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ORIGINAL ARTICLE

THE INFLUENCE OF SACCHARIN AND SORBITOL UPON THE BB-12® ACTIVITY IN MILK AND THE RHEOLOGICAL CHARACTERISTICS OF FERMENTED PRODUCTS

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The influence of an intense sweetener, saccharin (E954), and a bulk sweetener, sorbitol (E420), was studied related to the activity of the probiotic culture BB-12® in milk. Sweeteners concentrations used in the experiments were chosen based on the results of a preferential sensorial analysis.

The acidity and pH dynamics monitored during bifidobacteria incubation at 37°C showed that BB-12® behaves in a similar way in milk and milk sweetened with saccharin (0.05‰) or sorbitol (1.5%). Also it was noticed that the fermentation starts without a lag phase in the presence of sorbitol.

The bifidobacteria maintained their viability at a level of 10^9 viable count during 14 days of storage at 4°C. Sweetened fermented milk samples showed higher consistency index compared to the control sample. More than that, after 7 days of storage (4ºC) it was observed an increase of the k-value for the saccharin and sorbitol samples, while for the control sample the consistency index has a 85% decrease. After 7 days of storage the sweetened samples maintained the same viscoelastic behavior.

The study was performed in order to collect scientific evidences on the possibility to obtain probiotic dairy products sweetened with alternative sweeteners.

Keywords: probiotics, saccharin, sorbitol, BB-12®, *Bifidobacterium*

1. Introduction

According to the official site of the Danish company Chr. Hansen, BB-12® is the most widely researched probiotic bacteria. More than that, BB-12® is already available in numerous dairy products around the world as a result of the growing public awareness on diet-related health benefits, which is fuelling the demand for probiotic foods (www.chr-hansen.com).

Dairy foods, including in particular fermented milks and yoghurts, are among the best accepted food carriers for probiotic cultures (Hekmat, Soltani and Reid, 2009). This explains why the food industry produces a diversity of probiotic yoghurts and milk drinks and food stores have a wide range of such products on their shelves. Among fermented milk products, fruit yoghurts have been very popular also. The addition of fruit and sweeteners to yoghurt has made it more widely palatable (Van de Water and Naiyanetr, 2008). Also, the addition of sweeteners is to subdue the level of acidity produced, particularly when high acid/low sugar content fruits such as blackcurrant and raspberry are added to the cooled fermented base. The most popular method of adding is to include the sweetener in the fruit concentrate. Most fruits concentrates contain added sweeteners, the levels of which vary from 25-65%, the most common level being 30-35% (Early, 1998).

Today, as a consequence of the consumer's desire for a healthy diet that is also low in calories, a wide range of fruit yoghurts sweetened with non-caloric sweeteners can be found in food stores.

A reduced carbohydrate fermented dairy product has less than 4.9% carbohydrate, a viscosity ranging from 0.9-1.6 Pa·s and a pH ranging from 4.1 to 4.5 and it may be a fermented dairy product produced using ultrafiltered milk, a low carbohydrate or low glycemic sweetener, and a fruit preparation (Nguyen, 2008).

For the preparation of low caloric fruit yoghurts manufactures use artificial sweeteners, which have a significantly greater sweetness than sucrose, i.e. cyclamate is 30-80 times sweeter, saccharine 240-350 times sweeter, and can therefore be added at very small levels cost effectively (Early, 1998).

Based on their relative sweetness compared to sucrose, sweeteners are divided into intense or bulk sweeteners. In the past, the Scientific Committee on Food was the scientific guarantor for the safety of food additives (including sweeteners) in use within the European Union (EU). At present, this responsibility lies with the European Food Safety Authority. Extensive scientific research has demonstrated the safety of all sweeteners permitted for food use in the EU. Their safety is documented by the results of several *in vitro* and *in vivo* animal studies, tests in humans, and in some cases epidemiological studies. Their safety has been evaluated through a risk assessment process covering hazard identification, hazard characterization, exposure assessment and risk characterization (Mortensen, 2006). Permitted sweeteners have been allocated an acceptable daily intake (ADI), that was established on the basis of toxicity and safety studies (Pinheiro, 2005) representing the amount of a food additive, expressed as mg/kg body weight that can be ingested daily over a lifetime without incurring any appreciable health risk. ADI "acceptable" means that the expected exposure to the substance used in foods at the levels necessary to achieve desired technological effects does not represent a hazard to health. The consumption of sweeteners in the quantities within the ADI does not constitute a health hazard to consumers (Mortensen, 2006). The use of such sweeteners in fermented milks is regulated by Codex standard 243-2003.

Having in view all these facts, the influence of an intense sweetener, saccharin (E954), which is very low in calories and is safer for teeth than other sweeteners, and a bulk sweetener, sorbitol (E420), was studied related to the activity of the probiotic culture BB-12® in milk.

Hyvonen and Slotte, cited by Early R. (1998) reported potential inhibition of starter microorganisms with as low as 0.1% addition of saccharin and, for this reason, they advice dairy producers to add these sweeteners after fermentation.

This study aimed to collect scientific evidences on the possibility to obtain probiotic dairy products sweetened with alternative sweeteners, which are added into the milk before fermentation.

2. Materials and methods

The culture used in the experiments was *Bifidobacterium lactis,* a starter culture with the brand name of BB-12® (Chr.Hansen, Denmark). The culture, which was kindly provided by Chr. Hansen Romania, was activated in a liquid culture medium, MRS BROTH (Scharlan, Spain) and then innoculated in milk (10^6 cells/cm^3 milk).

All the tests were performed in UHT milk (La Dorna, Romania, 1.5% fat, batch no: 250309) in order to eliminate the raw material variability and the influence other bacteria, normally present in raw milk or pasteurised milk, could exert upon *Bifidobacterium lactis*.

As sweetners there were used:

- Saccharin (Importer: Ubimedia Raw material supplier, Galati, Romania)
- Diamant (Krüger GmBH&Co, Germany), a mixture of sorbitol (99,89%) and Na-saccharin (0,11%). Since this product contains 99.89% sorbitol, from now on it will be named sorbitol

The acidity was measured, during 24 hours incubation at 37°C, by titration with 0.1N NaOH solution using phenolphtaleine as indicator and expressed in Thörner degrees. It was prefered to express the titrable acidity in Thorner degrees instead of % lactic acid (% m/m), because it is known that bifidobacteria produce both lactic acid and acetic acid.

The pH was measured using a 315i pH meter (WTW GmbH, Germany) specially designed for measurements in meat, cheese, milk and viscous products.

Bacteria were counted directly in smears, in order to establish the inoculum quantity that have to be used for obtaining a concentration of 10^6 bacteria per cm^3 milk, or indirectly, by plate counting in MRS AGAR solid medium (Scharlan, Spain), in order to establish the bacteria viability.

The rheological measurements (flow behavior and viscoelasticity) were performed with a AR2000ex rheometer (TA Instruments, New Castle, USA), with a cone-plate geometry, cone angle 2^o. Fermented milk samples were placed carefully on the measuring system and left for 5 minutes to relax before testing. First a high shearing at $500s^{-1}$ for 60 s was applied, followed by a 300 s equilibration time. Fermented milks were subjected to controlled stress experiments. The stress was elevated from 0.1 to 100 Pa with a linear data acquisition time.

The flow curves showed shear rate ($\dot{\gamma}$) versus shear stress (σ) with up and down sweeps. All calculations were performed using the Rheology Advantage software. Samples were measured at 4 ± 1^0C. The hysteresis loop area between the upward and downward flow curves was integrated and calculated. Experimental data were fitted to the Ostwald de Waele model: $\sigma = K\dot{\gamma}^n$, where K ($mPas^n$) is the consistency index, and n the flow index. (González-Tomás, 2008)

Then a strain in the linear region (5%) was selected and a frequency sweep test was performed, frequency varied from 0.1-10 Hz. Storage modulus ($G^{/}$) which is a measure of elastic nature, loss modulus ($G^{//}$) which is related with the viscous nature of the material, and loss tangent (tan δ) were measured (Adeyeye and all., 2002).

Sensory analysis was conducted on a 10 member panel represented by students who attained a Sensory Analysis course and were selected as good panelists that only had to choose the sample they liked best. They performed an acceptance test using the selection technique in order to establish a concentration of sweetener in milk that will be considered acceptable by the consumers. Acceptance may be measured by preference or liking of a specific food item. The consumer acceptance test gives an estimate of product acceptance based on the product's sensory properties.(Resurreccion, 1998). The samples (30 cm^3) containing saccharin (0.05‰; 0.01%; 0.02%; 0.05%; 0.1%) or sorbitol (1%; 1.5%; 2%) were served in clear plastic cups and labeled with 2-digit codes. Samples were served at 4 ± 2 °C. Panelists individually evaluated 4 milks per session in an odor-free room dedicated to sensory analysis (Sensory Analysis Laboratory of the Faculty of Food Science and Engineering University Dunarea de Jos Galati, Romania). Ambient temperature spring water was available for palate cleansing. The panelists had to decide which concentration of saccharin and sorbitol is suitable for sweetening the milk.

3. Results and discussion

Based on the results obtained during the preferential sensorial analysis it was decided that the concentration of 0.05‰ saccharin and 1.5% sorbitol are suitable for producing sweetened fermented milk. So the fermentative capacity of BB-12® was comparatively evaluated in milk, milk with 0.05‰ saccharin, and milk with 1.5% sorbitol.

The graphs in Figure 1 show that the sample with sorbitol has a lag period much more reduced than the control sample and the sample with saccharin. For the control and the sweetened with saccharin sample this lag period is about 8 hours long, while for the sample with sorbitol is only 2 hours. This fact shows that the bacteria do not need an adaptation period to the presence of sorbitol and the fermentation process starts early. Also, the graphs in Figure 1 show that for all samples the final values of the acidity reaches close values and pH reaches 4.3 (isoelectric pH of milk casein) almost at the same time.

Recently, Klahorst, (2000), included polyols, such as sorbitol, to the prebiotics group (Tomasik, 2003). Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria (www.fao.org). So, the presence of sorbitol stimulated the growth of the BB-12® reducing the lag period, but because the amount of sorbitol added into the milk sample was very low, it was consumed rapidly and the fermentative process was not significantly influenced.

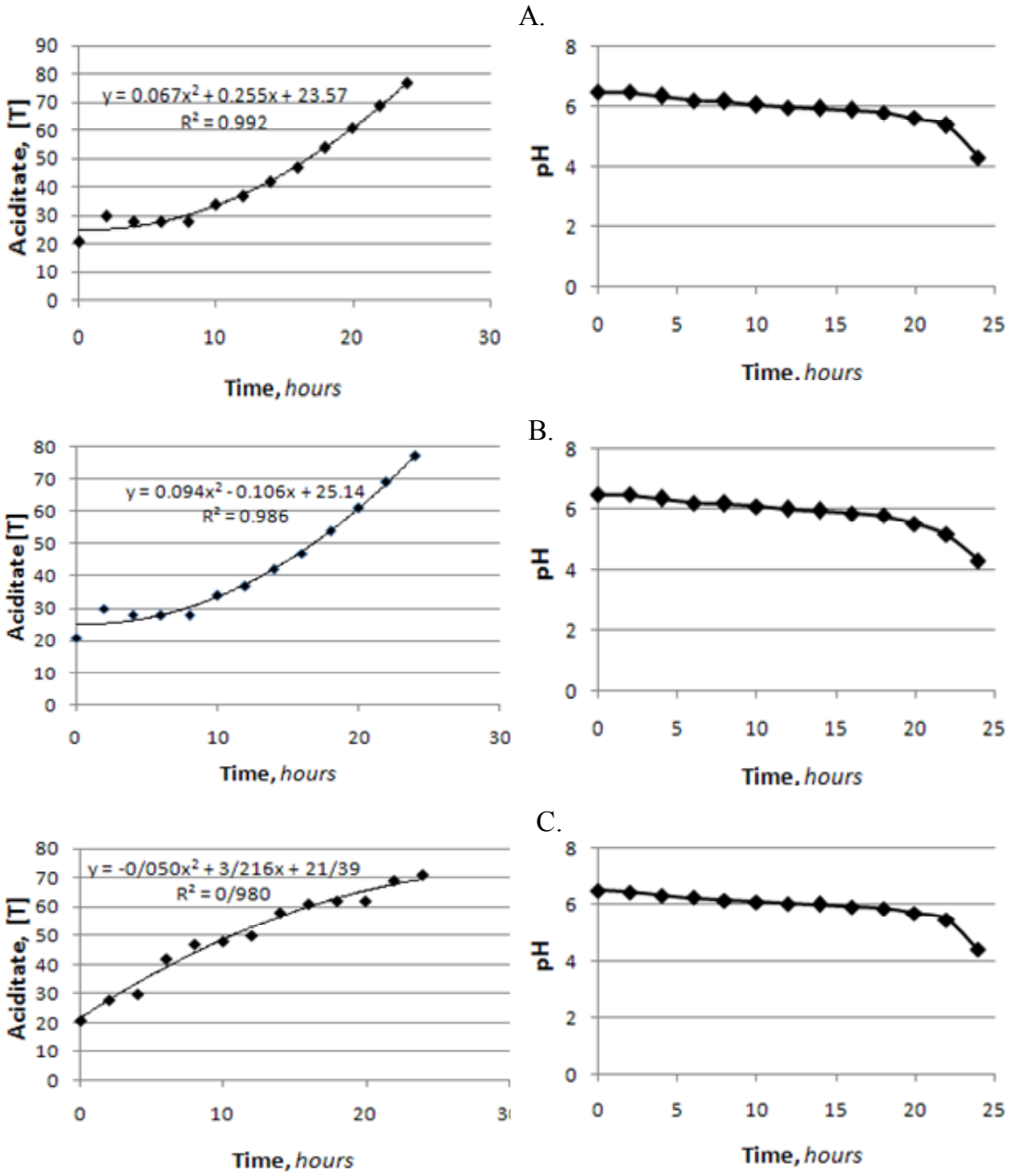


Figure 1. Changes in acidity and pH of milk samples with different concentrations of sweeteners under the action of BB-12® at 37°C: A. Control; B. 0.05‰ saccharin; C. 1.5% sorbitol.

The pH measurements indicated no adverse effect of the sweeteners on the growth of **BB-12®** cultures as the acidification process was consistent with normal pH development.

The viable cell counts reached in the end of the fermentative process 10^9 cfu/cm^3 for all samples (control and milk sweetened with saccharin or sorbitol).

All of the samples showed scarcely any change in pH value and maintained a refreshing and desirable sweetness upon storage both at refrigeration temperatures (4 – 6°C) and at 15 °C, for 2 weeks.

To establish whether the presence of the artificial sweetners in the milk affects the viability of the bacteria, counting was performed after 14 days of storage at 4°C. The aspect of all colonies was specific for the BB-12®.

Table 1. Viability of BB-12®cultures in fermented milk in the presence of sweeteners after 14 days of storage

Sweetener	cfu/cm^3
No added sweetener	$5.3 \cdot 10^9$
Saccharin 0.05‰	$2 \cdot 10^9$
Sorbitol 1.5%	$7.5 \cdot 10^9$

Probiotic cell counts remained above 10^9 colony forming units (cfu)/ cm^3 after the 14 days storage at 4°C (Table 1). This concentration is above the one required to produce therapeutic benefits: 106 and 108 viable cells/cm^3 (Bari and all., 2009).

The Ostwald de Waele model parameters, consistency index and flow behavior index (n) are presented in Table 2. After the first day of storage all samples indicated similar flow properties, while in the 7th days of storage the highest flow index was registered for the control sample.

Differences were noticed for the consistency of all samples with added sweeteners. An increase of the K-value with time was observed for the samples with sorbitol and saccharin that resulted in higher consistency index after 7 days when compared with the values after the first day of storage. Sweetened fermented milk samples showed higher consistency index after 1 day and after 7 days of storage compared to the control sample. More than that, after 7 days of storage (4ºC) it was observed an increase of the k-value for the saccharin and sorbitol samples, while for the control sample the consistency index has decreased from 0,9659 (Pa s^n) to 0,1241 (Pa s^n)

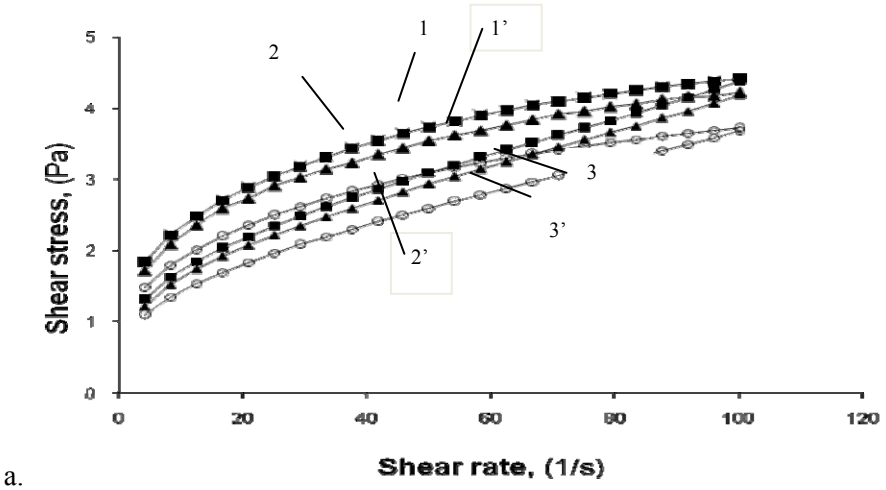
Table 2. Values of consistency index (K), flow behavior index (n) and relative thixotropic area (A_R) estimated using Ostwald de Waele model

Day 1	K(Pa s^n)	n	R^2	A_R
Control	0.9659	0.295	0.9992	40.255
Sorbitol	1.148	0.286	0.9991	53.106
Saccharin	1.230	0.281	0.9998	46.056
Day 7	K(Pa s^n)	n	R^2	A_R
Control	0.1241	0.4294	0.9016	5.668
Sorbitol	1.7450	0.2698	0.9956	39.990
accharin	1.5006	0.2579	0.9942	57.500

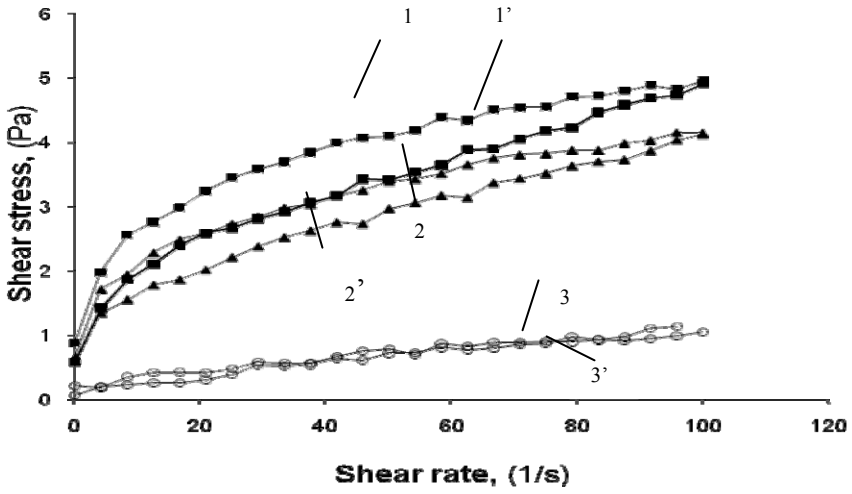
The decrease of the consistency index for the control sample can be attributed to higher fermentation rate. All samples showed observable hysteresis loops when they were sheared during a complete cycle, indicating that the sample flow was time-dependent. These products could be characterized as non-Newtonian fluids with thixotropic behavior resulting from the structural breakdown during the shearing cycle.

Assuming that the hysteresis loop area is an index of the energy needed to destroy the structure responsible for flow time dependence and according to the obtained values of the relative thixotropic area (González-Tomás, 2008) (Figure 2 a-b) the experimental data show that saccharin sample needed a higher energy to break this structure after 7 days of storage. For all the samples examined, except for the sample with saccharin a decrease in the hysteresis loop area, could be noticed within the storage time. This can be explained by the decrease in stability during the storage time. Moreover, control

sample demonstrated a higher decrease of the hysteresis loop after 7 days of storage (Figure 2b), so the energy needed to destroy the structure is reduced when compared with sweeteners samples.



a.



b

Control Saccharin Sorbitol

Figure 2. Flow curves of the samples with sweeteners after: 1 day (a) and 7 day (b) of storage.
1, 2, 3 – ascendent behavior 1', 2', 3' – descendent behavior

Tan δ , which is defined as the ratio of the loss modulus ($G^{/}$) to the storage modulus ($G^{//}$), is a measure of the viscous properties relative to the elastic properties of the viscoelastic material (Shoemaker and all., 1992). Looking at the values obtained for this parameter (Figure 3), it can be seen that after the first day of storage there are no important differences between the samples regarding the flow behavior. After 7 days of storage it can be noticed, for the control sample, an increase of the viscous behavior to the elastic behavior, while for the sweetened samples tan δ it varied in a narrow range

Similarly, Angelov and coworkers (2006) found no significant effect of saccharin on the growth of probiotic lactic acid bacteria used to obtain an oat-based probiotic drink. They concluded that the addition of artificial sweeteners (aspartame, sodium cyclamate, saccharine and Huxol) had no significant effect on the dynamics of the fermentation process and on the viability of the starter culture during the product storage. Studies regarding the effect of sorbitol upon different bacteria reported

different conclusions: Hyvonen and Slotte (2007) concluded that sorbitol, 15%, is not suitable for use as the only sweetener in pre-sweetened yoghurt retarding the growth of yoghurt culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*). On the other hand analysing the influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria Vinderola and all. (2002), studied the growth of 24 strains of probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and bifidobacteria) using sweeteners (aspartame and acesulfame) and concluded that sweeteners did not influence the growth of the strains in the concentrations commonly used in the dairy industry. Regarding sorbitol no study was found related with its effect upon BB-12® or other probiotic bacteria.

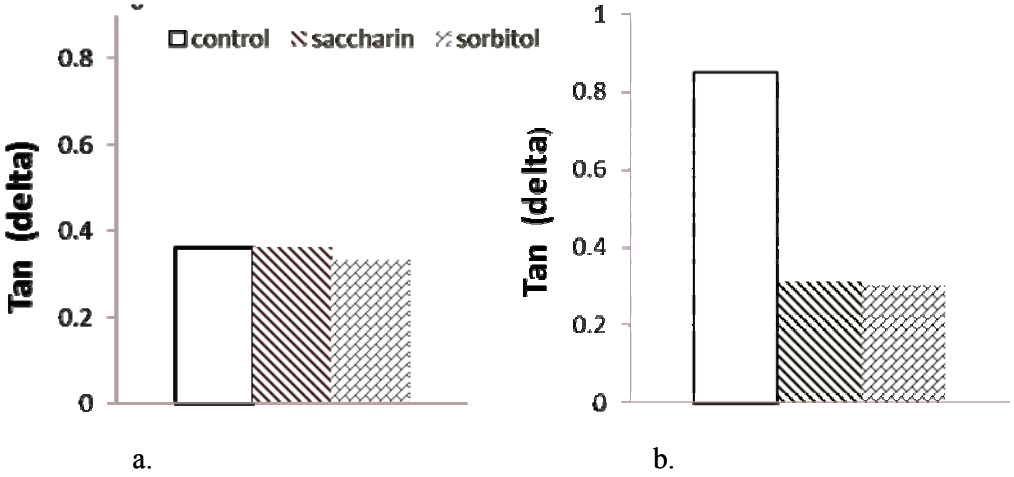


Figure 3. Tan δ values at 10 Hz for control and sweetened samples after 1(a) and 7(b) day.

4. Conclusions

Experiments confirmed the possibility of obtaining fermented milk from milk with added sweeteners.

The presence of saccharin 0.05‰ and sorbitol 1.5%, had no significant influence on the fermentative capacity of BB-12® due to the fact that the fermentative process ended at the same time and all samples reached closes pH values at the same time even if the presence of sorbitol reduces the lag period from 8 to 2 hours due to its prebiotic character.

The presence of saccharin 0.05‰ and sorbitol 1.5% do not significantly affect the bacteria viability after 14 days refrigeration of the fermented milk samples.

Sweetened fermented milk samples showed higher consistency index compared to the control sample. More than that, after 7 days of storage (4ºC) it was observed an increase of the k-value for the saccharin and sorbitol samples, while for the control sample the consistency index has decreased to15% of the initial value. So, the energy needed to destroy the structure for the control sample is reduced when compared with sweeteners samples, after 7 days of storage. The sweetened samples maintained the same viscoelastic behavior after storage at 4ºC for one week .

The results obtained may allow manufacturers to broaden their assortment range of fermented probiotic dairy products by introducing on market products low in calories, for people with diabetes or who care diets, adding sweeteners before fermentation stage.

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