with illuminant D65. The results were expressed in accordance to CIELab coordinate color space system; that is, the L^* component (lightness, it ranges from black to white), and the a^* (+, red; −, green) and b^* (+, yellow; −, blue) parameters were determined. Hue angle (h) and Chroma (C^*) values also were

presented. The total color difference (ΔE*), between values observed in the final storage time (day 30) with initial time (day 0) of each concentrated yogurts were calculated, as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL* is the difference of luminosity, Δa* is the difference of the parameter a*, and Δb* represents the difference of the parameter b*, for the same sample.

2.4 Statistics

The probiotic concentrated lactose-free yogurts were manufactured through the three experimental trials and were carried out in independent days. All samples analyses were done in triplicate. To evaluate significant differences ($P < 0.05$) between treatments, a one-way analysis of variance (ANOVA) and the *t*-test were used. The software STATISTICA version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) was employed for all statistical analyses, and the results were expressed as mean ± standard deviation.

3. Results and discussion

3.1 Survival of microencapsulated *Bifidobacterium* BB-12 during storage

On the day of obtaining the spray-dried powders, all samples showed viable cells count higher than 9 log CFU g^{-1}, which characterize them as a probiotic product. The characterization and the monitoring of the microparticle during storage at different

temperatures are also important approaches to improve and determine the optimal conditions when adding them to food products (Verruck et al., 2018).

The viable *Bifidobacterium* BB-12 cell counts throughout the storage time of 120 days at 4 ± 1°C are shown in Figure 1. Our findings suggest that lactose-free milk powder or its partial replacement with inulin or oligofructose in the spray drying process caused no negative effect on the bacteria survival. Verruck et al. (2018), who also studied the refrigerated storage of probiotic powders, did not observe a reduction in the viable cell count of *Bifidobacterium* BB-12 in a spray-dried powder based on full-fat goat's milk and inulin or oligofructose. However, in the present study, the greatest change was found for the spray-dried powder 1, in which there was a decrease in viability over time, varying from 1.95–6.83%, but like the others, remained within the probiotic pattern (equal to or greater than 6 log CFU g^{-1} of the product). As documented by our previous study (Dantas et al., 2021a), spray-dried powder 1 presented the largest initial moisture and a_w (7.67 g 100 g^{-1} and 0.396, respectively). Liu et al. (2016) stated that the higher the moisture content, the lower the bacterial survival during storage, and that therefore, an ideal moisture content would be around 4 g 100 g^{-1}. Himmetagaoglu and Erbay (2019) highlighted that in a water activity lower than 0.3, a better shelf-stability is achieved since microbiological growth and chemical reactivity (such as non-enzymatic browning) is at a minimum. Nevertheless, the good results for all samples may be justified by the fact that the bacterial cells are in a latent state in the conditions of storage at 4°C, and consequently, the rates of the chemical reaction are reduced (de Lara Pedroso, Thomazini, Heinemann, & Fávaro-Trindade, 2012). Muhammad, Ramzan, Huo, Tian, and Bian (2017) also affirmed that storage temperature and residual moisture content are crucial factors for the increase or decrease of the probiotic viability by influencing the lipid oxidation of the membrane. Finally, our results are in agreement

with those found by Rodriguez-Restrepo, Giraldo, and Rodriguez-Barona (2017), who studied the viability of *Bifidobacterium animalis* subsp. *lactis* encapsulated by spray drying with gum arabic and whole milk powder, after 140 days of storage at 6 ± 2 °C.

3.2 Characterization of the probiotic concentrated lactose-free yogurts

3.2.1 Viability of the probiotic and starter cultures

For all the formulations of yogurt, the viable cell count of *Bifidobacterium* BB-12 was more than 7 log CFU g^{-1}, as recommended to get healthy benefits (Table 2). Abd El-Salam et al. (2011) affirmed that the high viability of probiotics-free cells added in concentrated fermented milk (in their case, labneh) may be related to the high total solids content. They noted that the counts of *L. casei* and *L. acidophilus* remained above 8 log CFU g^{-1} throughout the storage period. According to Iravani, Korbekandi, and Mirmohammadi (2015), one of the factors that restrict the stability of probiotic bacteria-free cells in fermented products is the low pH. It was observed a decrease of the pH values, and at the same time, a slight decrease ($P < 0.05$) in the viability of *Bifidobacterium* BB-12 free cells, however, this not affected the probiotic properties of concentrated lactose-free yogurt. Similar behavior was observed by Pinto, Fritzen-Freire, Dias, and Amboni (2019), who investigated the incorporation of *Bifidobacterium* BB-12 free cells and encapsulated in Greek-style yogurt.

In the previous study, published by Dantas et al. (2021a), the focus was on the shelf-life stability of bifidobacteria using the same formulation of spray-dried powders employed in the present work. Moreover, the authors studied bifidobacteria's *in vitro* gastrointestinal survival, which is a more accurate predictor of performances studies for

probiotic bacterial survival. This study also confirmed that bifidobacteria free cells cannot be replaced by the spray-dried powders because after the under *in vitro* simulated gastrointestinal conditions assay, the free cells survival rate was minor than any other spray-dried powder (1, 2, or 3) formulated, prepared again, and used in the present study. Survival rates (%) of *Bifidobacterium* BB-12 after under simulated gastrointestinal for free cells and spray-dried powders 1, 2 and 3 were equals to 75.53%, 80.01%, 81.29%, and 87.59%, respectively (Dantas et al., 2021a). In turn, results presented by Dantas et al. (2021b) were necessary to understand the behavior of the wall materials employed on the survival of *Bifidobacterium* BB-12. If this study did not demonstrate good results, there would be no reason to apply these spray-dried powders formulations in skyr-style yogurt or any other product. Therefore, it was detected previously the additional protection guarantee of *Bifidobacterium* BB-12 microencapsulation process, using feed solutions with lactose-free skim milk powder, lactose-free skim milk powder and inulin, and lactose-free skim milk powder and oligofructose. Based on these previous results, it was possible to conclude that the potential protective effect of encapsulation occurs during the passage of the bifidobacteria through the gastrointestinal tract, and it is not only represented by survival rates (%) during 30 days of storage observed in the present study. These survival rates (%) were equals to 92.54%, 90.91%, 87.84%, and 86.03%, for yogurt added of *Bifidobacterium* BB-12 free cells, yogurt added of spray-dried powder 1, yogurt added of spray-dried powder 3, and yogurt added of spray-dried powder 2, respectively.

Viable *S. thermophilus* count showed high values and slight variations ($P < 0.05$) during storage time (Table 2). Dimitrellou et al. (2016), Pinto, Fritzen-Freire, Dias, and Amboni (2019), and Varga, Sule, and Nagy (2014) reported analogous results

for yogurt or similar fermented milk products stored under refrigeration for 4–6 weeks. *S. thermophilus* generally survives well (>10^8 CFU mL^{-1}) in these products. Moreover, De Souza Oliveira, Perego, De Oliveira, and Converti (2011) observed reduction in the generation time (t_g) for *S. thermophilus* and *L. bulgaricus* when inulin was used in skim milk fermented, suggesting an effect of the prebiotic on these pure cultures, and not just for the other bacteria (probiotic strains) used in the study. In this work, the viability of *L. bulgaricus* was higher ($P < 0.05$) on day of manufacture in the yogurt added with spray-dried powder 2 (microcapsule from milk and inulin) compared with the other yogurts. In summary, our findings showed satisfactory values of viability for starter cultures in all yogurt samples, since they were greater than 7 log CFU g^{-1}, and thus are in accordance with the quality parameters established by Brazilian legislation (Brasil, 2007) for fermented milks.

3.2.2 Color measurements

Table 3 shows the color parameters for the yogurts during the storage period. Overall, it was observed that the L^* values (lightness) were not different ($P > 0.05$) among the yogurts with spray-dried powders addition on day 1, being that all samples displayed high lightness values. However, for yogurt with free cells of bifidobacteria was observed a decrease in lightness values on day 15, followed by an increase on day 30. Similar results were verified by De Campo et al. (2019), who incorporated zeaxanthin nanoparticles in yogurt. Regarding L^* values, these authors observed a decrease on day 7 following by an increase on day 28. De Campo et al. (2019) related that the slight decrease in L^* values can be related to the casein present in milk. When used free cells, the protein interacts with the protease of the bacterial culture, and the

proteolysis reaction can reduce the *L** intensity (De Campo et al., 2019). Moreover, these authors also affirmed that fat globules and casein micelles contribute to the white appearance due to their capacity to scatter light, which also can explain the increase in *L** values on day 30 in the present study.

It was possible to verify a variation ($P < 0.05$) in the yellow coloration (*b** values) among the yogurts. Verruck, Dantas, and Prudencio (2019) discussed that the yellowish coloration is natural in dairy products prepared with cow's milk because cows transfer high carotenoid levels from their diet to milk. Since Yogurt SDP1 was added to the probiotic spray-dried powder made exclusively from milk, this explains its more yellowish color ($P < 0.05$) compared to the other samples. Concerning the *a** parameter, despite significant differences ($P < 0.05$) between all samples for any storage day, it was observed a general tendency to greenish coloration, since all showed negative values. Canella et al. (2018) commented that this coloration may be attributed to the content of riboflavin naturally present in the milk. Furthermore, our results agree with those noted by Debon et al. (2012), who studied color measurements of prebiotic fermented milk during 28 days of storage.

Since the Hue angle shows the location of the color in a diagram (where 0°, 90°, 180°, and 270° represent pure red, pure yellow, pure green, and pure blue, respectively) (Jha, 2010), the values obtained for this parameter corroborate with the values of *a** and *b**, as they indicate that all yogurts showed a tendency towards slightly greenish-yellow. Despite these similarities, yogurt SDP1 showed the lowest *h* value among all samples, during the entire storage period, reinforcing the fact that it is more yellow than the others yogurt samples. Although of these differences, it is important to highlight that all samples of concentrated lactose-free yogurt showed low ΔE* values. According to

Martínez-Cervera et al. (2011), it is expected a ΔE* result ≤ 3, so the human eye does not notice that color differences.

Sołowiej et al. (2015) reported that C* represents color saturation, i.e., it is the combination of the parameters a* and b*, which shows the proportions in which the color is mixed with white, black, or gray. Overall, an increase of *C** values was observed during storage. According to Rozycki, Buera, Piagentini, Costa, and Pauletti (2010), this phenomenon is related to the accumulation of oxidation products that can react with amino groups forming yellow products. Canella et al. (2018) also verified an increase of the parameter *C** after 30 days of storage in a symbiotic fermented lactic beverage.

3.2.3 pH, acidity and texture analyses

As disclosed in Table 4, the pH values decreased ($P < 0.05$) for all yogurt formulations during the storage period. The yogurt FC showed lower pH values ($P < 0.05$) than the other yogurts during the entire storage period. Pinto et al. (2017) and Zomorodi (2019) also reported lower pH values in yogurts containing free bifidobacteria in comparison with their microencapsulated forms. The inverse pattern was observed for titratable acidity. This behavior is called post-acidification, and according to Pinto, Fritzen-Freire, Dias, and Amboni (2019), it probably results from the residual activity of the starter cultures. It is well known that *L. bulgaricus* and *S. thermophilus* ferment glucose. Ribeiro et al. (2014) discussed that post-acidification is undesirable for both the microbial viability and sensory quality of yogurt. According to Pan, Liu, Luo, and Luo (2019), this phenomenon results in the rupture of casein strands,

size decrease of casein micelles aggregates, and protein rearrangement in the yogurt gel, this leads to its shrinkage and consequent whey separation, i.e., syneresis.

Table 5 shows the evolution of texture parameters (firmness, gumminess, and cohesiveness) during refrigerated storage of probiotic concentrated lactose-free yogurts. De Campo et al. (2019) and Kesenkaş et al. (2017) also observed similar changes in the texture parameters during cold storage of yogurts. However, the addition of spray-dried powders 1 and 2 contributed to an increase ($P < 0.05$) in the firmness of the yogurt. The incorporation of powders tends to increase the total solids content, which leads to an increase in the firmness values. These results corroborate those obtained by Karaca, Saydam, and Guven (2019), since the addition of persimmon and apple powders significantly affected the instrumental firmness of low-fat and fat-free probiotic yogurts. In the Yogurt SDP2, the presence of inulin in the spray-dried powder 2 probably contributed to the increase of firmness parameter. Gyawali and Ibrahim (2016) highlighted that inulin is a good stabilizer because it immobilizes the water molecules in the food matrix, contributing to the increase of the firmness parameter. Costa et al. (2019) noted that samples of Greek yogurt with inulin addition presented higher firmness values due to the increase in gel strength.

The yogurt FC and the yogurt SDP3 not show differences ($P > 0.05$) between the firmness values on day 1. Costa et al. (2019) found similar results for Greek yogurt, a type of concentrated yogurt, and highlighted that the oligofructose was not able to influence the firmness values. This is because the presence of this prebiotic reduced the interaction forces of the protein matrix when compared to the addition of other prebiotics like inulin, polydextrose, and galactooligosaccharide. Furthermore, they cited that the smaller particle size of the oligofructose (73 μm and 89 μm for oligofructose and inulin, respectively) results in a lubricating effect, contributing to the non-increase

of firmness value. As the gumminess is the product of the firmness and the cohesiveness, the gumminess showed fluctuating values between the values obtained for both parameters (firmness and cohesiveness).

A slight decrease ($P < 0.05$) in the cohesiveness was observed in the Yogurt FC during the storage period. Pinto et al. (2017) discussed that cohesiveness is a measurement of how well a product resists a second deformation relative to its resistance under the first deformation. It can be interpreted as how firm the linkage inside the gel needs to be to withstand deformation. Accordingly, a diminution in cohesiveness value could denote a lower association between protein-protein bindings after 15 days of storage. Bedani, Campos, Castro, Rossi, and Saad (2014) and Pinto et al. (2019) also reported similar results during storage of probiotic soy yogurts added with inulin and/or okara flour, and for lactose-free Greek yogurts with free or encapsulated cells, respectively.

4. Conclusions

Overall satisfactory viability of *Bifidobacterium* BB-12 was found for all spray-dried powders produced with lactose-free skim milk powder, lactose-free skim milk powder and inulin, and lactose-free skim milk powder and oligofructose, when stored under refrigerated conditions for 120 days. Concerning the yogurts, the viability of *S. thermophilus* and *L. bulgaricus* was not critically affected during storage of the samples. Bifidobacteria survival rates (%) during 30 days increased as follows: yogurt with free cells > yogurt with spray-dried powder 1 > yogurt with spray-dried powder 3 > yogurt with spray-dried powder 2. Despite that, for all the formulations of yogurt, the viable cell count of *Bifidobacterium* BB-12 was higher than recommended to exert

health benefits. Therefore, we continue to conclude that bifidobacteria free cells cannot be replaced by these spray-dried powders formulations, because after the *in vitro* simulated gastrointestinal conditions previously realized, the free cells survival rate was minor than of these spray-dried powders. Thus, given the higher post-acidification observed for yogurt added of free cells, we conclude that the spray-dried powders are the best choices for developing a probiotic lactose-free skyr-style yogurt.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

Abd El-Salam, M. H., Hippen, A. R., El-Shafie, K., Assem, F. M., Abbas, H., Abd El-Aziz, M., Sharaf, O., & El-Aassar, M. (2011). Preparation and properties of probiotic concentrated yoghurt (labneh) fortified with conjugated linoleic acid.

International Journal of Food Science & Technology, 46, 2103–2110. https://doi.org/10.1111/j.1365-2621.2011.02722.x.

Angelopoulou, A., Alexandraki, V., Georgalaki, M., Anastasiou, R., Manolopoulou, E., Tsakalidou, E., & Papadimitriou, K. (2017). Production of probiotic Feta cheese using *Propionibacterium freudenreichii* subsp. *shermanii* as adjunct. *International Dairy Journal*, *66*, 135–139. https://doi.org/10.1016/j.idairyj.2016.11.011.

AOAC. (2005). *Official Methods of Analysis of the Association Analytical Chemists* (18th ed.). Gaithersburg, United States: Association of Official Analytical Chemists.

Aspri, M., Leni, G., Galaverna, G., & Papademas, P. (2018). Bioactive properties of fermented donkey milk, before and after in vitro simulated gastrointestinal digestion. *Food Chemistry*, *268*(1), 476–484. https://doi.org/10.1016/j.foodchem.2018.06.119.

Bedani, R., Campos, M. M. S., Castro, I. A., Rossi, E. A., & Saad, S. M. I. (2014). Incorporation of soybean by-product okara and inulin in a probiotic soy yoghurt: texture profile and sensory acceptance. *Journal of the Science of Food and Agriculture*, *94*(1), 119–125. https://doi.org/10.1002/jsfa.6212.

Brasil. (2007). Instrução Normativa nº 46, 23 de outubro de 2007 - Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. In MAPA (Ed.). Diário Oficial da União. Brasília, DF, Brasil.

Brasil. (2017). Resolução da Diretoria Colegiada - RDC nº 135, de 8 de fevereiro de 2017. In Ministério da Saúde (Ed.). Diário Oficial da União. Brasília, DF, Brasil.

Canella, M. H. M., Muñoz, I. B., Pinto, S. S., de Liz, G. R., Müller, C. M. O., Amboni, R. D. M. C., & Prudencio, E. S. (2018). Use of concentrated whey by freeze

concentration process to obtain a symbiotic fermented lactic beverage. *Advance Journal of Food Science and Technology*, *14*(2), 56–68. http://dx.doi.org/10.19026/ajfst.14.5832.

Costa, M. F., Pimentel, T. C., Guimaraes, J. T., Balthazar, C. F., Rocha, R. S., Cavalcanti, R. N., Esmerino, E. A., Freitas, M. Q., Raices, R. S. L., Silva, M. C., & Cruz, A. G. (2019). Impact of prebiotics on the rheological characteristics and volatile compounds of Greek yogurt. *LWT- Food Science and Technology*, *105*, 371–376. https://doi.org/10.1016/j.lwt.2019.02.007.

Dantas, A., Verruck, S., de Liz, G. R., Hernandez, E., & Prudencio, E. S. (2021a). Lactose-free skim milk and prebiotics as carrier agents of *Bifidobacterium* BB-12 microencapsulation: physicochemical properties, survival during storage and *in vitro* gastrointestinal condition behaviour. *International Journal of Food Science and Technology*, *56*(5), 2132–2145. https://doi.org/10.1111/ijfs.14823.

Dantas, A., Verruck, S., Canella, M. H. M., Maran, B. M., Murakami, F. S., de Avila Junior, L. B., de Campos, C. E. M., Hernandez, E., & Prudencio, E. S. (2021b). Current knowledge about physical properties of innovative probiotic spray-dried powders produced with lactose-free milk and prebiotics. *LWT – Food Science and Technology*, 151, 112175. https://doi.org/10.1016/j.lwt.2021.112175.

Dave, R. I., & Shah, N. P. (1996). Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and bifidobacteria. *Journal of Dairy Science*, *79*(9), 1529–1536. https://doi.org/10.3168/jds.S0022-0302(96)76513-X.

De Campo, C., Assis, R. Q., da Silva, M. M., Costa, T. M. H., Paese, K., Guterres, S. S., Rios, A. O. & Flôres, S. H. (2019). Incorporation of zeaxanthin nanoparticles in yogurt: Influence on physicochemical properties, carotenoid stability and

sensory analysis. *Food Chemistry*, *301*(15), Article 125230. https://doi.org/10.1016/j.foodchem.2019.125230.

de Lara Pedroso, D., Thomazini, M., Heinemann, R. J. B., & Favaro-Trindade, C. S. (2012). Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by microencapsulation using spray-chilling. *International Dairy Journal*, *26*(2) 127–132. https://doi.org/10.1016/j.idairyj.2012.04.008.

De Souza Oliveira, R. P., Perego, P., de Oliveira, M. N., & Converti, A. (2011). Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. *LWT - Food Science and Technology*, *44*(2) 520–523. https://doi.org/10.1016/j.lwt.2010.08.024.

Debon, J., Prudêncio, E. S., Petrus, J. C. C., Fritzen-Freire, C. B., Müller, C. M. O., Amboni, R. D. de M. C., & Vieira, C. R. W. (2012). Storage stability of prebiotic fermented milk obtained from permeate resulting of the microfiltration process. *LWT - Food Science and Technology*, *47*(1), 96–102. https://doi.org/10.1016/j.lwt.2011.12.029.

Dimitrellou, D., Kandylis, P., Petrović, T., Dimitrijević-Branković, S., Lević, S., Nedović, V., & Kourkoutas, Y. (2016). Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk. *LWT - Food Science and Technology*, *71*, 169–174. https://doi.org/10.1016/j.lwt.2016.03.007.

Dos Santos, D. X., Casazza, A. A., Aliakbarian, B., Bedani, R., Isay Saad, S. M., & Perego, P. (2019). Improved probiotic survival to in vitro gastrointestinal stress in a mousse containing *Lactobacillus acidophilus* La-5 microencapsulated with inulin by spray drying. *LWT – Food Science and Technology*, *99*, 404–410. https://doi.org/10.1016/j.lwt.2018.10.010.

Gyawali, R., & Ibrahim, S. A. (2016). Effects of hydrocolloids and processing conditions on acid whey production with reference to Greek yogurt. *Trends in Food Science & Technology*, *56*, 61–76. https://doi.org/10.1016/j.tifs.2016.07.013.

Hansen, L. T., Allan-Wojtas, P. M., Jin, Y. L. & Paulson, A. T. (2002). Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiology, 19*, 35–45. https://doi.org/10.1006/fmic.2001.0452.

Harju, M., Kallioinen, H., & Tossavainen, O. (2012). Lactose hydrolysis and other conversions in dairy products: technological aspects. *International Dairy Journal*, *22*(2), 104–109. https://doi.org/10.1016/j.idairyj.2011.09.011.

Himmetagaoglu, A. B., & Erbay, Z. (2019). Effects of spray drying process conditions on the quality properties of microencapsulated cream powder. *International Dairy Journal*, *88*, 60–70. https://doi.org/10.1016/j.idairyj.2018.08.004.

IDF 117B. (1997). *Enumeration of Characteristic Microorganisms*. Brussels, Belgium: International Dairy Federation Standard.

Iravani, S., Korbekandi, H., & Mirmohammadi, S. V. (2015). Technology and potential applications of probiotic encapsulation in fermented milk products. *Journal of food science and technology*, *52*(8), 4679–4696. https://doi.org/10.1007/s13197-014-1516-2.

Jha, S. N. (2010). Colour measurements and modeling. In: Shyam N. Jha. (eds.). *Nondestructive evaluation of food quality: theory and practice* (pp. 17–40). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-15796-7_2.

Jungersen, M., Wind, A., Johansen, E., Christensen, J. E., Stuer-Lauridsen, & Eskesen, D. (2014). The Science behind the Probiotic Strain *Bifidobacterium animalis* subsp. *lactis* BB-12®. *Microorganisms, 2,* 92–110. https://doi.org/ 10.3390/microorganisms2020092.

Karaca, O. B., Saydam, I. B., & Güven, M. (2019). Physical, chemical, and sensory attributes of low-fat, full-fat, and fat-free probiotic set yogurts fortified with fiber-rich persimmon and apple powders. *Journal of Food Processing and Preservation*, *43*(6), Article e13926. https://doi.org/10.1111/jfpp.13926.

Kesenkaş, H., Karagozlu, C., Yerlikaya, E., Özer, E., Akpinar, A., & Akbulut, N. (2017). Physicochemical and sensory characteristics of winter yoghurt produced from mixtures of cow's and goat's milk. *Tarim Bilimleri Dergisi-Journal of Agricultural Sciences*, *23*(1), 53–62.

Körzendörfer, A., Schäfer, J., Hinrichs, J., & Nöbel, S. (2019). Power ultrasound as a tool to improve the processability of protein-enriched fermented milk gels for Greek yogurt manufacture. *Journal of Dairy Science*, *102*(9), 7826–7837. https://doi.org/10.3168/jds.2019-16541.

Liu, H., Gong, J., Chabot, D., Miller, S. S., Cui, S. W., Ma, J., Zhong, F., & Wang, Q. (2016). Incorporation of polysaccharides into sodium caseinate-low melting point fat microparticles improves probiotic bacterial survival during simulated gastrointestinal digestion and storage. *Food Hydrocolloids*, *54*(Part B), 328–337. https://doi.org/10.1016/j.foodhyd.2015.10.016.

Machado, T. A. D. G., de Oliveira, M. E. G., Campos, M. I. F., de Assis, P. O. A., de Souza, E. L., Madruga, M. S., Pacheco, M. T. B., Pintado, M. M. E., & do Egypto Queiroga, R. C. R. (2017). Impact of honey on quality characteristics of

goat yogurt containing probiotic *Lactobacillus acidophilus*. *LWT - Food Science and Technology*, *80*, 221–229. https://doi.org/10.1016/j.lwt.2017.02.013.

Macori, G., & Cotter, P. D. (2018). Novel insights into the microbiology of fermented dairy foods. *Current Opinion in Biotechnology*, *49*, 172–178. https://doi.org/10.1016/j.copbio.2017.09.002.

Meira, Q. G. S., Magnani, M., de Medeiroa Júnior, F. C., do Egypto Queiroga, R. C. R., Madruga, M. S., Gullón, B., Gomes, A. M. P., Pintado, M. M. E., & de Souza, E. L. (2015). Effects of added *Lactobacillus acidophilus* and *Bifidobacterium lactis* probiotics on the quality characteristics of goat ricotta and their survival under simulated gastrointestinal conditions. *Food Research International*, *76*(Part 3), 828–838. https://doi.org/10.1016/j.foodres.2015.08.002.

Moineau-Jean, A., Champagne, C. P., Roy, D., Raymond, Y., & LaPointe, G. (2019). Effect of Greek-style yoghurt manufacturing processes on starter and probiotic bacteria populations during storage. *International Dairy Journal*, 93, 35–44. https://doi.org/10.1016/j.idairyj.2019.02.003.

Muhammad, Z., Ramzan, R., Huo, G.-C., Tian, H., & Bian, X. (2017). Integration of polysaccharide-thermoprotectant formulations for microencapsulation of *Lactobacillus plantarum*, appraisal of survivability and physico-biochemical properties during storage of spray dried powders. *Food Hydrocolloids*, *66*, 286–295. https://doi.org/10.1016/j.foodhyd.2016.11.040.

Nadelman, P., Frazão, J. V., Vieira, T. I., Balthazar, C. F., Andrade, M. M., Alexandria, A. K., Cruz, A. G., Fonseca-Gonsalves, A., & Maia, L. C. (2017). The performance of probiotic fermented sheep milk and ice cream sheep milk in

inhibiting enamel mineral loss. *Food Research International*, *97*, 184–190. https://doi.org/10.1016/j.foodres.2017.03.051.

Pan, L., Liu, F., Luo, S., & Luo, J. (2019). Pomegranate juice powder as sugar replacer enhanced quality and function of set yogurts: Structure, rheological property, antioxidant activity and in vitro bioaccessibility. *LWT - Food Science and Technology*, *115*, Article 108479. https://doi.org/10.1016/j.lwt.2019.108479.

Pereira, C. M. T., Pereira, D. M., Medeiros, A. C. de, Hiramatsu, E. Y., Ventura, M. B., & Bolini, H. M. A. (2021). Skyr yogurt with mango pulp, fructooligosaccharide and natural sweeteners: physical aspects and drivers of liking. *LWT – Food Science and Technology*, 150, 112054, https://doi.org/10.1016/j.lwt.2021.112054.

Pinto, S. S., Cavalcante, B. D. M., Verruck, S., Alves, L. F., Prudêncio, E. S., & Amboni, R. D. M. C. (2017). Effect of the incorporation of *Bifidobacterium* BB-12 microencapsulated with sweet whey and inulin on the properties of Greek-style yogurt. *Journal of Food Science & Technology*, *54*, 2804–2813. https://doi.org/10.1007/s13197-017-2717-2.

Pinto, S. S., Verruck, S., Vieira, C. R. W., Prudêncio, E. S., Amante, E. R., & Amboni, R. D. M. C. (2015). Influence of microencapsulation with sweet whey and prebiotics on the survival of *Bifidobacterium* BB-12 under simulated gastrointestinal conditions and heat treatments. *LWT – Food Science and Technology*, *64*(2), 1004–1009. https://doi.org/10.1016/j.lwt.2015.07.020.

Pinto, S., Fritzen-Freire, C. B., Dias, C. O., & Amboni, R. D. M. C. (2019). A potential technological application of probiotic microcapsules in lactose-free Greek-style yoghurt. *International Dairy Journal, 97*, 131–138. https://doi.org/10.1016/j.idairyj.2019.05.009.

Ranadheera, C. S., Evans, C. A., Adams, M., & Baines, S. K. (2016). Co-culturing of probiotics influences the microbial and physico-chemical properties but not sensory quality of fermented dairy drink made from goats' milk. *Small Ruminant Research*, *136*, 104–108. https://doi.org/10.1016/j.smallrumres.2016.01.016.

Ribeiro, M. C. E., Chaves, K. S., Gebara, C., Infante, F. N. S., Grosso, C. R. F., & Gigante, M. L. (2014). Effect of microencapsulation of *Lactobacillus acidophilus* LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. *Food Research International*, *66*, 424–431. https://doi.org/10.1016/j.foodres.2014.10.019.

Rodriguez-Restrepo, Y. A., Giraldo, G. I., & Rodriguez-Barona, S. (2017). Solubility as a fundamental variable in the characterization of wall material by spray drying of food components: application to microencapsulation of *Bifidobacterium animalis* subsp *lactis*. *Journal of Food Process Engineering*, *40*(6), 1–8. https://doi.org/10.1111/jfpe.12557.

Rozycki, S. D., Buera, M. P., Piagentini, A. M., Costa, S. C., & Pauletti, M. S. (2010). Advances in the study of the kinetics of color and fluorescence development in concentrated milk systems. *Journal of Food Engineering*, *101*(1), 59–66. https://doi.org/10.1016/j.jfoodeng.2010.06.009.

Roškar, I., Švigelj, K., Štempelj, M., Volfand, J., Štabuc, B., Malovrh, Š., & Rogelj, I. (2017). Effects of a probiotic product containing *Bifidobacterium animalis* subsp. *Animalis* IM386 and *Lactobacillus plantarum* MP2026 in lactose intolerant individuals: Randomized, placebo-controlled clinical trial. *Journal of Functional Foods*, *35*, 1–8. https://doi.org/10.1016/j.jff.2017.05.020.

Sheu, T. Y., Marshall, R. T., & Heymann, H. (1993). Improving survival of culture bacteria in frozen desserts by microentrapment. *Journal of Dairy Science*, *76*(7) 1902–1907. 10.3168/jds.S0022-0302(93)77523-2.

Silva, V. L. M., Costa, M. P., Vieira, C. P., & Conte-Junior, C. A. (2019). Short communication: Biogenic amine formation during fermentation in functional sheep milk yogurts. *Journal of Dairy Science*, *102*(10), 8704–8709. https://doi.org/10.3168/jds.2019-16379.

Sołowiej, B., Glibowski, P., Muszyński, S., Wydrych, J., Gawron, A., & Jeliński, T. (2015). The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. *Food Hydrocolloids*, *44*, 1–11. https://doi.org/10.1016/j.foodhyd.2014.08.022.

Steingrímsdóttir, L., Thorkelsson, G., & Eythórsdóttir, E. (2018). Food, nutrition, and health in Iceland. In Andersen, V., Bar, E., & Wirtanen, G. (Eds.). *Nutritional and health aspects of food in Nordic countries* (pp 145–177). London: Academic Press. https://doi.org/10.1016/B978-0-12-809416-7.00006-8.

Suri, S., Kumar, V., Prasad, R., Tanwar, B., Goyal, A., Kaur, S., Gat, Y., Kumar, A., Kaur, J., & Singh, D. (2019). Considerations for development of lactose-free food. *Journal of Nutrition & Intermediary Metabolism*, *15*, 27–34. https://doi.org/10.1016/j.jnim.2018.11.003.

Trani, A., Gambacorta, G., Loizzo, P., Cassone, A., Fasciano, C., Zambrini, A.V., & Faccia, M. (2017). Comparison of HPLC-RI, LC/MS-MS and enzymatic assays for the analysis of residual lactose in lactose-free milk. *Food Chemistry*, *233*, 385–390. https://doi.org/10.1016/j.foodchem.2017.04.134.

Varga, L., Sule, J., & Nagy, P. (2014). Short communication: survival of the characteristic microbiota in probiotic fermented camel, cow, goat, and sheep milks during refrigerated storage. *Journal of Dairy Science*, *97*(4), 2039–2044. https://doi.org/10.3168/jds.2013-7339.

Verruck, S., Barreta, C., Miotto, M., Canella, M. H. M., de Liz, G. R., Maran, B. M., Garcia, S. G., Silveira, S. M., Vieira, C. R. W., Cruz, A. G., & Prudencio, E. S. (2020). Evaluation of the interaction between microencapsulated *Bifidobacterium* BB-12 added in goat's milk Frozen Yogurt and *Escherichia coli* in the large intestine. *Food Research International*, *127*, Article 108690. https://doi.org/10.1016/j.foodres.2019.108690.

Verruck, S., Dantas, A., & Prudencio, E. S. (2019). Functionality of the components from goat's milk, recent advances for functional dairy products development and its implications on human health. *Journal of Functional Foods*, *52*, 243–257. https://doi.org/10.1016/j.jff.2018.11.017.

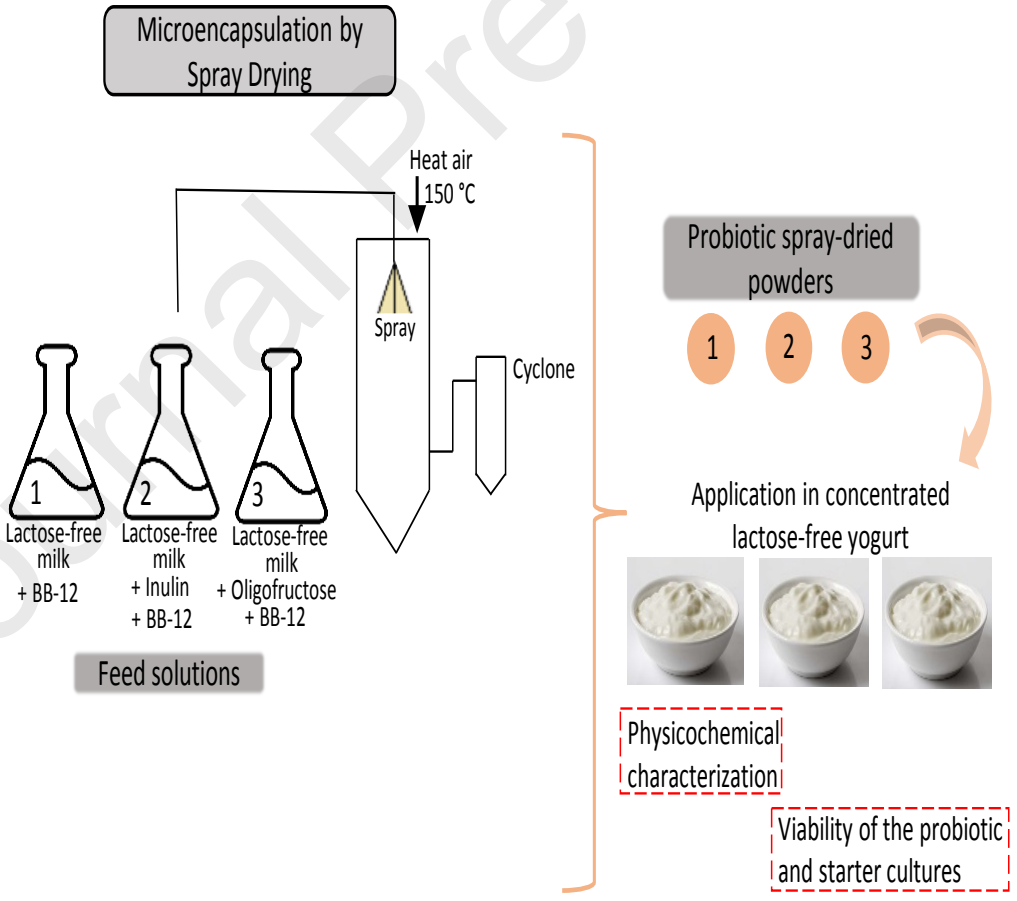
Verruck, S., de Carvalho, M. W., de Liz, G. R., Amante, E. R., Vieira, C. R. W., Amboni, R. D. de M. C., & Prudencio, E. S. (2017). Survival of *Bifidobacterium* BB-12 microencapsulated with full-fat goat's milk and prebiotics when exposed to simulated gastrointestinal conditions and thermal treatments. *Small Ruminant Research*, *153*, 48–56. https://doi.org/10.1016/j.smallrumres.2017.05.008.

Verruck, S., de Liz, G. R., Dias, C. O., Amboni, R. D, M. C., & Prudencio, E. S. (2018) Effect of full-fat goat's milk and prebiotics use on Bifidobacterium BB-12 survival and on the physical properties of spray-dried powders under storage conditions. *Food Research International*, *119*, 643-652 https://doi.org/10.1016/j.foodres.2018.10.042.

Vinderola, C. G., & Reinheimer, J. A. (1999). Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. *International Dairy Journal*, *9*(8), 497–505. https://doi.org/10.1016/S0958-6946(99)00120-X.

Wang, A., Lin, J., & Zhong, Q. (2020). Physical and microbiological properties of powdered *Lactobacillus salivarius* NRRL B-30514 as affected by relative amounts of dairy proteins and lactose. *LWT – Food Science and Technology*, *121*, Article 109044. https://doi.org/10.1016/j.lwt.2020.109044.

Zomorodi, S. (2019). Survival of free and microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* in Doogh (a yoghurt drink). *Pharmaceutical and Biomedical Research*, *5*(1), 19–24. https://doi.org/10.18502/pbr.v5i1.760.



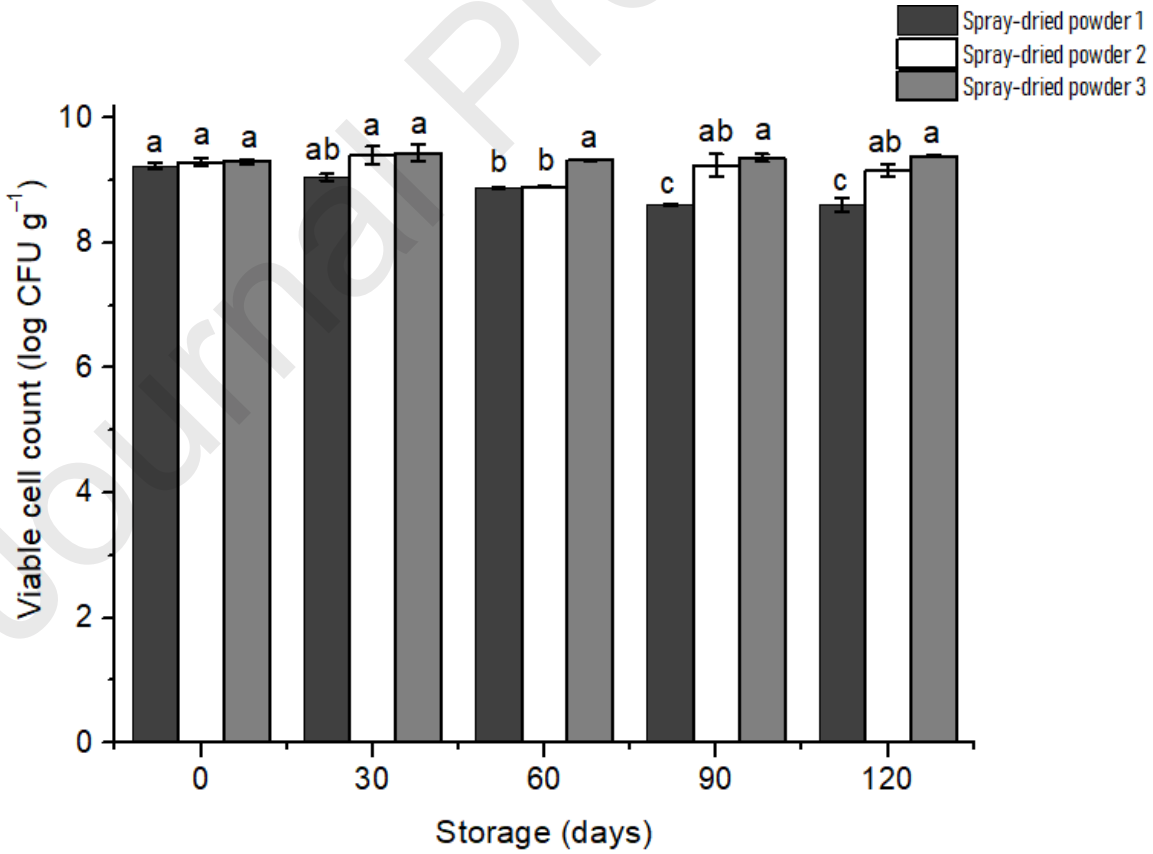
- Lactose-free milk and/or prebiotic spray-dried powders showed probiotic stability.

- Lactose-free milk and/or prebiotic spray-dried powders showed BAL stability.

- The probiotic survival rate was higher for the yogurt with free cells.

- Encapsulated probiotic addition with lactose-free milk showed the highest survival rate.

- Physicochemical behavior was favorable to probiotic skyr-style yogurts elaboration.



[a, b, c]Different letters in the top denote significant differences ($P < 0.05$) for a same sample during the storage period.

Figure 1. Results of the viable *Bifidobacterium* BB-12 cells count during 120 days at temperature of 4 ± 1 °C of spray-dried powders 1, 2 and 3 from feed solutions prepared with lactose-free skim milk powder (200 g L^{-1}); lactose-free skim milk powder (100 g L^{-1}) and inulin (100 g L^{-1}); and lactose-free skim milk powder (100 g L^{-1}) and oligofructose (100 g L^{-1}), respectively.

Table 1. Description of the different types of probiotic concentrated lactose-free yogurt.

Product	Definition
Yogurt FC	Concentrated lactose-free yogurt added of free *Bifidobacterium* BB-12
Yogurt SDP1	Concentrated lactose-free yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced only with lactose-free milk powder
Yogurt SDP2	Concentrated lactose-free yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced with lactose-free milk powder and inulin
Yogurt SDP3	Concentrated lactose-free yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced with lactose-free milk powder and oligofructose

Table 2. Viable cells count of *B. lactis* BB-12 and starter cultures in the concentrated lactose-free yogurt.

		Number of viable cells (log CFU g^{-1})		
Product	Day	*Bifidobacterium* BB-12	*Streptococcus thermophilus*	*Lactobacillus bulgaricus*
Yogurt FC	0	8.887 ± 0.130^{Aa}	9.261 ± 0.032^{Aa}	8.328 ± 0.016^{Ba}
	15	8.498 ± 0.031^{Ab}	9.175 ± 0.010^{Bab}	8.392 ± 0.160^{Ba}
	30	8.224 ± 0.278^{Ab}	8.987 ± 0.088^{ABb}	8.447 ± 0.022^{ABa}
Yogurt SDP1	0	8.866 ± 0.180^{Aa}	9.357 ± 0.001^{Ab}	8.335 ± 0.013^{Bb}
	15	8.504 ± 0.159^{Ab}	9.460 ± 0.003^{Aa}	8.618 ± 0.007^{ABa}
	30	8.060 ± 0.145^{Ac}	9.166 ± 0.023^{Ac}	8.572 ± 0.057^{Aa}
Yogurt SDP2	0	9.123 ± 0.092^{Aa}	9.379 ± 0.084^{Aa}	8.557 ± 0.001^{Aa}
	15	8.553 ± 0.087^{Ab}	9.629 ± 0.005^{Aa}	8.597 ± 0.008^{ABa}
	30	7.849 ± 0.298^{Ac}	8.786 ± 0.075^{Bb}	7.910 ± 0.128^{Bb}
Yogurt SDP3	0	8.975 ± 0.087^{Aa}	9.274 ± 0.003^{Aab}	8.411 ± 0.073^{Ba}
	15	8.731 ± 0.164^{Aa}	9.534 ± 0.131^{Aa}	8.703 ± 0.018^{Aa}
	30	7.884 ± 0.092^{Ab}	8.998 ± 0.022^{ABb}	8.248 ± 0.211^{ABa}

[A,B,C,D]Within a column, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different yogurts for the same storage period.

[a,b,c]Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different storage day, for each sample.

Yogurt FC: yogurt added of free *Bifidobacterium* BB-12; Yogurt SDP1: yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced only with lactose-free milk powder; Yogurt SDP2: yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced with lactose-free milk powder and inulin; Yogurt SDP3: yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced with lactose-free milk powder and oligofructose.

Table 3. Color parameters of the yogurt with free probiotic and concentrated lactose-free yogurt added of spray-dried powders during 30 days of storage at 5 °C.

	Day	Yogurt FC	Yogurt SDP1	Yogurt SDP2	Yogurt SDP3
*L**	0	93.85 ± 0.89^{Ab}	93.32 ± 0.48^{Aa}	94.50 ± 0.82^{Aa}	93.47 ± 1.07^{Aa}
	15	79.70 ± 0.96^{Cc}	91.60 ± 0.65^{Bb}	93.85 ± 0.45^{Aa}	93.29 ± 0.17^{Aa}
	30	95.49 ± 0.33^{Aa}	92.41 ± 0.78^{Bab}	93.43 ± 0.51^{Ba}	93.10 ± 0.60^{Ba}
*a**	0	-2.06 ± 0.01^{Dc}	-1.27 ± 0.01^{Ab}	-1.69 ± 0.03^{Cac}	-1.56 ± 0.02^{Bb}
	15	-1.41 ± 0.08^{Ca}	-1.08 ± 0.01^{Aa}	-1.21 ± 0.09^{Ba}	-1.19 ± 0.02^{ABa}
	30	-1.91 ± 0.01^{Db}	-1.10 ± 0.06^{Aa}	-1.37 ± 0.02^{Cb}	-1.19 ± 0.02^{Ba}
*b**	0	8.39 ± 0.15^{Cb}	10.57 ± 0.04^{Ac}	9.74 ± 0.04^{Bb}	9.80 ± 0.11^{Bb}
	15	10.68 ± 0.30^{Ba}	11.20 ± 0.06^{Ab}	10.28 ± 0.31^{Ba}	9.72 ± 0.10^{Cb}
	30	8.21 ± 0.18^{Db}	11.62 ± 0.07^{Aa}	9.82 ± 0.02^{Cb}	10.12 ± 0.10^{Ba}
*C**	0	8.64 ± 0.15^{Cb}	10.65 ± 0.04^{Ac}	9.89 ± 0.04^{Ba}	9.92 ± 0.12^{Bb}
	15	10.62 ± 0.29^{Ba}	11.25 ± 0.06^{Ab}	10.19 ± 0.30^{BCa}	9.79 ± 0.11^{Cb}
	30	8.40 ± 0.21^{Db}	11.68 ± 0.07^{Aa}	9.92 ± 0.02^{Ca}	10.19 ± 0.10^{Ba}
h	0	103.77 ± 0.16^{Aa}	96.86 ± 0.07^{Ca}	99.50 ± 0.54^{Ba}	98.89 ± 0.13^{Ba}
	15	97.15 ± 0.80^{Ab}	95.45 ± 0.12^{Bb}	97.10 ± 0.68^{Ab}	96.94 ± 0.13^{Ab}
	30	103.14 ± 0.38^{Aa}	95.88 ± 0.47^{Db}	97.92 ± 0.12^{Bb}	96.70 ± 0.16^{Cb}
ΔE*		1.68	1.40	1.12	0.61

[A,B,C,D]Within a line, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different yogurts for the same storage period.

[a,b,c]Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different storage days, for each sample.

L^*: lightness value, it defines black at 0 and white at 100; a^*: it is relative to the green–red opponent colors, with negative values toward green and positive values toward red; b^*: it represents the blue–yellow opponents, with negative numbers toward blue and positive toward yellow; C^*: polar coordinate (chroma, relative saturation); h: polar coordinate (hue angle); ΔE^*: the total color difference observed between the final storage time (day 30) and initial time (day 0).

Table 4. pH and titratable acidity of the concentrated lactose-free yogurts containing free or microencapsulated *Bifidobacterium* BB-12 during storage.

Product	Day	pH	Titratable acidity (g/100 g)
Yogurt FC	0	4.18 ± 0.04^{Ca}	1.52 ± 0.01^{Ab}
	15	4.10 ± 0.02^{Cb}	1.62 ± 0.02^{Ab}
	30	4.02 ± 0.01^{Cc}	1.89 ± 0.07^{Aa}
Yogurt SDP1	0	5.06 ± 0.01^{Aa}	1.44 ± 0.01^{Ba}
	15	4.89 ± 0.05^{Ab}	1.52 ± 0.04^{Ba}
	30	4.47 ± 0.02^{Ac}	1.44 ± 0.04^{Ba}
Yogurt SDP2	0	4.79 ± 0.01^{ABa}	1.46 ± 0.03^{ABa}
	15	4.60 ± 0.02^{Bb}	1.49 ± 0.01^{Ba}
	30	4.41 ± 0.03^{Bc}	1.49 ± 0.02^{Ba}
Yogurt SDP3	0	4.68 ± 0.18^{Ba}	1.48 ± 0.02^{ABa}
	15	4.60 ± 0.03^{Bab}	1.48 ± 0.01^{Ba}
	30	4.39 ± 0.01^{Bb}	1.50 ± 0.04^{Ba}

[A,B,C]Within a column, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different yogurts for the same storage period.

[a,b,c]Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different storage day, for each sample.

Yogurt FC: yogurt added of free *Bifidobacterium* BB-12; Yogurt SDP1: yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced only with lactose-free milk powder; Yogurt SDP2: yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced with lactose-free milk powder and inulin; Yogurt SDP3: yogurt added of spray-dried powder which contain *Bifidobacterium* BB-12 microcapsules produced with lactose-free milk powder and oligofructose.

Table 5. Textural properties of the concentrated lactose-free yogurts.

Product	Day	Firmness (g)	Gumminess (g)	Cohesiveness
Yogurt FC	1	55.61 ± 1.14^{Cb}	36.30 ± 1.23^{Cb}	0.653 ± 0.009^{Ba}
	15	44.50 ± 1.87^{Bc}	28.07 ± 1.18^{Bc}	0.631 ± 0.000^{ABa}
	30	71.85 ± 1.95^{Ba}	42.52 ± 0.86^{Ca}	0.592 ± 0.004^{Bb}
Yogurt SDP1	1	85.49 ± 2.36^{Aab}	51.66 ± 1.11^{Aa}	0.715 ± 0.021^{Aa}
	15	71.79 ± 13.43^{ABb}	62.23 ± 10.62^{Aa}	0.649 ± 0.013^{Aa}
	30	119.52 ± 6.02^{Aa}	72.17 ± 0.31^{ABa}	0.651 ± 0.033^{ABa}
Yogurt SDP2	1	62.98 ± 1.14^{Bb}	51.66 ± 0.99^{Bc}	0.683 ± 0.004^{ABa}
	15	95.68 ± 1.95^{Aab}	56.54 ± 1.63^{Ab}	0.610 ± 0.004^{Ba}
	30	129.42 ± 15.80^{Aa}	78.91 ± 2.63^{Aa}	0.614 ± 0.054^{ABa}
Yogurt SDP3	1	54.58 ± 0.98^{Cc}	37.43 ± 0.01^{Cb}	0.671 ± 0.008^{ABb}
	15	76.11 ± 2.60^{Ab}	49.45 ± 2.61^{ABab}	0.641 ± 0.000^{Ab}
	30	94.70 ± 6.76^{ABa}	60.42 ± 5.71^{Ba}	0.726 ± 0.013^{Aa}

[A,B,C]Within a column, different superscript uppercase letters denote significant differences ($P < 0.05$) among the different yogurts for the same storage period.

[a,b,c]Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the different storage day, for each sample.

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Florianópolis, May 12, 2021.

To Editor-in-Chief

Dr. Anderson Sant'Anna

Food Research International

Dear Editor,

Declaration of interest: Manuscript entitled: **"Microencapsulated *Bifidobacterium* BB-12 added in a concentrated lactose-free yogurt: its survival during storage and effects on the product's properties"**

The authors declare that they have no conflict of interest.

Best regards,

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CRediT author statement

The CRediT author statement of the manuscript intitled "**Microencapsulated *Bifidobacterium* BB-12 added in a concentrated lactose-free yogurt: its survival during storage and effects on the product's properties**" is described below:

Adriana Dantas: Conceptualization, Investigation, Validation, Formal analysis, Resources, Data curation, Writing – Original draft, Writing – Review and Editing, Visualization. **Silvani Verruck**: Conceptualization, Resources, Data curation, Writing – Original draft. **Maria Helena Machado Canella**: Resources, Data curation, Writing – Original draft. **Eduard Hernandez**: Conceptualization, Validation, Formal analysis, Resources, Data curation, Writing – Original draft, Writing – Review and Editing, Visualization, Supervision. **Elane Schwinden Prudencio**: Conceptualization, Investigation, Validation, Formal analysis, Resources, Data curation, Writing – Original draft, Writing – Review and Editing, Visualization. Writing – Review & Editing. Supervision, Project administration, Funding acquisition.

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