Interference of lactose and sucrose in refrigerated storage of firm consistency yogurts

Interferência da lactose e sacarose no armazenamento refrigerado de iogurtes de consistência firme

ABSTRACT
This work consisted of a comparative analysis of four formulations of yogurts (with lactose/sucrose-free, with lactose/with sucrose, lactose-free/sucrose-free and lactose-free/with sucrose) in relation to changes occurring as a function of the presence or absence of sucrose and lactose. Chromatographic analysis (quantification of lactose), pH analysis and counting of viable lactic acid bacteria were carried out over 42 days. Coliforms, molds and yeasts were analyzed in a complementary manner. The pH values of all formulations were significantly reduced by comparing day 0 to day 42. In the formulations with lactose, there was a significant reduction in the concentration of this sugar throughout the storage. Higher stability of lactic acid bacteria was observed in the formulation with lactose and sucrose-free. In all formulations, counts of Streptococcus salivarius subsp. thermophilus were higher than the counts of Lactobacillus delbrueckii subsp. bulgaricus. There was no presence of coliforms, molds and yeasts in any formulation. In conclusion, lactic acid bacteria remain viable in yogurt throughout storage, even under refrigeration, and the presence or absence of lactose and sucrose interferes with S. thermophilus and L. bulgaricus counts.

Key words: Intolerance, lactic acid bacteria, lactose, storage, yogurt
RESUMO
Este trabalho consistiu em uma análise comparativa de quatro formulações de iogurtes (com lactose / sem sacarose, com lactose / com sacarose, sem lactose / sem sacarose e sem lactose / com sacarose) em relação às alterações ocorridas em função da presença ou ausência de sacarose e lactose. Análises cromatográficas (quantificação de lactose), análises de pH e contagem de bactérias ácido-lácticas viáveis foram realizadas durante 42 dias. De forma complementar, também foram realizadas análises de coliformes, bolores e levaduras. Os valores de pH de todas as formulações foram significativamente reduzidos comparando o dia 0 ao dia 42. Nas formulações com lactose, houve uma redução significativa na concentração desse açúcar durante o armazenamento. Foi observada maior estabilidade das bactérias ácido-lácticas na formulação com lactose e sem sacarose. Em todas as formulações, contagens de Streptococcus salivarius subsp. thermophilus foram superiores às contagens de Lactobacillus delbrueckii subsp. bulgaricus. Não houve presença de coliformes, bolores e levaduras em nenhuma formulação. Em conclusão, as bactérias ácido-lácticas permanecem viáveis no iogurte durante o armazenamento, mesmo sob refrigeração, e a presença ou ausência de lactose e sacarose interfere nas contagens de S. thermophilus e L. bulgaricus.

Palavras-chave: Intolerância, bactérias ácido-lácticas, lactose, armazenamento, iogurte

1 INTRODUCTION

Yogurt is the most popular fermented milk (Magenis et al., 2006; De Oliveira, 2014), being produced from milk fermentation by protosymbiotic cultures of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, and other lactic acid bacteria can also be used, in order to increase the characteristics of the final product (Brasil, 2007).

During the fermentation process, lactic acid bacteria consume 20-30% of lactose present in milk (Buttriss, 1997) producing volatile compounds and lactic acid, which is responsible for the acidification of the product and consequently for the coagulation of milk proteins (Fellows, 2006; Wijesinha-Bettoni and Burlingame, 2013; Gezginc et al., 2015), giving rise to yogurt.

Yogurt consumption has been growing worldwide (Shiby and Mishra, 2013, De Oliveira, 2014), mainly due to its practicality (De Oliveira, 2014), nutritional value, flavor and low cost (Tamime and Robson, 2007 apud Kwon et al., 2014). This trend has motivated the dairy industry to innovate in the production of yogurt, meeting an increasingly demanding and specific demand (Tamime and Robson, 2007 apud Vénica et al., 2018).

A major problem related to the consumption of foods containing lactose, such as yogurt, is the difficulty of digestion of this sugar by a considerable part of the population. It is estimated that approximately 70% of the world population presents one of the forms of lactose maldigestion, known as primary lactase deficiency (Heyman, 2006). In this case, even in childhood, generally from 3 years of age, the concentration of lactase begins to decrease, reducing the absorption capacity of lactose (Heyman, 2006; Koblitz, 2013). Other forms of maldigestion are secondary lactase deficiency, caused by some lesion of the intestine that damages the mucosa, being more common in children, and there
are also cases of children who are already born with lactase deficiency, but this case is extremely rare (Heyman, 2006).

In cases of digesting lactose problems, lactose is not fully absorbed and accumulates in the intestine, where it is fermented by the intestinal microbiota, causing uncomfortable symptoms, a condition known as lactose intolerance (Weaver, 2013).

People with lactose maldigestion can tolerate yogurt better than milk, which is due to longer gastric emptying, slower gastrointestinal transit, and lactase production by viable fermenters microorganisms after yogurt ingestion (Buttriss, 1997; Labayen et al., 2001; Jay, 2005). In cases where there is no addition of milk solids, it is possible to relate this better tolerance also to the lower concentration of lactose in comparison to milk, due to the considerable consumption of this sugar during the fermentation process (Buttriss, 1997). However, due to the great variation of the individual tolerance towards lactose, it is not possible to guarantee that the consumption of yogurt does not cause the discomfort of intolerance in all consumers with maldigestion of this sugar (Heyman, 2006).

Therefore, in the face of such individualism, to ensure that everyone can consume the product without symptoms, the food industry produces Zero Lactose yogurt, leaving consumers with lactose maldigestion more secure in their consumption.

The main form of producing lactose-free milk and dairy products is the enzymatic hydrolysis of this sugar, using the enzyme \( \beta \)-galactosidase (E.C. 3.2.1.23), popularly known as lactase (Morlock et al., 2014). This enzyme, predominantly of microbial origin, hydrolyzes the lactose, forming the monosaccharides glucose and galactose, easily absorbed by the intestine (Ordóñez, 2005; Koblitz, 2013).

In the case of sweetened yogurts, there is another drawback, which concerns the addition of sucrose. High sugar intake has been associated with obesity and a greater chance of chronic diseases, such as type 2 diabetes, for example. Thus, the reduction of its consumption has been treated as a way of promoting health (Morenga et al., 2013, World Health Organization, 2018). A growing public of people, have been seeking to reduce the consumption of sugar, by worrying each day more with health and nutrition, generating a great demand for products without adding sugar. From this new demand, added to the demand of the public of consumers with diabetes, a great variety of products without sucrose addition has appeared in the current market.

The market for specialty dairy products, such as Zero Lactose, and without added sucrose, has been growing exponentially, which does not occur with the same speed in research in this area. The objective of the present study was to compare yogurts prepared with different formulations (with lactose/with sucrose, with lactose/sucrose-free, lactose-free/with sucrose, lactose-free/sucrose-free),
following the modifications that occurred during refrigerated storage and relating them to the carbohydrate compositions of the dairy bases used to produce the yogurts.

2 MATERIALS E METHODS

All the analyzes were carried out at the laboratories of the Food Engineering course, from the Federal University of Uberlândia, in Patos de Minas.

Production of yogurts

Four firm yogurt formulations were produced: with lactose/sucrose-free, with lactose/with sucrose, lactose-free/sucrose-free and lactose-free/with sucrose. Each formulation was replicated three times.

Yogurts were produced from commercial ultra high-temperature milk (UHT) (carbohydrates: 5 g/100 mL, proteins: 3 g/100 mL, fats: 3 g/100 mL), following the methodology proposed by Vénica et al. (2018) with some modifications.

Each of the formulations was made from 1 L of milk and 0.1 g of lyophilized mixed YOMIX 885 culture (Danisco France SAS, Sassenage, FR).

For the lactose-free formulations, the milk was first subjected to a lactose hydrolysis process, by the addition of 2 mL of the enzymatic preparation LACTLOW L lactase base (Granolab | Granotec, Araucária, PR) and stirring in a heating plate and magnetic stirring, model C-MAG HS 7 S32 (Ind. and Com. Electro-Eletrônica Gehaka Ltda., São Paulo, SP) adjusted to 45°C for a period of 1 hour.

For the sucrose formulations, 80 g of sugar was added to the milk, with and without lactose.

Then, the four formulations were heat treated at 85 °C for 30 minutes in a micro-processed mouth water bath, model Q334M-28 (Quimis, Diadema, SP) to ensure the sterility of the product.

After this period, the milk bases were cooled to 43 °C at which temperature the lyophilized mixed culture was added. The formulations were shaken and packed in sterile individual glass jars, which were incubated in a germination chamber, model SL-225/364 (Solab, Piracicaba, SP) at 43 ± 1 °C until reaching a pH of 4.7 ± 1. The yogurts were stored at 4 °C ± 1 for 42 days.

pH analyses

The pH was measured using an mPA-210 model pH meter (Tecnopon, Piracicaba, SP) calibrated with buffer solutions 4.0 and 7.0. The analyses were performed in three repetitions for each yogurt replicate.

Quantification of lactose by high performance liquid chromatography (HPLC)

The lactose was quantified by HPLC using high efficiency liquid chromatograph (model LC-20 AT Prominence), with RID detector (Shimadzu Corporation, Kyoto, Japan) Supelcogel K column
Clarification of samples

Before being injected into the chromatography system, the samples were clarified following the methodology of Cataldi et al. (2003) and methodology 997.05 of AOAC International (2003), both adapted.

For each of the formulations, 15.625 g of sample was diluted in 50 mL of deionized water. After homogenization, 0.620 mL of Carrez I solution was added, with subsequent stirring. Then, 0.620 mL of Carrez II solution was also added, stirring again.

Carrez solutions were prepared according to AOAC International (2003) methodology 997.05, as described below:

- Carrez I: 1.5 g of K$_4$[Fe(CN)$_6$].3H$_2$O (potassium ferrocyanide trihydrate) P.A ACS (Vetec brand) for a total of 10 ml of solution;
- Carrez II: 3.0 g Zn(OAc)$_2$.2H$_2$O (zinc acetate bihydrate) P.A. crystallized (brand Proquimios) for a total of 10 ml of solution.

After this step, the samples were centrifuged at 3300 rpm for 10 minutes and the supernatants were filtered in a syringe filter with 0.20 μm pores.

Lactic acid bacteria count

The acid-lactic bacteria count was performed according to a methodology proposed by Akgun, Yazici & Gulec (2016). The counts of *L. bulgaricus* were measured on MRS agar (Oxoid, pH 5.2) incubated at 45 °C for 72 hours under anaerobic conditions. The counts of *S. thermophilus* were measured on M17 agar (Sigma-Aldrich, pH 7.1) supplemented with 10% (w/v) lactose solution (Sigma-Aldrich) incubated at 37 °C for 48 hours under aerobic conditions. In both cases, the pour plate technique was used. Counts were expressed in colony forming units per gram of product (cfu g$^{-1}$).

Analysis of the most probable number of total and thermotolerant coliforms

The analysis of coliforms was performed according to the methodology set forth in Chapter VI of Annex I of Normative Instruction No. 62 of August 26, 2003 (Brasil, 2003).

For the presumptive test dilutions $10^{-1}$ at double concentration (relative to dilution $10^{0}$) and dilutions $10^{-1}$ and $10^{-2}$ at single concentration were inoculated into Lauryl Sulfate broth (Ionlab), in tubes with threads containing Durhan tubes and incubated in BOD at 36 ± 1 °C for 48 hours. In the confirmatory test, tubes with bubble presence were peaked in tubes containing EC broth (Biolog) with Durhan tube and incubated at 45 ± 0.2 °C for 48 hours.
Mold and yeast counts

For the yeast and mold counting analyzes, YMR Compact Dry plates (Nissui Pharma, Japan) were used. Incubation occurred at 25 °C for 4 days, conditions used in a study by Akgun et al. (2016).

Statistical Analysis

Three replicate assays were conducted in the yogurt manufacturing, for greater representativeness of the results. The values obtained in the analyzes were expressed as the mean ± standard deviation (SD). One-way ANOVA and Tukey averages comparison tests, with a significance level of 95%, were used to verify if there was a significant difference between the results obtained, as a function of the yogurt composition and storage time. The software Microsoft Excel (2013 version, Microsoft, Redmond, Washington) and BioEstat (version 5.3, Instituto Mamirauá, Tefé, AM) were used.

3 RESULTS AND DISCUSSION

pH analyses

The results obtained in the pH analyzes are shown in Figure 1.

Figure 1 - pH values of yogurts at the end of production and during storage at 4 ± 1 °C.

For all formulations, pH values were significantly reduced until day 14, increasing slightly from day 21 and falling back on day 35. There was a statistical difference between the formulations only on day 7.

Mohammadi-Gouraji et al. (2019) also observed an increase in the pH of yogurts on day 21, attributing such behavior to the production of possible metabolites, such as amino acids, bacteriocins
and vitamins at the end of the product's useful life. However, since day 21 was the last day analyzed by the authors, it is not possible to predict the pH behavior from this moment onwards.

Vénica et al. (2018) analyzed the pH of four yogurt formulations over 28 days: with lactose (with and without sucrose) and without lactose (with and without sucrose). For all formulations analyzed, there was a reduction of pH up to day 21, and from day 14 the differences were very discrete. On day 28, the values were slightly higher than on day 21, but there was no statistical difference. Despite this trend of increasing at day 28, it is not possible to predict if the profile presented after that day would be similar to that found in this study.

Analyzing the pH change of unflavored yogurt over 8 days under storage at 4 °C, Wei et al. (2017) reported a decrease from ~ 4.57 to 4.24 from day 2 to day 8, consistent with this study.

Akgun et al. (2016) followed up changes in 4 formulations of unflavored buffalo milk yogurt over 20 days of storage at 4 °C and also observed a reduction in pH over the storage period, ranging from 4.60 - 4.64 to 4.35 - 4.39. The authors attributed this drop to the production of lactic acid even at refrigeration temperatures.

Even at a storage temperature of 4 °C used in this work, it is normal for there to be a drop in pH values of the yogurts over the days as a function of the residual metabolic activity of the viable lactic acid bacteria present (Beal et al., 1999; Wei et al., 2017), as occurred until the 14th day. This reduction in pH in the refrigerated storage period is related to the increase in lactic acid concentration, known as post acidification, since its production is not interrupted after refrigeration (Kneifel et al., 1993; Akgun et al., 2016). However, according to Shahbandari et al. (2016), over time, with the reduction of available carbohydrates, lactic acid bacteria can degrade proteins, promoting the increase of pH, as observed between days 14 and 28.

No studies were found in the literature with sufficient analysis time, which reported a drop in pH value, followed by an increase and subsequent drop again, as found in this work. Therefore, it is not possible to state the reason why pH values dropped again after day 35. One hypothesis for future studies would be the degradation of the compounds possibly responsible for the pH increase, formed in the previous moment, and the continuous production of lactic acid associated with the viability of lactic acid bacteria present in yogurts.

**Quantification of lactose by high performance liquid chromatography (HPLC)**

The lactose concentrations present in the yogurts, on days 1 and 42, are shown in Table 1.
Table 1 - Carbohydrate concentrations (g L\(^{-1}\)) during storage at 4 ± 1 ºC (mean ± standard deviation; n = 3).

| Day | Formulation | WLWS | WLSF | LFSF | LFWS |
|-----|-------------|------|------|------|------|
| Lactose | 1 | 29.36 ± 1.30\(^{aA}\) | 29.58 ± 0.42\(^{aA}\) | 0.00 ± 0.00\(^{aC}\) | 0.00 ± 0.00\(^{aC}\) |
| | 42 | 25.90 ± 0.43\(^{bA}\) | 23.61 ± 1.06\(^{bB}\) | 0.00 ± 0.00\(^{aC}\) | 0.00 ± 0.00\(^{aC}\) |

\(^{a}\) Means followed by the same uppercase letter in the same line or the same lowercase letter in the same column for the samples do not differ according to Tukey’s test. LFWS: lactose-free/with sucrose, WLWS: with lactose/with sucrose, WLSF: with lactose/sucrose-free, LFSF: lactose-free/sucrose-free.

As observed, there were significant changes in lactose concentrations over the refrigerated storage period for with lactose/with sucrose and with lactose/sucrose-free yogurts promoted by the residual metabolic activity of viable lactic acid bacteria (Beal et al., 1999), as found in a study by Vénica et al. (2018).

Lactose consumption was higher in the with lactose/sucrose-free sample, indicating that the carbohydrate composition in the formulations affects the metabolic activity of lactic acid bacteria, as concluded by Vénica et al. (2018).

In a study conducted by Vénica et al. (2018), lactose consumption over time was also higher for yogurt with lactose and sucrose-free, compared to yogurt with lactose and with sucrose. The authors suggested that the lower consumption of lactose in sweetened yogurt may be due to the consumption of sucrose as a source of carbon, even though it has not quantified this sugar.

According to Sobowale et al. (2011), the presence or absence of one sugar may interfere with the absorption of another sugar, which may justify the difference in the rate of consumption of lactose in the different formulations.

Some people with maldigestion of lactose tolerate the consumption of up to 12 g of lactose or 200 mL of milk in a single dose, with few or no symptoms (De Vrese et al., 2001; European Food Safety Authority, 2010). This tolerance can become even greater if the product to be consumed is yogurt (Hutkins and Goh, 2014; Tong and Berner, 2016; Aryana and Olson, 2017). As a considerable reduction in lactose concentration has been observed throughout the refrigerated storage of the yogurts produced in this research, it is concluded that for these consumers, consuming the yogurt in the last days of its useful life will bring even lower risks of the manifestation of symptoms.

**Microbiological analyses**

The results of the counts of lactic acid bacteria are shown in Table 2.
Table 2 - Change in lactic acid bacteria counts during storage at 4 ± 1 ºC (mean ± standard deviation; n = 3).

| Days | Streptococcus thermophilus | Lactic acid bacteria count (log cfu g⁻¹) | Lactobacillus bulgaricus |
|------|--------------------------|------------------------------------------|-------------------------|
|      | LFWS | WLWS | WLSF | LFSF | LFWS | WLWS | WLSF | LFSF |
| 1    | 9.31 ± 0.02ₐ   | 9.13 ± 0.03ₐ   | 9.18 ± 0.03ₐ   | 9.03 ± 0.00ₐ   | 5.08 ± 0.14ₐ   | 4.51 ± 0.03ₐ   | 5.25 ± 0.25ₐ   | 5.59 ± 0.01ₐ   |
| 7    | 9.17 ± 0.00ₐ   | 9.29 ± 0.02ₐ   | 9.23 ± 0.10ₐ   | 9.28 ± 0.08ₐ   | 5.29 ± 0.20ₐ   | 4.56 ± 0.07ₐ   | 5.23 ± 0.25ₐ   | 5.31 ± 0.4ₐ    |
| 14   | 9.31 ± 0.1ₐ    | 9.21 ± 0.06ₐ   | 9.23 ± 0.04ₐ   | 9.23 ± 0.04ₐ   | 5.41 ± 0.08ₐ   | 4.61 ± 0.10ₐ   | 5.29 ± 0.22ₐ   | 5.59 ± 0.04ₐ   |
| 21   | 9.14 ± 0.09ₐ   | 9.30 ± 0.03ₐ   | 9.31 ± 0.04ₐ   | 9.18 ± 0.05ₐ   | 4.81 ± 0.09ₐ   | 4.41 ± 0.09ₐ   | 5.12 ± 0.1ₐ    | 3.34 ± 0.03ₐ   |
| 28   | 9.17 ± 0.00ₐ   | 9.16 ± 0.04ₐ   | 9.19 ± 0.0ₐ    | 9.23 ± 0.04ₐ   | 4.59 ± 0.0ₐ    | 4.40 ± 0.0ₐ    | 5.15 ± 0.0ₐ    | 5.22 ± 0.07ₐ   |
| 35   | 9.13 ± 0.00ₐ   | 9.08 ± 0.0ₐ    | 9.21 ± 0.0ₐ    | 9.20 ± 0.0ₐ    | 4.33 ± 0.0ₐ    | 4.28 ± 0.0ₐ    | 5.15 ± 0.0₂ₐ   | 4.38 ± 0.3₀ₐ   |
| 42   | 9.15 ± 0.0ₐ    | 9.18 ± 0.0ₐ    | 9.22 ± 0.0ₐ    | 9.11 ± 0.0ₐ    | 3.94 ± 0.0ₐ    | 4.19 ± 0.1₁ₐ   | 5.06 ± 0.0ₐ    | 4.02 ± 0.0ₐ    |

*Means followed by the same uppercase letter in the same line or the same lowercase letter in the same column for the samples do not differ according to Tukey’s test. LFWS: lactose-free/with sucrose, WLWS: with lactose/with sucrose, WLSF: with lactose/sucrose-free, LFSF: lactose-free/sucrose-free.

As can be observed, in general, there was lower stability of the L. bulgaricus culture throughout the refrigerated storage period, being also the most affected by the different treatments in relation to the S. thermophilus culture.

Regarding the S. thermophilus counts, there was only statistical difference over time for the with lactose/with sucrose sample, with a minimum count on day 35, and for the lactose-free/sucrose-free sample, with an increase in counting on day 7.

Regarding the counts of L. bulgaricus, there was no significant difference over time for the with lactose/sucrose-free sample. For the other samples, different profiles were observed, but on day 42, both lactose-free/with sucrose yogurt and lactose-free/sucrose-free presented significantly lower counts compared to day 1. The highest drop in counting occurred in lactose-free/with sucrose yogurt.

The counts of S. thermophilus were statistically the same among the different treatments on all the days of analysis, except for day 1. The counts of L. bulgaricus were statistically different among the treatments in all the days of analysis, except for day 7.

Yogurts produced with milk without lactose hydrolysis and without added sugar (with lactose/sucrose-free) showed more stable lactic acid bacteria counts over time in relation to the others.

In the work done by Vénica et al. (2018) there was no significant difference between the formulations analyzed: with lactose (with and without sucrose) and without lactose (with and without sucrose), indicating that carbohydrate compositions of the different milk bases did not affect the viability of lactic acid bacteria. There was also no significant difference between the counts of S. thermophilus and L. bulgaricus throughout the refrigerated storage, indicating good stability of the same in the different matrices. These authors found values varying from 9.1 to 9.3 log CFU / g and...
from 2.2 to 2.8 log CFU / g for *S. thermophilus* and *L. bulgaricus*, respectively. The *S. thermophilus* counts were consistent with the present study, but the counts of *L. bulgaricus* were lower.

Mohammadi-Gouraji et al. (2019), observed a significant drop in counts of *L. bulgaricus* and *S. thermophilus* over 21 days of analysis, of approximately two log cycles for both cultures in natural traditional yogurt made with skim milk, which is not in accordance with the present work, since for the lactose and sucrose-free samples there was no significant difference in the counts over the storage period.

Akgun et al. (2016) analyzed the population of *S. thermophilus* and *L. bulgaricus* in traditional 3% fat (w/w) buffalo milk yogurts over 20 days of refrigerated storage. The *S. thermophilus* count ranged from 8.25 to 8.60 log CFU / g, showing no significant difference, indicating good stability of the culture over the life of the yogurt. The authors reported counts of *L. bulgaricus* ranging from 6.33 to 5.20 log CFU/g, being therefore lower than the counts of *S. thermophilus*, which is consistent with the present work. Despite a reduction in the counts of the two lactic acid bacteria, only for *L. bulgaricus*, there was a significant statistical difference, indicating a lower stability of this culture throughout the storage period, which is not in agreement with the present study, in relation to the similar formulation (with lactose/sucrose-free), since in this case, the two cultures remained stable throughout the 42 days.

Oliveira et al. (2015) purchased at the market, low-fat natural yogurt at the beginning of their useful lives and added different concentrations of a strawberry mix of own production, producing different formulations of strawberry flavored yogurts. Control yogurts (without addition of the mix) showed *L. bulgaricus* counts ranging from ~6 to ~3.5 log CFU / mL, while *S. thermophilus* counts ranged from ~7.5 to ~7 log CFU / mL, indicating greater stability of the *S. thermophilus* culture throughout the refrigerated storage period, as observed by Akgun et al. (2016).

The presence of coliforms was not detected in any of the formulations produced, as well as of molds and yeasts, indicating good microbiological quality and efficacy in hygienic-sanitary care in the production and conditioning stages (Akgun et al., 2016; Barkallah et al., 2017).

Birollo et al. (2000), Akgun et al. (2016) and Barkallah et al. (2017) also did not detect the presence of coliforms in yogurts produced in their studies. Among these authors, molds and yeasts were grown only in Akgun et al. (2016), while in the others these microorganisms were not detected even after 4 weeks of storage at 4 °C.

Akgun et al. (2016) found mold and yeast counts ranging from 0.4 log cfu g⁻¹ to 5.7 log cfu g⁻¹ from day 1 to day 20 of storage for yogurt with lactose, produced from milk of buffalo with 3% (w/w) of fat and without sucrose. Despite the significant growth of these microorganisms, the *S. thermophilus* count did not vary statistically during the storage, however, the population of *L.
bulgaricus reduced in approximately 1 logarithmic cycle. Some authors have related the reduction in the population of L. bulgaricus to the growth of the yeast population (Canganella et al., 1998; Viljoen et al., 2003). This relationship is consistent considering the results obtained in the present work, since there was no growth of molds and yeasts in any of the formulations and both the S. thermophilus and L. bulgaricus populations in the similar sample (with lactose/sucrose-free) remained statistically invariant throughout the storage.

4 CONCLUSIONS

It is possible to conclude that, even under refrigeration, lactic acid bacteria remain viable in yogurts, since they promote the reduction of both pH and lactose in these products. In the present study, it was possible to prove that the presence or absence of lactose and sucrose interfered in the counts of S. thermophilus and L. bulgaricus and the formulation with lactose/sucrose-free promoted greater stability to the cultures throughout the storage.

ACKNOWLEDGMENTS

The authors thank Fundação de Apoio Universitário (FAU-UFU), Pró-Reitoria de Pesquisa e Pós-graduação (ProPP-UFU), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP) and technicians of the Laboratories of Food Engineering of the Federal University of Uberlandia, Patos de Minas campus for their support.

REFERENCES

Akgun, A., Yazici, F., & Gulec, H. A. (2016). Effect of reduced fat content on the physicochemical and microbiological properties of buffalo milk yoghurt. LWT - Food Science and Technology. https://doi.org/10.1016/j.lwt.2016.08.015.

AOAC International. (2003). Official Methods of Analysis (15 (rev 2)). Gaithersburg, MD: AOAC International.

Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. Journal of Dairy Science, 100(12), 9987–10013. https://doi.org/10.3168/jds.2017-12981.
Barkallah, M., Dammak, M., Louati, I., Hentati, F., Hadrich, B., Mechichi, T., … Abdelkafi, S. (2017). Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage. *LWT - Food Science and Technology*, 84, 323–330. https://doi.org/10.1016/j.lwt.2017.05.071.

Beal, C., Skokanova, J., Latrille, E., Martin, N., & Corrieu, G. (1999). Combined Effects of Culture Conditions and Storage Time on Acidification and Viscosity of Stirred Yogurt. *Journal of Dairy Science*, 82(4), 673–681. https://doi.org/10.3168/jds.S0022-0302(99)75283-5.

Birollo, G. A., Reinheimer, J. A., & Vinderola, C. G. V. (2000). Viability of lactic acid microflora in different types of yoghurt. *Food Research International*, 33(9), 799–805. https://doi.org/10.1016/S0963-9969(00)00101-0.

Brasil. (2003). Ministério da Agricultura, Pecuária e Abastecimento. Normative Instruction No. 62, August 26, 2003. Oficializa os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. *Diário Oficial da União*, Brasília, DF, september 18, 2003, Section 1, p. 14.

Brasil. (2007). Ministério da Agricultura, Pecuária e Abastecimento Normative Instruction n° 46, october 23, 2007. Adota o Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. *Diário Oficial da União*, Brasília, DF, october 24, 2007, Section 1, p. 4.

Bunim, J. (2013). *Quantity of Sugar in Food Supply Linked to Diabetes Rates*. Retrieved May 14, 2019, from: https://www.ucsf.edu/news/2013/02/98777/quantity-sugar-food-supply-linked-diabetes-rates.

Buttriss, J. (1997). Nutritional properties of fermented milk products. International *Journal of Dairy Technology*, 50(1), 21–27. https://doi.org/10.1111/j.1471-0307.1997.tb01731.x.

Canganella, F., Ovidi, M., Paganini, S., Vettraino, A. M., Bevilacqua, L., & Trovatelli, L. D. (1998). Survival of undesirable micro-organisms in fruit yoghurts during storage at different temperatures. *Food Microbiology*, 15(1), 71–77. https://doi.org/10.1006/fmic.1997.0142.
Cataldi, T. R. I., Angelotti, M., & Bianco, G. (2003). Determination of mono- and disaccharides in milk and milk products by high-performance anion-exchange chromatography with pulsed amperometric detection. *Analytica Chimica Acta*, 485, 43–49. https://doi.org/10.1016/S0003-2670(03)00405-7.

Cutrim, C. S., de Barros, R. F., da Costa, M. P., Franco, R. M., Conte-Junior, C. A., & Cortez, M. A. S. (2016). Survival of *Escherichia coli* O157: H7 during manufacture and storage of traditional and low lactose yogurt. *LWT - Food Science and Technology*, 70, 178–184. https://doi.org/10.1016/j.lwt.2016.02.047.

De Oliveira, M. N. (2009). Características funcionais de leites fermentados e outros produtos lácteos. In M. N. Oliveira (Ed.), *Tecnologia de produtos lácteos funcionais* (pp. 21–84). São Paulo: Artheneu.

De Oliveira, M. N. (2014). Fermented Milks and Yogurt. *Encyclopedia of Food Microbiology*, 1. https://doi.org/10.1016/B978-0-12-384730-0.00121-X.

European Food Safety Authority. (2010). Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia. *EFSA Journal*, 8(9:1777), 1–29. https://doi.org/10.2903/j.efsa.2010.1777.

Fellows, P. J. (2006). *Tecnologia do processamento de alimentos*: Princípios e prática (2nd ed.). Porto Alegre: Artmed.

Gezginc, Y., Topcal, F., Comertpay, S., & Akyol, I. (2015). Quantitative analysis of the lactic acid and acetaldehyde produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains isolated from traditional Turkish yogurts using HPLC. *Journal of Dairy Science*, 98(3), 1426–1434. https://doi.org/10.3168/jds.2016-99-2-1694.

Heyman, M. B. (2006). Lactose Intolerance in Infants, Children, and Adolescents. *Pediatrics*, 118(3), 1279–1286. https://doi.org/10.1542/peds.2006-1721.
Hutkins, R., & Goh, Y. (2014). *Streptococcus thermophilus*. *Encyclopedia of Food Microbiology*, 3, 554–559. https://doi.org/10.1016/B978-0-12-384730-0.00325-6.

Jay, J. M. (2005). *Microbiologia de alimentos* (6th ed.). Porto Alegre: Artmed.

Kneifel, W., Jaros, D., & Erhard, F. (1993). Microflora and acidification properties of yogurt and yogurt-related products fermented with commercially available starter cultures. *International Journal of Food Microbiology*, 18(3), 179–189. https://doi.org/10.1016/0168-1605(93)90043-G.

Koblitz, M. G. B. (2013). Carboidrases. In M. G. B. Koblitz (Ed.), *Bioquímica de Alimentos*: Teoria e aplicações práticas (pp. 19–76). Rio de Janeiro: Guanabara Koogan.

Kwon, D. Y., Nyakudya, E., & Jeong, Y. S. (2014). Fermentation: Food Products. *Encyclopedia of Agriculture and Food Systems*, 3, 113–123. https://doi.org/10.1016/B978-0-444-52512-3.00155-8.

Labayen, I., Forga, L., González, A., Lenoir-Wijnkoop, I., Nutr, R., & Martínez, J. A. (2001). Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. *Alimentary Pharmacology and Therapeutics*, 15(4), 543–549. https://doi.org/10.1046/j.1365-2036.2001.00952.x.

Lefebvre, S., Hasford, J., & Wang, Z. (2019). The effects of guilt and sadness on sugar consumption. *Journal of Business Research*, 100, 130–138. https://doi.org/10.1016/j.jbusres.2019.03.023.

Magenis, B.M., E.S. Prudêncio, R.D.M.C. Amboni, N.G. Cerqueira Júnior, R.V.B. Oliveira, V. Soldi, and H.D. Benedet. 2006. Compositional and physical properties of yogurts manufactured from milk and whey cheese concentrated by ultrafiltration. *International Journal of Food Science & Technology*. 41:560–568. doi:10.1111/j.1365-2621.2005.01100.x.

Mohammadi-Gouraji, E., Soleimanian-Zad, S., & Ghiaci, M. (2019). Phycocyanin-enriched yogurt and its antibacterial and physicochemical properties during 21 days of storage. *LWT - Food Science and Technology*, 102, 230–236. https://doi.org/10.1016/j.lwt.2018.09.057.

Morenga, L. Te, Mallard, S., & Mann, J. (2013). Dietary sugars and body weight: Systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ (Online)*, 346(7492), 1–25. https://doi.org/10.1136/bmj.e7492.
Morlock, G. E., Morlock, L. P., & Lemo, C. (2014). Streamlined analysis of lactose-free dairy products. *Journal of Chromatography A*, 1324, 215–223. https://doi.org/10.1016/j.chroma.2013.11.038.

Oliveira, A., Alexandre, E. M. C., Coelho, M., Lopes, C., Almeida, D. P. F., & Pintado, M. (2015). Incorporation of strawberries preparation in yoghurt : Impact on phytochemicals and milk proteins. *Food Chemistry*, 171, 370–378. https://doi.org/10.1016/j.foodchem.2014.08.107.

Ordóñez, J. A. (2005). *Tecnologia de Alimentos*: Alimentos de origem animal (v. 2). Porto Alegre: Artmed.

Saint-Eve, A., Lévy, C., Le Moigne, M., Ducruet, V., & Souchon, I. (2008). Quality changes in yogurt during storage in different packaging materials. *Food Chemistry*, 110(2), 285–293. https://doi.org/10.1016/j.foodchem.2008.01.070.

Shahbandari, J., Golkar, A., Taghavi, S. M., & Amiri, A. (2016). Effect of Storage Period on Physicochemical, Textural, Microbial and Sensory Characteristics of Stirred Soy Yogurt. *International Journal of Farming and Allied Sciences*, 5(6), 476–484. Retrieved from: http://ijfas.com/wp-content/uploads/2016/09/476-484.pdf.

Shiby, V. K., & Mishra, H. N. (2013). Fermented Milks and Milk Products as Functional Foods-A Review. *Critical Reviews in Food Science and Nutrition*, 53(5), 482–496. https://doi.org/10.1080/10408398.2010.547398.

Sobowale, A. A., Efuntoye, M. O., & Adesetan, O. O. (2011). Energy sources of yoghurt bacteria and enhancement of their galactose uptake. *African Journal of Biotechnology* 10(21), 4457–4463. https://doi.org/10.5897/AJB10.2600.

Tong, P. S., & Berner, L. A. (2016). Dairy Processing and Products. *Reference Module in Food Science*. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.02935-8.

Vénica, C. I., Wolf, I. V., Suárez, V. B., Bergamini, C. V., & Perotti, M. C. (2018). Effect of the carbohydrates composition on physicochemical parameters and metabolic activity of starter culture
in yogurts. *LWT - Food Science and Technology*, 94, 163–171. https://doi.org/10.1016/j.lwt.2018.04.034.

Viljoen, B. C., Lourens-Hattingh, A., Ikalafeng, B., & Peter, G. (2003). Temperature abuse initiating yeast growth in yoghurt. *Food Research International*, 36(2), 193–197. https://doi.org/10.1016/S0963-9969(02)00138-2.

Vrese, M. De, Stegelmann, A., Richter, B., Fenselau, S., Laue, C., & Schezenmeir, J. (2001). Probiotics-compensation for lactase insufficiency 1–3. *The American Journal of Clinical Nutrition*, 73(2), 421S–429S. https://doi.org/doi: 10.1093/ajcn/73.2.421s.

Weaver, C. (2013). Milk and dairy products as part of the diet. In E. Muehlhoff, A. Bennett e D. Mcmahon (Eds), *Milk and dairy products in human nutrition* (pp. 103-206). Roma: FAO - Food and Agriculture Organization of the United Nations. Retrieved from online: http://www.fao.org/docrep/018/i3396e/i3396e.pdf, last accessed May 2019.

Wei, Z., Zhang, W., Wang, Y., & Wang, J. (2017). Monitoring the fermentation, post-ripeness and storage processes of set yogurt using voltammetric electronic tongue. *Journal of Food Engineering*, 203, 41–52. https://doi.org/10.1016/j.jfoodeng.2017.01.022.

Wijesinha-Bettoni, R., & Burlingame, B. (2013). Milk and dairy product composition. In E. Muehlhoff, A. Bennett, & D. Mcmahon (Eds), *Milk and dairy products in human nutrition* (pp. 41–102). Roma: FAO - Food and Agriculture Organization of the United Nations. Retrieved from online: http://www.fao.org/docrep/018/i3396e/i3396e.pdf, last accessed May 2019.

World Health Organization. (2018). *Obesity and overweight*. Retrieved May 14, 2019, from: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.