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EFFECT OF MODIFIED CHITOOLIGOSACHARIDES ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF A PROBIOTIC FERMENTED BEVERAGE

Efeito de quito-oligosacarídeos modificados nas características físico-químicas e microbiológicas em uma bebida probiótica fermentada

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ABSTRACT

The effect of the addition of chitooligosaccharides modified with glucose (COS-Glc), with potential prebiotic activity, on a smoothie beverage was evaluated during the storage. Two probiotic smoothie formulations made with probiotic yogurt (60%) and mango pulp (40%) were prepared, as follows: formulation F1 containing 2.0 % COS-Glc, and formulation F2 without the addition of COS-Glc (control). Commercial probiotic culture of *Bifidobacterium lactis* ssp. was used in both formulations. The products were evaluated for hygienic-sanitary quality, physicochemical composition, titratable acidity, syneresis, and bacteria viability (selective enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium* spp.) after one day of manufacture. The viability of the microorganisms, the hygienic-sanitary quality and the physicochemical characteristics (titratable acidity and syneresis) of the beverages were evaluated throughout 30 days of storage at 8 ± 1 °C. The hygienic-sanitary quality was satisfactory, and the beverages were suitable for consumption. The addition of

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modified chitooligosaccharides to the probiotic beverage led to an increase in titratable acidity and syneresis. The formulation F1 presented significantly higher total solids, total dietary fiber, and carbohydrates when compared to F2. The addition of COS-Glc did not affect the viability of the probiotics in the beverage along with the storage, which remained within the recommended dose to exert its therapeutic effect. The combination of COS-Glc and probiotics can be an interesting tool to produce smoothie type dairy beverages without adversely affecting the characteristics of the product, besides conferring added value to the product due to the incorporation of an ingredient with potential prebiotic activity.
Keywords: chitosan**;** bifidobacteria; viability; yogurt; fruit pulp; prebiotic.

RESUMO

O efeito da adição de quito-oligossacarídeos modificados com glucose (COS-Glc), com potencial atividade prebiótica em uma bebida *smoothie* foi avaliado durante a estocagem. Duas formulações de bebida probiótica foram elaboradas com iogurte probiótico (60%) e polpa de manga (40%), sendo a formulação F1 adicionada de 2,0% de COS-Glc e a formulação F2 (controle) sem COS-Glc. Uma cultura probiótica comercial (*Bifidobacterium lactis* ssp.) foi utilizada em ambas as formulações. Os produtos foram avaliados quanto à qualidade higiênico-sanitária, composição físico-química, acidez titulável, sinérese e viabilidade dos micro-organismos (contagem seletiva de *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus e Bifidobacterium* spp.) após 1 dia de fabricação. Verificou-se a viabilidade dos microrganismos, a qualidade higiênico-sanitária e as características físico-químicas (acidez titulável e sinérese) das bebidas ao longo de 30 dias mantidas à 8 ± 1 °C. A qualidade higiênico-sanitária foi satisfatória e as bebidas se mostraram aptas para o consumo. A adição de quito-oligossacarídeos modificados na bebida probiótica favoreceu o aumento da acidez titulável e da sinérese. A formulação F1 apresentou valores significativamente superiores de sólidos totais, fibras totais e carboidratos quando comparados à F2. A adição de COS-Glc não afetou a viabilidade dos micro-organismos probióticos na bebida ao longo da estocagem, os quais permaneceram em doses recomendadas para exercer o efeito terapêutico. A combinação de COS-Glc e probióticos podem ser utilizados juntos para a produção de bebidas lácteas tipo *smoothie* sem afetar as caraterísticas do produto, conferindo ainda valor adicional ao produto devido a incorporação de um ingrediente com potencial atividade prebiótica.

Palavras-chave: quitosana; bifidobactéria; viabilidade; iogurte; polpa de fruta; prebiótico.

INTRODUCTION

Functional foods provide health benefits beyond meeting basic nutritional needs, once they have bioactive compounds, such as dietary fiber, prebiotic oligosaccharides, antioxidants and active "friendly" bacteria that promote the equilibrium of intestinal bacterial strains (PERRICONE *et al.*, 2015).

Functional beverage consists of a drink product supplemented or enriched with functional ingredients such as vitamins,

minerals, bio-active peptides, probiotics, prebiotics, etc. The first commercialized functional dairy beverages were probiotic/prebiotic/symbiotic dairy-based beverages, which still dominates the market of functional dairy beverages (TURKMEN *et al.*, 2019).

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (TURKMEN *et al.*, 2019). The probiotic strains most used for the development of functional foods usually belong to the *Bifidobacterium* and *Lactobacillus* genus. To provide health benefits, the probiotic bacteria must reach the intestines alive in sufficient quantities, around 6 to 7 log CFU/g (KUMAR; KUMAR, 2016), and according to the International Dairy Federation (IDF), a minimum dose 10^7 CFU per g of product is recommended (LOURENS-HATTINGH; VILJOEN, 2001; KAILASAPATHY *et al.*, 2008; KAUR *et al.*, 2014). However, this amount may vary between species and strains, once several factors can affect the viability of probiotics in the food matrix including pH, organic acids, storage conditions, oxygen levels, presence of microorganisms and inhibitors (KAILASAPATHY *et al.*, 2008; TRIPATHI; GIRI, 2014).

Prebiotics are non-digestible food ingredients that are selectively fermented in the gut, which stimulates the growth and/or activity of one or a limited number of a microbial genus(era)/species, thus conferring health benefits to the host. Although the probiotic market has grown rapidly worldwide, the use of products has not been validated, thus there is a great interest in finding novel prebiotics (RASTALL; MAITIN, 2002).

In this context, modified chitooligosaccharides (COS) are an interesting alternative to improve the functional impact of fermented milk, since they have a structure similar to glycooligosaccharides. A synbiotic product is defined as a product containing both prebiotics and probiotics, which can stimulate and increase the survival of probiotic and autochthonous specific strains in the intestinal tract (GIBSON; ROBERFROID, 1995). Since the word "synbiotic" alludes to synergism, this term should be used for products in which the prebiotic compound selectively favors the probiotic compound (SCHREZENMEIR; VRESE, 2001).

COS are promising substrates considered as sources of new prebiotic ingredients. However, COS must be modified, once the amino groups present in their structure can cause an antimicrobial effect on the intestinal microbiota, with possible negative health outcomes. The partial substitution of the amino groups for carbohydrates via the Maillard reaction can convert COS into a substrate used by probiotic bacteria, thus demonstrating that these derivatives can be good candidates to be used as prebiotics (MONTENEGRO, 2013). Some authors have also reported that the unmodified COS cannot present specific beneficial effect upon the gastrointestinal microbiota (FERNANDES *et al.*, 2012), while the modified COS such as chitosan oligosaccharide lactate (Mw < 5000 Da) may act as microbiota modulator due to the increased butyrate levels during the fermentation process (VERNAZZA *et al.*, 2005).

Yogurt is produced by fermentation of lactose into lactic acid by the thermophilic starter bacteria *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. It is widely consumed throughout the world due to its sensory characteristics and nutritional benefits (ALMEIDA *et al.*, 2008). In recent years, yogurts have been reformulated to include *L. acidophilus* and *Bifidobacterium* strains together with the starter cultures. Bio-yoghurt contains live and active probiotic microorganisms, which confers health benefits to consumers, being a potential vehicle of

probiotic cells (LOURENS-HATTINGH; VILJOEN, 2001).

Among the different functional dairy beverages, smoothies are blended forms of beverages which are typically semiliquid, with a smooth consistency, containing fruit, juice, fruit and vegetable combinations, and other complements such as yogurt, milk, or honey (KEENAN *et al.*, 2012).

Fruit and vegetables play an important role in the prevention of chronic diseases due to their high contents of bioactive compounds, such as vitamins, minerals, antioxidants, and fibers (MELO *et al.*, 2008). In this context, mango (*Mangifera indica L.*) stands out as a tropical fruit very appreciated and consumed. It possesses excellent sensory properties (bright color, sweet taste, and luscious flavor) and nutritional composition (vitamins, minerals, fibers, and phytochemicals), and is considered a good source of dietary antioxidants, such as ascorbic acid, carotenoids, and phenolic compounds (PALAFOX-CARLOS *et al.*, 2012). The fortification of yogurt with mango pulp can increase the nutrient content of the final product, including minerals and vitamins (KUMAR; MISHRA, 2003).

Therefore, this study aimed to elaborate two smoothie beverages made with probiotic yogurt and mango pulp, with and without the addition of modified COS, and to investigate the potential effect of the prebiotic ingredient and the viability of the probiotic cultures in this novel food matrix.

MATERIAL AND METHODS

Synthesis of modified COS

Commercial chitooligosaccharides (COS) with medium molecular weight (Mw) of 1500 Da was purchased from Nicechem (Shanghai, China). Glucose (Glc) was purchased from Sigma-Aldrich Co. (Steinheim, Germany).

The modification of chitooligosaccharides was carried out by the Maillard reaction by mixing 2% (w/v) COS solution and 1% (w/v) Glc, which was allowed to react for 20 min at 121 °C. Then, the reaction product was purified by dialysis (molecular weight cut-off of 500 Da, Spectrum Laboratories, USA) against distilled water for 3 days at 4 °C. The samples were subjected to freeze-drying (freeze dryer model FT33, Armfield, UK) at 100 mTorr, and temperature of the freezing chamber and sample chamber of -46 °C and 15 °C, respectively.

Starter and probiotic cultures

The following commercial cultures were used in the preparation of the smoothie beverages: yogurt starter culture consisting of *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* (JOINTEC X3, CSL, Italy) and a probiotic mixed culture consisting of *Bifidobacterium longum*, *B. infantis,* and *B. breve* (KIT BIFI, CSL, Italy), both provided by Kerry do Brasil. The freeze-dried cultures were activated according to the manufacturer's instructions. The cultures were aliquoted and stored at -20 °C for further use.

Manufacture of fermented smoothie beverages

Two beverage formulations were prepared for the study, in duplicate. For the formulation 1 (F1), reconstituted skimmed milk powder (SMP), sugar, and COS-Glc complex was used in the concentrations of 14%, 10%, and 2%, respectively. The mixture was heat-treated at 85 °C for 20-30 minutes using a thermostatic bath and cooled to 42-44 °C. Then, the starter and probiotic cultures were added and the fermentation was carried out until reaching pH 4.8-4.7. At the end of the fermentation, the yogurt was cooled to 20-25 °C, and mixed with the

pasteurized mango pulp at a concentration of 60/40 (yogurt/pulp, w/w) in an aseptic environment (laminar flow hood). Potassium sorbate was added at a concentration of 0.03%. Whereas the mango pulp has no added sugar, an additional amount of sugar was previously dissolved in water and heat-treated at 85 °C for 5-10 min, which was added to the formulation to reach 8% sugar in the beverage. The control formulation (F2) was prepared according to the procedure used in F1, with no addition of modified COS. Both formulations were stored at 8 ± 2 °C for 30 days. The beverages were evaluated immediately after manufacture and during storage (1, 15, and 30 days) for the hygienic-sanitary quality (molds and yeast counts, coliforms at 30 °C, and coliforms at 45 °C), titratable acidity, syneresis, and bacterial viability (selective counts of S. *thermophilus, L. bulgaricus,* and bifidobacteria).

Physicochemical characterization

The moisture contents, ash, lipids, protein, total fiber, and dietary fiber were determined according to standard procedures (LATIMER, 2012). The total solids (TS) were calculated by difference (TS = 100 – moisture). The total carbohydrates were calculated by difference, according to the formula: total carbohydrates (including fibers) = [100 – (% moisture + ash + lipids + proteins)].

Physicochemical characterization during storage

The pH was determined using a previously calibrated digital pH meter (Micronal B-375). The titratable acidity was determined by titration with 0.1 N sodium hydroxide solution to pH 8.3 (ZENEBON *et al.*, 2008), and the result was expressed in g lactic acid per 100 g sample. The spontaneous syneresis or whey separation of the beverages was determined by measuring the volume (in centimeters) of whey released from 10 mL of sample maintained in conical tubes at 8 ± 2 °C, according to Silva *et al.* (2010).

Microbiological characterization

Aliquots of 1 mL of sample were aseptically transferred to tubes containing 0.1% sterile peptone water for the microbiological determinations.

For the enumeration of *L. bulgaricus*, acidified MRS (Difco) agar (pH = 5.4) was used, with incubation at 37 ± 1 °C for 72 h. The enumeration of *S. thermophilus* was performed using M17 agar (Difco) supplemented with glucose solution (5%), with incubation at 37 ± 1 °C for 48 h (FRANK; YOUSEF, 2004). The methodology proposed by the International Dairy Federation (IDF, 2007) was used for the selective enumeration of the probiotic microorganisms (*Bifidobacterium* spp.), with modifications. For that, MRS agar was supplemented with lithium chloride (0.1%), L-cysteine (0.05%) and dicloxacillin (0.5 mg/L), using depth plating technique and incubation under anaerobic conditions at 37 ± 1 °C for 72 ± 3 h.

The coliforms at 30 °C and thermotolerant coliforms were determined by the most probable number using lauryl sulfate tryptose broth (LST). The samples were incubated at 30 ± 1 °C for 24 ± 2 h (ISO 4831, 2006). Aliquots from the tubes presenting microbial growth and gas production were transferred to tubes containing 2% brilliant green bile (BGB, Difco) broth and *Escherichia coli* broth (EC, Difco). The BGB tubes were incubated at 30 ± 1 °C for 24 ± 2 h to confirm the presence of coliforms at 30 °C and the EC tubes were maintained for up to 48 ± 2 h at 44 ± 1 °C for confirmation of the presence of coliforms at 45 °C (ISO 7251, 2005). Molds and yeasts were enumerated

using dichloran rose bengal chloramphenicol agar (DRBC, Difco), incubated for 5 days at 25 ± 1 °C (FRANK; YOUSEF, 2004).

Statistical analysis

The t-test at the 5% probability level was used to compare the formulations at the same storage time, and ANOVA followed by Tukey's test was used to evaluate the effect of the storage time. Data were analyzed using Minitab® Statistical software (version 16.1.1), (MINITAB®, 2011).

RESULTS AND DISCUSSION

Physicochemical characterization

The mango pulp used in the manufacture of the beverages presented pH of 4.0, acidity of 0.41 g citric acid per 100 g pulp, and 14.5 °Brix. These values are in compliance with the current legislation for mango pulp (pH between 3.3 and 4.5; minimum acidity of 0.32 g citric acid/100 g pulp, and minimum soluble solids content of 11 °Brix) (BRASIL, 2000).

Figure 1 shows the average titratable acidity values of the beverages during 30 days of storage.

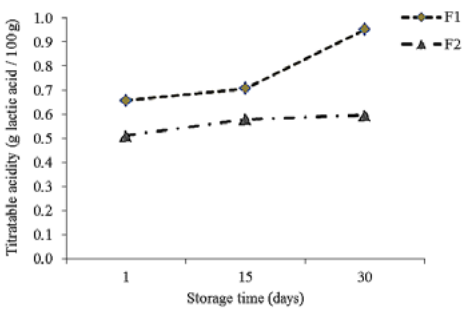

Figure 1 – Titratable acidity (g lactic acid /100 g) of the beverages during the refrigerated storage (8 ± 2 °C)

A significant increase (P≤0.05) in the acidity levels of the beverages (0.5 to 0.6 g /100 g) was observed throughout the storage, as expected for fermented dairy products (GALLINA *et al.*, 2012). This increase is due to the lactose fermentation by the bacteria even under refrigeration conditions, with consequent production of lactic acid, reducing the pH of the products. *L. delbrueckii* subsp. *bulgaricus* is the main responsible for the increase in titratable acidity during the shelf life of yogurts (KAILASAPATHY *et al.*, 2008). Significant differences (P≤0.05) in acidity values were observed between the formulations F1 and F2 for the same storage period, which was more pronounced at 30 days of storage, demonstrating that the addition of modified COS (COS-Glc) significantly affected the titratable acidity of the fermented beverages, when compared to the control (F2).

Other authors also reported an increase in acidity of yogurts with the addition of fruit pulp throughout the storage (GALLINA *et al.*, 2018; BARBOSA; GALLINA, 2017; HOSSAIN *et al.*, 2012). However, the formulations F1 and F2 are considered low-acid products when compared to the traditional yogurts, which must have between 0.6 and 1.5 g lactic acid/100 g, thus they may be a favorable environment for maintaining the viability of probiotic microorganisms.

The whey syneresis of the formulations F1 and F2 is shown in Figure 2. Significant differences (P≤0.05) were observed between F1 and F2 for all periods studied, with a significant increase (P≤0.05) in syneresis during the refrigerated storage, which was more pronounced in the formulation made with COS-Glc (F1). Barat; Ozcan (2018) also reported that the syneresis during the refrigerated storage of yogurts was significantly affected by the addition of fruit juice and the storage time.

Whey syneresis or spontaneous separation of whey on the surface of

the yogurt is regarded as an undesirable phenomenon (AMATAYAKUL *et al.*, 2006), and is considered an important aspect for the consumers' acceptance. This phenomenon is characterized by the spontaneous release of water from the gel, which can be improved by changes in temperature, pH, and mechanical damage to the gel during fermentation (SILVA *et al.*, 2010). A significant increase in acidity was observed during storage, especially for the formulation F1 made with COS-Glc, which may have led to a higher syneresis rate of the beverages throughout the storage. Barbosa; Gallina (2017) also observed a significant increase in syneresis during the storage of yogurts, especially at 30 days, with similar values to the formulation F2 (without COS-Glc) containing 40% mango pulp.

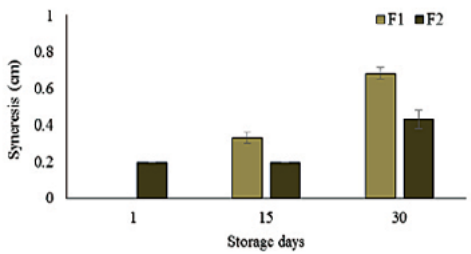

Figure 2 – Whey syneresis (cm) of the beverages during the storage (8 ± 2 °C)

Physicochemical characterization

Table 1 shows the results of the physicochemical characterization of the beverages (F1 and F2). The formulation F1 presented significantly higher total solids, total fiber, and carbohydrates when compared to the F2, probably due to the addition of 2% COS-Glc. The protein levels were lower than that expected for fermented milk, due to the addition of fruit pulp (40%), and F1 exhibited significantly higher protein contents when compared to F2, probably due to the nitrogen content of COS, which may have contributed to this increase.

The ash contents were similar to the values found by Januário *et al.* (2017) in yogurt flavored with organic vegetable juices. Regarding the fat content, values below 0.5% were expected in both formulations, due to the use of reconstituted skimmed milk powder.

Microbiological quality

The enumeration of molds and yeasts, coliforms at 30 °C, and coliforms at 45 °C are important parameters for the hygienic-sanitary quality of a product (GALLINA *et al.*, 2018). The beverages were analyzed for

Table 1 – Physicochemical characterization of the probiotic beverages*

Components (g/100g)	F1	F2
Total solids	$19.98^{a} \pm 0.13$	$17.31^{b} \pm 0.001$
Proteins	$2.67^{a} \pm 0.01$	$1.99^{b} \pm 0.001$
Lipids	$0.18^{b} \pm 0.01$	$0.58^{a} \pm 0.01$
Ash	$0.81^{a} \pm 0.005$	$0.83^{a} \pm 0.002$
Total fiber	$0.59^{a} \pm 0.01$	$0.36^{b} \pm 0.025$
Carbohydrates	$15.74^{a} \pm 0.08$	$13.88^{b} \pm 0.05$

* Values expressed as mean ± standard deviation. Means followed by the same letter on the same line do not differ statistically (P>0.05).

the presence of microorganisms after 1 day of manufacture, and the results are shown in Table 2.

Brazilian law has established no specific requirements for smoothies. Thus, the microbiological parameters of the beverages of this study were compared with those required for fermented milk (BRASIL, 2007). The molds and yeast counts of formulation F1 were within the acceptable limits, while no counts were observed for F2. Coliforms were not detected in the beverages. These microbiological results demonstrate the excellent hygienic-sanitary quality of the samples and good food handling practices.

Viability of yogurt and probiotic cultures

Figure 3 shows the *S. thermophilus* (gram-positive cocci) counts, which ranged from 7.52 to 8.98 log CFU/mL. A decrease in the number of this microorganism was observed throughout the storage, which was significant only for the formulation F1, at 30 days of storage. Although a significant difference was observed in *S. thermophilus* counts between F1 and F2 at 15 and 30 days of storage, there was a tendency in maintaining the *S. thermophilus* viability for both formulations, remaining around 8 log CFU/mL.

The *L. delbrueckii* subsp. *bulgaricus* (gram-positive bacilli) counts of the beverages varied from 3.45 to 4.50 log CFU/mL, with a significant increase (F1 formulation) during the storage, which was not observed for *S. thermophilus* counts. This result may be due to the pH reduction throughout the storage, once lower pH values can favor the growth of *L. delbrueckii* ssp. *bulgaricus* in yogurt,

Table 2 – Microbiological counts of the probiotic beverages (F1 and F2)*

Microorganisms	F1	F2
Coliforms at 30 °C (MPN/mL)	<0.3	<0.3
Coliforms at 45 °C (MPN/mL)	<0.3	<0.3
Molds and Yeasts (CFU/mL)	2.0 x 10^1	<10

* mean values from duplicate determinations.

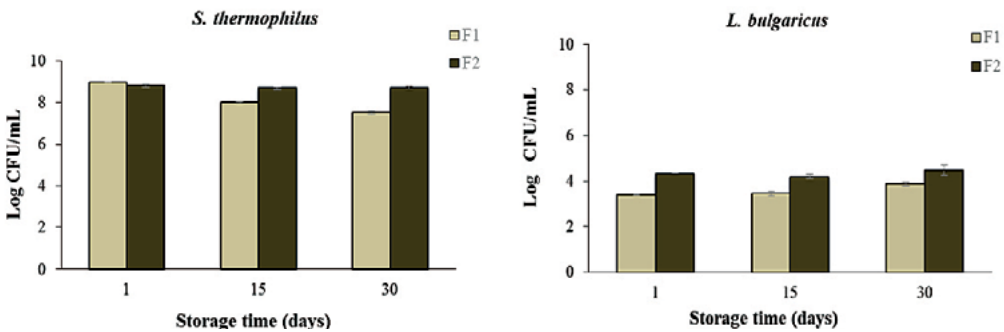

Figure 3 – Viable microbial counts (log CFU/mL) of *S. thermophilus* and *L. bulgaricus* of the beverages throughout the storage (8 ± 2 °C)

as reported by other authors (FARIAS *et al.*, 2016). No changes in *L. bulgaricus* counts were observed for the formulation F2.

Barbosa; Gallina (2017) found similar viable counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in smoothie beverages made with probiotic yogurt and mango pulp. In the present study, lower *L. delbrueckii* subsp. *bulgaricus* counts were observed for all formulations when compared to the *S. thermophilus* counts. According to Gallina *et al*. (2011), the lower *L. bulgaricus* counts may be due to an imbalance between strains in the starter culture or the interruption of fermentation at a slightly higher pH, which can affect the growth stage of *L. bulgaricus*. However, lower counts may favor the maintenance of probiotics, once *L. delbrueckii* ssp. *bulgaricus* has acidifying activity (FARIAS *et al.*, 2016), thus excess acidification is undesirable and harmful to bifidobacteria. The Brazilian legislation recommends minimum lactic acid bacteria counts of 10^7 CFU/g and 10^6 CFU/g for yogurts and fermented milk, respectively (BRASIL, 2007). Therefore, the present results showed that the beverages had total lactic acid bacteria counts within the desired threshold value throughout the storage.

Figure 4 shows the viability of *Bifidobacterium* spp (Gram-positive) throughout the storage of the beverages, which ranged from 6.24 to 6.81 log CFU/mL. Although a small reduction of the probiotics counts was observed for the formulations F1 and F2 during the storage, the reduction was significant ($P \leq 0.05$) only for the formulation F2 at 30 days of storage. No significant differences were observed between the formulations, demonstrating that despite the modified COS had no prebiotic effect, it did not affect the maintenance of the probiotics. However, it is expected that the modified COS will allow the product to have a prebiotic effect on the consumer.

Studies have shown that different factors can affect the viability of probiotics in food, such as the presence of organic acids and low pH values. Ozcan *et al.* (2015) emphasized that pH 4.5 or lower negatively affected the cell viability of probiotic bacteria due to the sensitivity of these bacteria to environmental stresses (low pH and high titratable acidity).

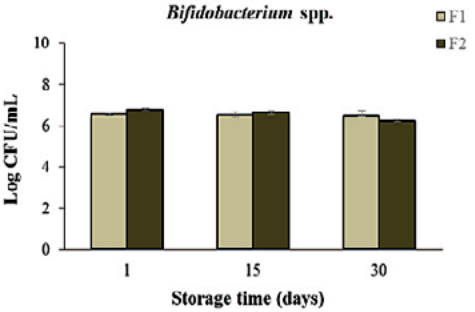

Figure 4 – Viable *Bifidobacterium* spp. counts (log CFU/mL) during the storage

The nature of organic acids in foods (juice or fruit pulp) can also negatively affect the growth of probiotics. The addition of cranberry juice to yogurt formulations can have adverse effects due to its higher content of benzoic acid when compared to pineapple juice at the same pH conditions (PAQUIN, 2009). Kailasapathy *et al.* (2008) evaluated the addition of mango (5 g/100 g) to probiotic yogurts and found no reduction of the viability of the probiotics.

When comparing the probiotic counts of the formulations F1 and F2, no significant differences were observed ($P>0.05$) during the storage, which remained around 6 log CFU/mL. Other authors reported similar bifidobacteria counts (between 10^6 and 10^7 CFU/mL) in probiotic yogurts and smoothie beverages with the addition of fruit pulp during 30 days of refrigerated storage, even under unfavorable pH conditions (GALLINA *et al.*, 2011; GALLINA *et al.*, 2012; BARBOSA; GALLINA, 2017;

GALLINA *et al.*, 2018). The Brazilian legislation recommends minimum probiotic counts of 10^6 CFU of bifidobacteria per gram of product for yogurts and fermented milk, thus, the smoothie formulations of this study meet the requirements of the legislation (BRASIL, 2007).

Some authors have proposed a minimum daily dose of probiotics from 10^8 to 10^9 CFU/g to be considered therapeutic, which corresponds to the consumption of 100 g of product containing 10^6 to 10^7 CFU/g (LOURENS-HATTINGH; VILJOEN, 2001; KAILASAPATHY *et al.*, 2008; KAUR *et al.*, 2014). Therefore, both smoothie beverages elaborated in this study (F1 and F2) presented counts in accordance with this recommendation.

CONCLUSIONS

Both beverage formulations presented satisfactory hygienic-sanitary quality and were adequate for consumption. The incorporation of modified chitooligosaccharides significantly affected the titratable acidity, whey syneresis, and *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* counts during the storage of the formulation made with modified COS when compared to the control (without chitooligosaccharides). In addition, the formulation with COS-Glc presented higher total solids, total fiber, and carbohydrates when compared to the control. The addition of modified COS-Glc did not affect the viability of bifidobacteria during 30 days of storage, which remained within the recommended dose to exert its therapeutic effect.

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