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FOOD TECHNOLOGY

# Development and characterization of light yoghurt elaborated with *Bifidobacterium animalis* subsp. *Lactis* Bb-12 and fructooligosaccharides

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**ABSTRACT**: *This study aimed to evaluate the effects of adding probiotic culture (Bifidobacterium animalis subsp. Lactis Bb-12) and prebiotics (fructooligosaccharide - FOS) to yoghurt formulations stored at 4°C for 28 days, using an experimental design (independent variables: (0–3% of FOS and probiotic starter cultures 0-3%). The pH, acidity, fat, syneresis, protein, ºBrix, sugars, FOS and probiotic bacteria count were analyzed. The probiotic- and prebiotic-added yoghurt formulations showed lower acidity, syneresis and glucose than the control yoghurt and compared to formulations containing probiotic and prebiotic separately. The 3% probiotic and prebiotic formulation showed a lower loss of concentration of FOS, and after 28 days presented 1.5g of FOS per 100g (0.3% kestose, 0.7% nystose, 0.5% fructosyl-nystose). Furthermore, the addition of prebiotics exerted a protective effect on probiotic bacteria, enhancing their survival.*
**Key words**: *yoghurt, probiotic, Bifidobacterium, prebiotics, fructooligosaccharides.*

## Desenvolvimento e caracterização de iogurte *light* elaborado com *Bifidobacterium animalis* subsp. *Lactis* Bb-12 e fruto-oligossacarídeos

**RESUMO**: *Este estudo teve como objetivo avaliar os efeitos da adição de cultura probiótica (Bifidobacterium animalis subsp. Lactis Bb-12) e prebióticos (fructooligosacarídeo - FOS) a formulações de iogurte armazenadas a 4°C por 28 dias, utilizando um planejamento experimental (variáveis independentes: (0-3% de FOS e cultura probiótica starter 0-3%). Foram analisados pH, acidez, gordura, sinérese, proteína, ºBrix, açúcares, FOS e contagem de bactérias probióticas. As formulações de iogurte adicionado de probiótico e prebiótico apresentaram menor acidez, sinérese e glicose quando comparados ao iogurte controle e também em comparação com as formulações contendo probiótico e prebiótico sozinhas. A formulação com 3% de probiótico e prebiótico apresentou menor perda de concentração de FOS e, após 28 dias, apresentou 1,5g de FOS por 100g (0,3% de kestose, 0,7% de nystose , 0,5% de fructosil-nistose). Além disso, a adição de prebióticos exerceu um efeito protetor sobre as bactérias probióticas e aumentou a sua sobrevivência.*
**Palavras-chave**: *iogurte, probiótico, Bifidobacterium, prebióticos, fructooligosacarídeos.*

## INTRODUCTION

Yoghurt is a healthy food due to the beneficial aspects of its high protein and calcium contents. It has normally been produced using skimmed milk as raw material in developed countries with variably adjusted low fat level. The excessive consumption of satured fats in the diet can cause some disorders, such as cardiovascular diseases, obesity, cancer and diabetes (RAMIREZ et al., 2010; GRANATO et al. 2017; KAYANUSH &OLSON, 2017).

The physicochemical attributes of yoghurt gels are considered by consumers as important aspects of the quality of the product. In this regard, one way of improving these characteristics is the addition of prebiotics, as a fat substitute. According to GIBSON et al. (2004), a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers wellbeing and health benefits to the host. Prebiotics in bakery products have also attracted a lot of interest as fat substitutes (HOPPERT et al., 2013),

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also have been shown to improve microbiological, chemical, and sensory properties of yogurt, even though there are some challenges during incorporation of prebiotics in yogurt (PRASANNA & RASTALL, 2017). On prebiotics, the Fructooligosaccharide (FOS) presents health benefits (VEGA & ZUNIGA-HANSEN, 2015) associated with consumption of dairy products (yoghurt). The dairy industry is looking for alternatives to increase the commercial value of its products in addition to having functional properties, such an example is the addition of probiotics.

Desirable bacteria (*Bifidobacterium* and *Lactobacillus* genera) have thus become more prominent in yoghurt, and this is beneficial to the human host. In addition, some of the fermentation products such as short chain fatty acids help to promote human health. Overall, prebiotics enable a beneficial modification of the host microflora composition. Strains belonging to the *Lactobacillus* and *Bifidobacterium* genera are among the most known probiotic microorganisms. *Bifidobacterium animalis* subsp. *Lactis* Bb-12 has been used with functional properties either alone or together with other bacteria such as *Lactobacillus acidophilus* La-5 or *Streptococcus thermophilus* (SORO & BABA, 2015). Several beneficial effects have been attributed to *L. acidophilus* La-5 and *B. animalis* Bb-12, among them: prophylactic activity against infectious rotavirus in children (WEICHERT et al., 2012), relief of clinical symptoms of atopic dermatitis in children and intestinal microbiota modulation (SAVARD et al., 2011). These strains are potential probiotics that are commercially available.

Yoghurt presents great acceptance potential in all age groups and social classes. It constitutes a concentrated source of dairy nutrients, with calcium and proteins of high nutritional value present in its composition. Furthermore, the addition of probiotics in combination with prebiotics results in a less acid symbiotic product, thereby improving the sensory characteristics and conferring more resistance to lactic cultures in the product. In this sense, the present work aimed to evaluate the effects of adding prebiotic (fructooligosaccharide) and probiotic culture (*Bifidobacterium animalis* subsp. *Lactis* Bb-12) on physicochemical characteristics of low-fat yoghurt.

## MATERIAL AND METHODS

*Development of yoghurt formulations*

For the preparation of yoghurt formulations, traditional thermophilic starter cultures were used, containing strains of *Streptococcus thermophilus*; *Lactobacillus delbrueckii* subsp. *bulgaricus* (DVS Yeast YF L812, LC® Bologna); thermophilic probiotic lactic acid culture (*Bifidobacterium animalis* subsp. *Lactis* Bb-12 -Christian Hansen, Hørshoolm, Denmark); Commercial fructooligosaccharides - FOS (Fibre FOS®); pasteurized skimmed milk (1.0% fat); milk powder (Elects®); dye powder pink (Regina®); and liquid raspberry flavour (Two Wheels®).

The yoghurts were prepared according to the methodology described by RIBEIRO & KROLOW (2006) with modifications. Initially, a pre-inoculum (0.01% w/v) of probiotic lactic acid bacterial cultures using hydrated pasteurized skimmed milk (1.0 % fat), and inoculated at 37°C for 6h were prepared. The remaining ingredients, milk powder (4% w/v), FOS and sugar (1% w/v) were mixed, subjected to pasteurisation (85–90°C for 30s) and cooled to 40–45°C. Pre-inoculum culture (4% v/v) and the probiotic mixture were then added and inoculated at 45°C for a period of 4 to 6h (pH 4.6–4.8). Thereafter, the flavour (0.26g $L^{-1}$) and the dye (0.8g $L^{-1}$) were added, and the formulations were then packaged in 500 to 1000mL plastic bottles (sterilized), and stored at 4°C for subsequent physicochemical, rheological and microbiological analysis at 1, 7, 14, 21 and 28 days of storage. The concentrations of probiotic culture and prebiotic (FOS) have been defined, following a $2^2$ factorial design with the independent variables FOS (0, 1.5 and 3.0%) and Probiotic cultures (0, 1.5 and 3.0%). The variable concentration of sugar, milk powder, aroma, flavour and traditional culture lactic fermentation time were fixed.

*Characterization of yoghurts*

To evaluate the characteristics of the product developed, the physicochemical determinations (pH, acidity, fat, syneresis, ºBrix, protein, lactose, sucrose, glucose, fructose, kestose, nystose and fructosyl-nystose), rheological (viscosity) and microbiological (count was made of probiotic culture) were performed at 1, 7, 14, 21 and 28 days of storage. The pH was determined with a digital potentiometer and acidity in terms of lactic acid and fat according to AOAC (2000). The syneresis was determined by the method of drainage (MANZANO et al., 2008), total soluble solids (°Brix) were determined by the refractometric method – Refractometre of Abbé (BEL® Equipamentos Ltda, Brazil) and protein content was determined by the Kjeldahl method – Marconi and MA-036® (AOAC, 2000). The concentrations of sugars (lactose, fructose, glucose and sucrose) and FOS were determined by high performance liquid chromatography (HPLC Agilent

1100 Series Detector, RID Column Phenomenex $NH_2$ 100 Å). The chromatographic conditions were: mobile phase consisting of acetonitrile/water (70:30, v/v); $NH_2$ (1mL $min^{-1}$); column temperature 20°C; detector temperature 25°C; running time 15min. For the quantification of sugars and FOS, the standards of lactose (Difco Ltda.), D-fructose (Synth), D-glucose (Vetec), sucrose (Fmaia), kestose (GF2), nystose (GF3) and fructosyl-nystose (GF4) at different concentrations (500, 1000, 2000, 5000 and 10,000ppm) were used (KUHN et al., 2013).

*Survival of probiotic culture in yoghurt*

Yoghurt samples (25mL) were blended with 225mL of peptone water in a Bag Mixer 400 (Interscience, St. Nom, France) and dilutions made. For the enumeration of *B. lactis* Bb-12, MRS agar with the addition of 0.2% (w/v) lithium chloride and 0.3% (w/v) sodium propionate was used in accordance with VINDEROLA & REINHEIMER (2003). Dilutions were plated and incubated in anaerobic jars (AnaeroGen) at 37 ± 1°C for 72h. After the incubation period, colonies were counted and expressed as log colony-forming units per gram (log CFU $mL^{-1}$). All analyses were performed in triplicate.

*Statistical treatment*

The results of physicochemical and microbiological analyses were analysed according to the methodology of design of experiments and analysis of variance (ANOVA) followed by Tukey's run to compare the differences between the means, with the aid of STATISTICA software (Statsoft, v.5.0 for Windows), with a significance level of 90 and 95% confidence.

## RESULTS AND DISCUSSION

*Physicochemical characterization*

According to table 1, the runs that contain probiotics are less acidic. These results can be better viewed by Pareto charts (Figure 1), which show the estimated values of the variables tested (FOS and probiotic culture) on the effects of pH response. After 7 and 28 days (Figure 1a and 1b), it is observed that the increase of probiotic concentration led to an increase in pH and consequently decreased the acidity. Similar behaviour was observed after 14 and 21 days of storage (Figure not shown). In relation to acidity at 21 days of storage (Figure 1d), it is shown that all variables and interaction were significant ($p<0.10$), and that the probiotic and FOS had negative effects, indicating that increasing the concentration led to a decrease in acidity. The decline shown in the pH and the increase in acidity are the result of post-acidification of the products and production of lactic acid by lactic bacteria during the storage period. Furthermore, the addition of FOS contributes to the survival of *Bifidobacterium* in the end of the shelf life of the product after 28 days, with counts between 7.0 and 10 log10CFU $mL^{-1}$ in formulations added with 1.5% (run 5) and 3.0% (run 4) of FOS. The addition of the probiotic and prebiotic combination (synbiotic product) results in a less acidic product, thereby, improving the flavour and assisting in the maintenance of probiotic cultures in the product.

Table 1 - Matrix of factorial design $2^2$ (real and coded values) for the responses pH and acidity of the yoghurt formulations during the days of storage.

| Run | ----Variables*---- | | ----------pH---------- | | | | | ----------Acidity (% of lactic acid)---------- | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | 1st | 7th | 14th | 21st | 28th | 1st | 7th | 14th | 21st | 28th |
| 1 | -1(0) | -1 (0) | 4.50$^{a}$ (0.01) | 4.52$^{a}$ (0.01) | 4.45$^{a}$ (0.01) | 4.18$^{b}$ (0.08) | 4.12$^{b}$ (0.12) | 0.95$^{c}$ (0.01) | 0.98$^{cb}$ (0.05) | 1.05$^{b}$ (0.01) | 1.23$^{a}$ (0.02) | 1.22$^{a}$ (0.02) |
| 2 | 1(3.0) | -1 (0) | 4.84$^{a}$ (0.01) | 4.50$^{b}$ (0.01) | 4.37$^{c}$ (0.01) | 4.25$^{d}$ (0,01) | 4.09$^{e}$ (0.02) | 0.85$^{b}$ (0.02) | 0.90$^{b}$ (0.03) | 0.94$^{b}$ (0.02) | 1.15$^{b}$ (0.07) | 1.24$^{a}$ (0.03) |
| 3 | -1(0) | 1(3.0) | 4.97$^{a}$ (0.02) | 4.70$^{b}$ (0.01) | 4.39$^{c}$ (0.01) | 4.32$^{c}$ (0.05) | 4.20$^{d}$ (0.03) | 0.82$^{c}$ (0.01) | 0.94$^{b}$ (0.02) | 1.00$^{b}$ (0.02) | 1.14$^{a}$ (0.05) | 1.17$^{a}$ (0.07) |
| 4 | 1(3.0) | 1(3.0) | 4.77$^{a}$ (0.01) | 4.50$^{b}$ (0.01) | 4.45$^{c}$ (0.01) | 4.35$^{d}$ (0.01) | 4.20$^{e}$ (0.01) | 0.86$^{c}$ (0.06) | 0.95$^{bc}$ (0.06) | 0.99$^{b}$ (0.04) | 0.98$^{bc}$ (0.04) | 1.21$^{a}$ (0.03) |
| 5 | 0(1.5) | 0(1.5) | 4.82$^{a}$ (0.01) | 4.46$^{b}$ (0.01) | 4.50$^{b}$ (0.01) | 4.34$^{c}$ (0.04) | 4.23$^{d}$ (0.01) | 0.80$^{c}$ (0.01) | 1.00$^{b}$ (0.02) | 1.00$^{b}$ (0.05) | 0.98$^{b}$ (0.02) | 1.20$^{a}$ (0.01) |

*$X_1$= fructooligosaccharides (%), *$X_2$ = Probiotic starter cultures (%); **Average (standard deviation) followed by the same letters in rows indicate no significant difference between days of storage at 5% level (Tukey test).

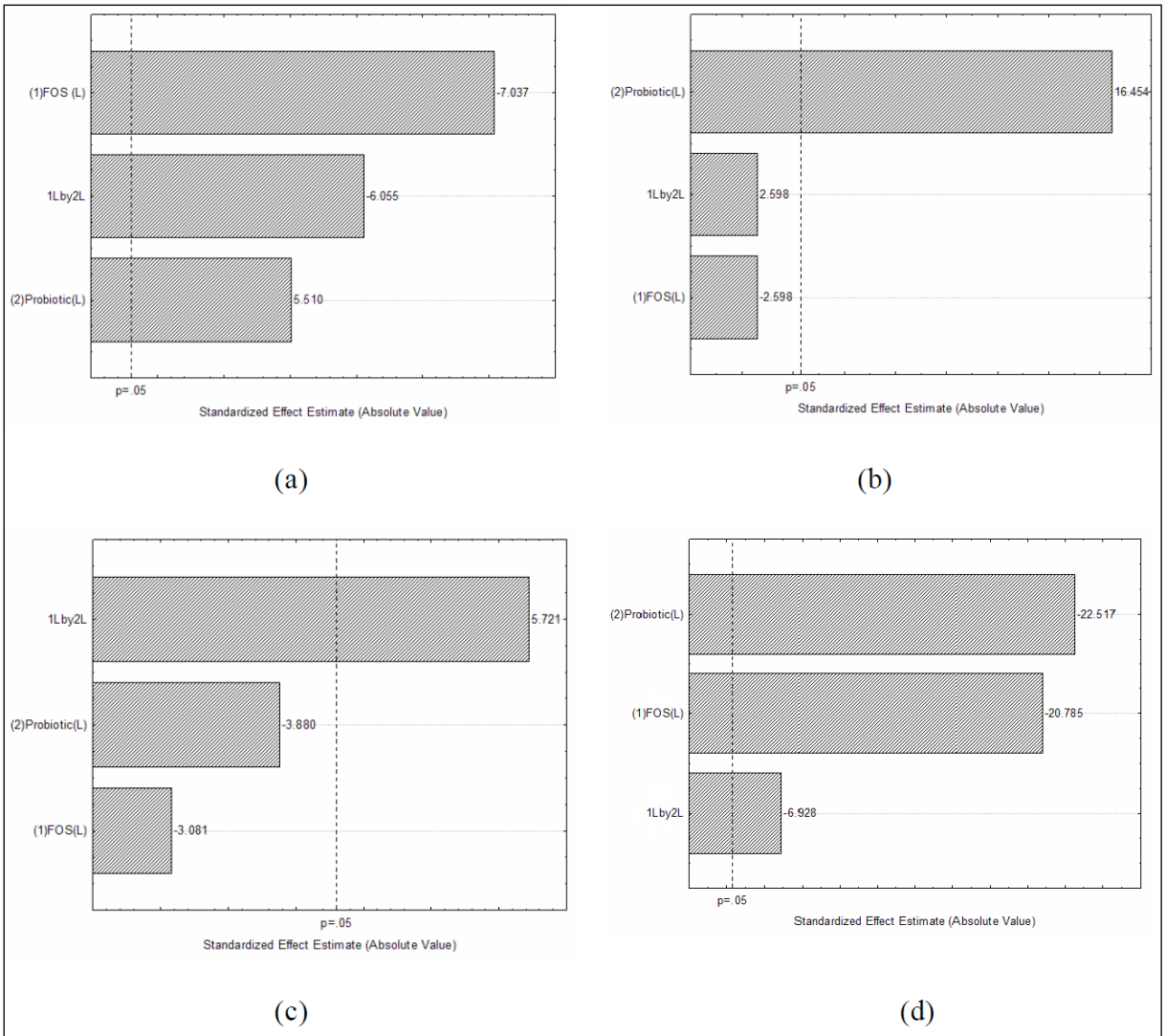


Figure 1 - Pareto chart for pH values on the 7th (a) and 28th (b) day of storage and acidity on the 1st (c) and 21st (d) day of storage, respectively.

The syneresis (Table 2) increased significantly ($p<0.05$) from the 21st day of storage. Formulation 2 with 3.0% of FOS showed high syneresis. However, in runs 3 and 4, the syneresis was lower until 21 days of storage and increased significantly after this period ($p<0.05$). Possibly, the lowest values of syneresis are related to the production of exopolysaccharides (EPS) by the probiotics, which can act as stabilizers in foods, contributing to the structure of yoghurt gel (LEIVERS et al., 2011). The results show that increasing the concentration levels of probiotic culture and/or reducing the FOS ($p<0.05$), the syneresis in the product was reduced significantly (Figures not shown). This is also confirmed by BEDANI et al. (2013), who found that with the addition of probiotic culture, the rate of syneresis tends to decrease or remain stable.

The yoghurt formulations presented approximately 3.5% of protein and 1.5% of fat. The values of total soluble solids (ºBrix) ranged from 17 to 21.7, whereas the highest levels were observed in formulations with higher concentrations of FOS (runs 2 and 3).

Figure 2 shows the consumption behaviour of lactose, sucrose and glucose by bacteria of the yoghurt samples during storage. Regarding consumption of lactose (Figure 2a), after processing, the formulations presented a content of 4.06%

Table 2 - Matrix of factorial design $2^2$ (real and coded values) for the response syneresis of the yoghurt formulations during the days of storage.

| Run | Variables | | Syneresis**(%) | | | | |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | 1st | 7th | 14 th | 21st | 28 th |
| 1 | -1(0) | -1(0) | 28.43$^{ab}$ (1.00) | 26.04$^{b}$ (1.25) | 25.43$^{b}$ (0.99) | 32.17 (2.12) | 32.81$^{a}$ (0.26) |
| 2 | 1(3.0) | -1 (0) | 33.39$^{a}$ (0.00) | 29.19$^{a}$ (1.43) | 29.20$^{a}$ (0.33) | 33.80$^{a}$ (1.07) | 35.49$^{a}$ (0.00) |
| 3 | -1(0) | 1(3.0) | 25.65$^{b}$ (1.00) | 27.08$^{b}$ (1.53) | 24.97$^{b}$ (1.36) | 24.69$^{b}$ (0.44) | 33.71$^{a}$ (1.15) |
| 4 | 1(3.0) | 1(3.0) | 28.25$^{b}$ (1.00) | 28.29$^{b}$ (1.00) | 26.62$^{b}$ (0.54) | 26.24$^{b}$(1.00) | 34.14$^{a}$ (0.20) |
| 5 | 0(1.5) | 0(1.5) | 31.44$^{b}$ (0.48) | 31.67$^{b}$ (1.42) | 31.18$^{b}$ (0.44) | 34.82$^{a}$ (0.56) | 34.99$^{a}$ (0.34) |

*$X_1$= fructooligosaccharides (%), *$X_2$ = Probiotic starter cultures (%); **Average (standard deviation) followed by the same letters in rows indicate no significant difference between days of storage at 5% level (Tukey test).

(w/v), being higher after 28 days of storage in run 4 (26.04%). The samples of runs 2, 5, 3 and 1 presented a reduction of 21.80, 15.30, 15.13 and 5.71%, respectively. Thus, the formulations added with FOS allowed a higher consumption of lactose by bacteria.

Figure 2b shows the behaviour of sucrose during storage of yoghurt. The yoghurt with the highest sucrose content was of run 2 with a consumption of 54.4% (initial content 11%, w/v), followed by runs 1, 5, 4 and 3, with consumptions of 28.7, 22.1, 17.1 and 4.9%, respectively. It is noteworthy that there is a lower consumption of sucrose in the sample of run 3; which, besides the traditional lactic bacteria, contains probiotic culture, which provide a lower consumption of sucrose. A similar result was also observed in samples from runs 4 and 5. Therefore, it appears that probiotic bacteria do not consume sucrose preferably in the fermentation.

Figure 2c shows the glucose concentration in the formulations of yoghurt during storage. After processing (day 1), the samples from run 2 showed the highest content of glucose (6.15%, w/v), due to the addition of 3% FOS, composed of 5. % glucose. This formulation showed a distinct behaviour compared to the others, especially from the 14th day and 28th day, where the glucose concentration in the formulation was 29.69%. Possibly, this is related to the fact that run 2 did not include the addition of probiotic culture and the traditional bacteria of yoghurt do not use FOS as a substrate, so the FOS was converted into glucose.

Regardless, the samples containing FOS and probiotic culture (Figure 2c, Runs 4 and 5) presented lower glucose values. In run 4, the initial value was 4.22%, and on the 28th day of storage, this was 4.62% (w/v). In run 5, the glucose concentration on the 1st day was 1.55% (w/v) and after 28 days this was 9.49% (w/v), showing an increase in the glucose content of this sample. Possibly, the increase on the 28th day of storage is related to lower concentration of probiotic (1.5%) and so there was less consumption and greater conversion of FOS in glucose. The glucose concentration of the control formulation (run 1) remained close to constant (~4.0% w/v) during storage, indicating that there was no glucose consumption by traditional bacteria.

Regarding fructose, for the samples containing FOS (run 2, 4 and 5), initial content of fructose was approximately 0.6%. In run 2, there was an increase in fructose content on the 28th day of storage, at 7.1% (w/v). It is known that prolonging the storage life of yoghurt, there are acidic conditions involved and consequent lowering of the pH, i.e., lactic acid production in larger quantities may increase the fructose. Furthermore, run 2 did not have probiotic culture added, which is another reason as to why there was no consumption of FOS by traditional bacteria of yoghurt, and this is converted into fructose, which was not observed in runs 4 and 5 during storage, in which it was totally consumed.

Figure 3 shows the concentration of the FOS component (kestose, nystose and fructosyl-nystose). When the sample of commercial FOS Fiber® (Mix-FOS) was analysed, it was observed that the same had 56.1% (w/w) of fructooligosacharides (14.7% of kestose, 23.8% of nystose and 17.6% of fructosyl-nystose), 8.0% of fructose, 5.6% of glucose and other isomers (not quantified). Run 2 (Figure 3a) was added with 3% of Mix-FOS, corresponding to 1.7% of FOS w/w (0.44% of kestose, 0.71% of nystose and 0.53% fructosyl-nystose). In this run, it is observed that there is a decline of kestose, nystose and fructosyl-nystose, over the period of storage of yoghurt. There was a greater decline for fructosyl-nystose (reduction of ~ 60%) and kestose (reduction of ~ 40%) on the 7th day of storage

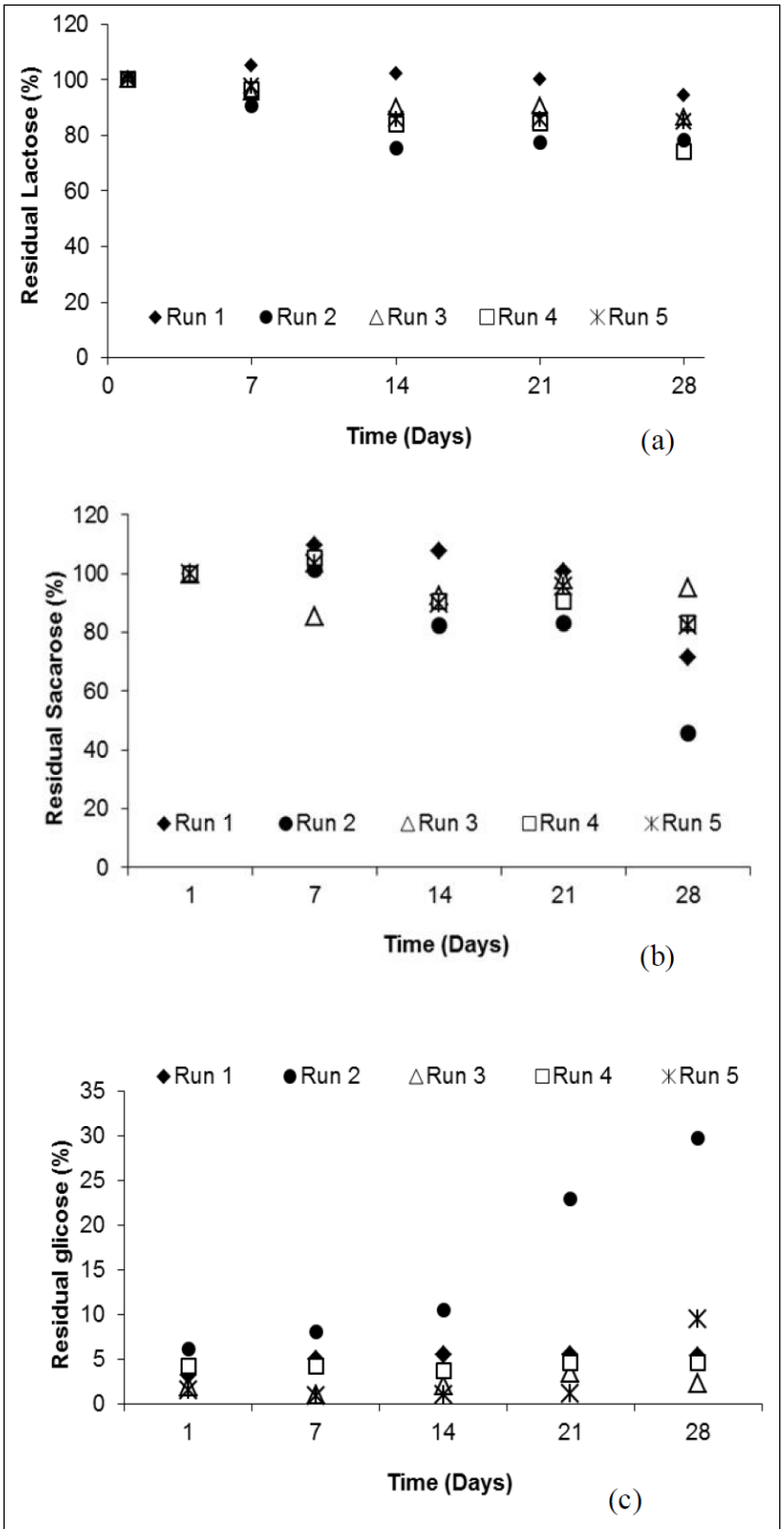


Figure 2 - Behaviour of lactose (a), sucrose (b) and glucose (c) consumption of yoghurt by bacteria during storage.

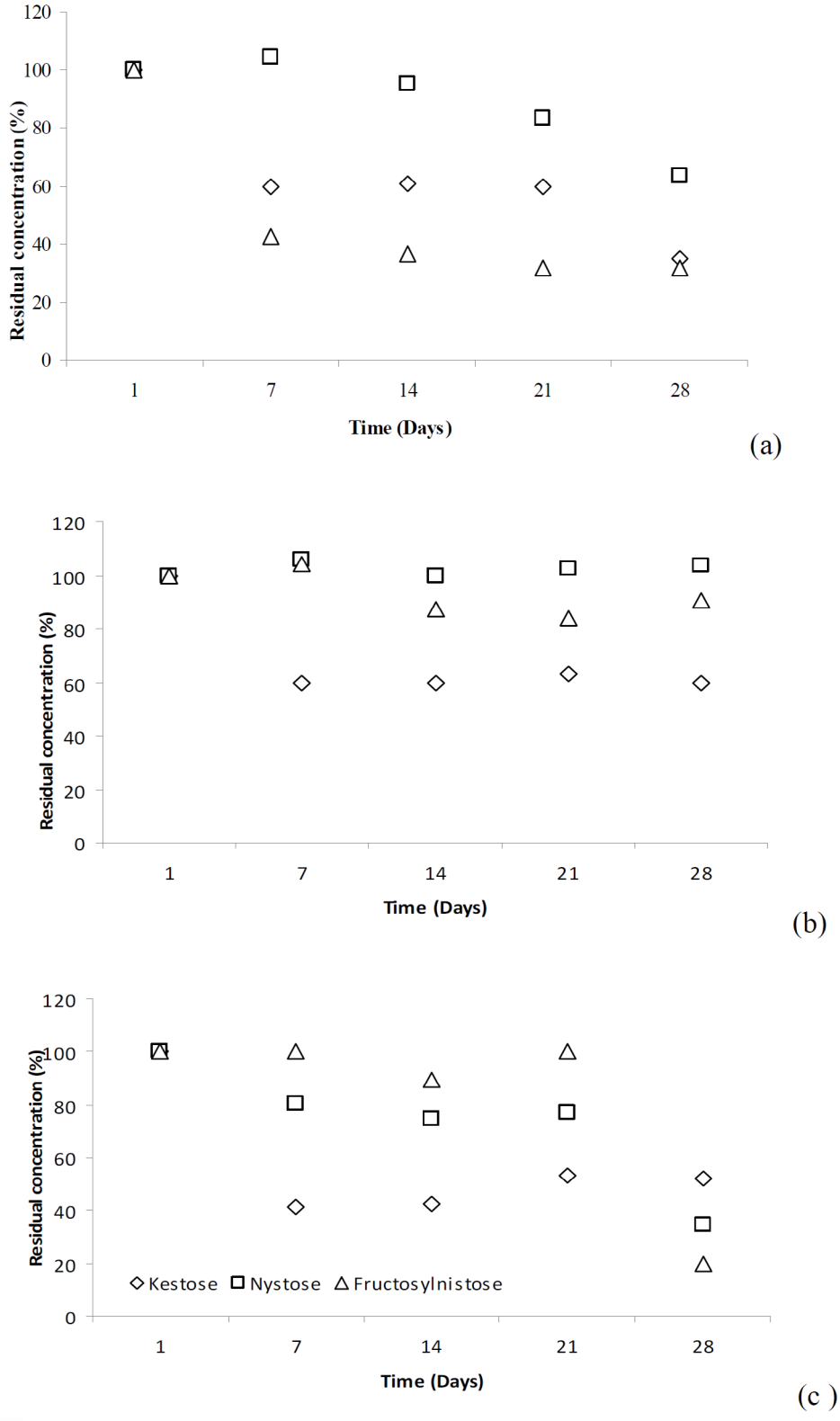


Figure 3 - Content of kestose, nystose fructosyl-nystose in yoghurt for run 2 (a), run 4 (b) and run 5 (c) as a function of storage time.

and then it remained almost constant until the 21st day, and then had a slight decline on the 28th day. Nystose remained almost constant until the 7th day of storage, with a gradual decline until the 28th day of storage. On the 21st day, the sample had 1.07g of FOS 100g$^{-1}$ of yoghurt, with 0.27% of kestose, 0.6% of nystose and 0.2% of fructosyl-nystose. A decline of almost 65% for the kestose, 36.6% for the nystose and 68.5% for the fructosyl-nystose was observed after 28 days of storage. This reduction is possibly because of the hydrolysis of FOS and fructose formation that occurred in the sample that *B. lactis* Bb-12 was not added.

By analysing the behaviour of run 4 (Figure 3b) with respect to the components of FOS, there is a lower reduction when compared to run 2 (Figure 3a), where for the kestose a larger decline was observed on the 7th day (reduction of 40%) and remained constant until the 28th day of storage. Nystose and fructosyl-nystose had similar behaviour until the 14th day; after this period the fructosyl-nystose was reduced by 9.3 %. In this same run, on the 1st day there was 1.7g of FOS 100g$^{-1}$ of yoghurt (3% FOS). In this case, there was smaller loss of FOS over time, remaining at 1.5g 100g$^{-1}$ yoghurt (0.3% kestose, 0.7% nystose and 0.5% fructosyl-nystose) at 21 and 28 days of storage. Thus, run 4, containing 1.5g of FOS 100g$^{-1}$ of product, is within the acceptable range for functional foods in accordance with BRASIL (2008).

On the other hand, run 5 (Figure 3c), added with 1.5% of commercial FOS, showed no characteristics of functional food, because after 21 and 28 days of storage, there were 1.32g and 0.26g of FOS 100g$^{-1}$ of yoghurt (0.24% and 0.1% of kestose, 0.1% and 0.55% of nystose, 12.53% and 12.06% fructosyl-nystose), respectively.

*Survival of probiotic culture in yoghurt*

Table 3 shows the probiotic bacteria count during the different storage times. It can be seen that the addition of FOS resulted in higher counts of probiotic bacteria. The addition of fibers (FOS) have a stimulating effect on the probiotics, being considered a symbiotic product (keeping its count in the range of $10^9$ to $10^{10}$ CFU mL$^{-1}$, with 3.0% (run 4) and 1.5% (run 5) of FOS, after 28 and 21 days of storage, respectively. According to Brazilian law (BRASIL, 2008), a food is considered a probiotic when it contains a minimal amount of viable probiotics in the range $10^8$ to $10^9$CFU mL$^{-1}$. This is the daily recommendation for consumption. According to WHO (2002), when consumed in appropriate amounts ($10^6$CFU mL$^{-1}$), result in a health benefit to the host.

It is known that keeping the count of probiotic bacteria within what is recommended for fermented milk products during shelf-life is no easy task, because their survival depends on a variety of factors such as the interaction between the species, growing conditions, final acidity, dissolved oxygen level of inoculation, storage temperature, among other factors.

## CONCLUSION

It can be concluded that the yoghurt added with prebiotic (fructooligosaccharide-FOS) and probiotic (*B. lactis* Bb-12) showed the lowest acidity, glucose and syneresis compared to the control yoghurt (without probiotic and prebiotic) and compared to formulations containing prebiotic only and probiotic only. The formulation containing 3% probiotic and prebiotic was the one that presented the smallest loss of FOS concentration, and was considered a

Table 3 – Matrix of $2^2$ factorial design (real and coded values) for the response count of probiotic bacteria (log10 CFU/mL) on the 1st, 7th, 14th, 21st and 28th day of storage.

| Run | Independent variable* | | **Probiotic bacteria ($log_{10}$ CFU/mL) | | | | |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | 1st day | 7th day | 14th day | 21st day | 28th day |
| 1 | -1 (0) | -1 (0) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 1 (3.0) | -1 (0) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 3 | -1 (0) | 1 (3.0) | 12.151$^a$ (±0.28) | 11.55$^b$ (±0.07) | 9.55$^b$ (±0.16) | 8.26$^c$ (±0.07) | 7.42$^c$ (±0.22) |
| 4 | 1 (3.0) | 1 (3.0) | 13.34$^a$ (±0.00) | 12.88$^a$ (±0.05) | 10.59$^b$ (±0.00) | 10.39$^b$ (±0.04) | 10.04$^b$ (±0.10) |
| 5 | 0 (1.5) | 0 (1.5) | 10.97$^a$ (±0.15) | 10.65$^a$ (±0.01) | 9.26$^b$ (±0.05) | 8.68$^b$ (±0.03) | 7.04$^c$ (±0.03) |

*$X_1$= fructooligosaccharides (%), *$X_2$= Probiotic starter cultures (%); **Average (standard deviation) followed by the same letters in rows indicate no significant difference between days of storage at 5% level (Tukey test).

functional food (1.5g of FOS per 100g yoghurt: 0.3% kestose, 0.7% nystose, and 0.5% fructosyl-nystose) at 21 and 28 day of storage at 4ºC. The addition of 3% of FOS in the yoghurt exerted a protective effect on probiotic bacteria and enhanced their survival.

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