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Impact of partial and total replacement of milk by water-soluble soybean extract on fermentation and growth parameters of kefir microorganisms

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ABSTRACT

In this study, the use of soybean extract on production and physicochemical characteristics of kefir was assessed. Also, the growth parameters of yeasts and lactic acid bacteria (LAB) during fermentation were evaluated. Experiments were carried out combining water-soluble soybean extract (S) and milk (M), leading to the evaluation of total or partial replacement of milk (formulations 100%S, 50%S50%M) a control formulation (100% M). During kefir fermentation, physicochemical analysis carried out as well as enumeration of yeasts and LAB. In all formulations, lactic acid concentration increased due to carbohydrates consumption by kefir microorganisms resulting in decrease of soluble solids content and increase of acidity. The final acidity of beverages varied from 0.600 to 0.738 g of lactic acid/100 mL and soluble solids ranged from 6.40 to 5.67 °Brix. Formulations 100%S and 100%M presented LAB counts of 8 $\log_{10}$ CFU/mL. LAB lag time increased in formulation 50%S50%M compared to formulations 100%S and 100%M (2.20 h, 1.03 h and 1.06 h, respectively). LAB and yeasts growth parameters were not affected in beverages prepared with milk and soybean kefir. This is the first study that examined the yeast and LAB growth parameters using water-soluble soybean extract fermented with kefir.

1. Introduction

Currently, consumers seek for foods that may help to prevent nutrition-related illnesses and enhance well-being. These fundamentals emerged from the concept of "*functional food*," which means "foods that may provide health benefits beyond basic nutrition" (Siró, Kápolna, Kápolna, & Lugasi, 2008). Among the different functional food available, those which probiotics are added stand out. According to FAO/WHO (2002), probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." The most common microorganisms used as probiotics are species of *Lactobacillus*, and *Bifidobacterium* (Williams, 2010) and fermented foods are suitable products for the vehiculation of probiotics, especially fermented dairy products.

Kefir is an acidic and mild alcoholic fermented dairy product with a distinct flavor, viscosity and slightly effervescence. The acid taste is a result of lactic acid production by lactic acid bacteria (LAB), and the slightly alcoholic aspect is due to the presence of yeasts, both microorganisms added to the milk as kefir grains. The effervescent effect is provided by the production of carbon dioxide (CO_2) after fermentation (Ferreira, 2005; Mistry, 2004). While lactic acid, ethanol, and CO_2 are the main products of kefir fermentation, other minor components also contribute to the flavor, like diacetyl, acetaldehyde, compounds belonging to the ethyl group and amino acids (Leite et al., 2013).

Kefir grains are a symbiotic association of different microorganisms wrapped in a polysaccharide matrix called kefiran, and the microbial ecology depends on the origin and cultivation method of the grains (Ferreira & Santos, 2008). *Lactobacillus brevis*, *Lactobacillus helveticus*, *Lactobacillus kefir*, *Leuconostoc mesenteroides*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus* and *Pichia fermentans* are the main LAB and yeast species identified in kefir grains (FAO/WHO, 2003; Liu, Wang, Chen, Yueh, & Lin,

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2006). Over time, in some countries, especially in those of Eastern Europe, kefir consumption has been suggested for the treatment of some diseases, including hypertension, ischemic heart disease, hypercholesterolemic effects and allergies (Farnworth, 2006; Leite et al., 2013; Liu et al., 2006).

Cow's milk allergy is an immunological intolerance to milk proteins or lactose (Hill, Murch, Rafferty, Wallis, & Green, 2007). The impossibility of allergic people to milk proteins or lactose to consume dairy products has urged the development of alternative products. According to Fiorda et al. (2017), the use of other matrices (such as fruits, vegetables, and molasses) as a substrate for kefir fermentation is still limited compared with their dairy counterpart. However, the large microbial diversity prevailing in kefir grains and its easy adaptation to different substrates are advantages when compared to single-species starter cultures. Soy-based formulations represent a valid nutritional option as replacement of milk, as soybean is an excellent source of low-cost protein (Liu et al., 2006). Also, beneficial effects associated to soybeans consumption on nutrition and health have been observed, including cancer prevention, plasma cholesterol reduction, obesity, diabetes, and protection against bowel and kidney disease (Friedman & Brandon, 2001). The production of fermented kefir beverage using soybean extract, the different human health effects (antimutagenic, antioxidant and anti-allergenic properties) related to its consumption and also the beneficial effects on the intestinal microflora have been studied (Baú, Garcia, & Ida, 2014; Kesenkas, Dinkçi, Seçkin, Kinik, & Gönç, 2011a; Liu, Chen, & Lin, 2005; Liu et al., 2006; McCue & Shetty, 2005). However, papers focus on nutritional, healthy or sensory characteristics of the product and do not evaluate the microbial evolution on different subtracts. Moreover, so far no predictive models were fitted to assess the microbial growth of kefir microorganisms. Thus, the objective of this study was to appraise the effect of the addition of different ratios of soybean extract in the composition of a water-soluble soybean extract beverage fermented with kefir grains and to obtain the parameters of multiplication of kefir microorganisms during fermentation.

2. Material and methods

2.1. Formulations

The preparation of a fermented beverage with water-soluble soybean extract and kefir grains included three initial bases with different concentrations of soybean extract and cow's whole milk (Italac® - Goiasminas Indústria de Laticínios Ltda., Jaru/RO/Brazil) – 100% soybean extract (100%S), 50% soybean extract + 50% milk (50%S50%M), and 100% milk (100%M). After testing different types of commercial soybean extracts and different proportions of ingredients, the soybean extract Sollys Original® (Nestlé Brasil Ltda., Três Rios/RJ/Brazil) was chosen because the final product presented a lower syneresis level after fermentation.

Formulation of fermented beverage was prepared as follow: initial liquid bases (100%S or 50%S50%M or 100%M) were added of 7.7% of culture containing the water kefir grains (w/v), following incubation at 37 °C for 19 h. Then, the inoculum was removed by sieving, and 2.6% of sucrose and 0.2% of thickener were added. Formulations 100%S and 50%S50%M were also formulated with 1.5% of soybean extract powder. The beverages were finally stored at 4 °C for 4 h.

2.2. Physicochemical analysis

Physicochemical analyses included the determination of pH using a digital potentiometer (Model K39-1014B, Kasvi – Curitiba/PR/Brazil); soluble solids at 25 °C using a digital refractometer (Model PAL-1, Atago – Tokyo/Japan); and acidity (as g lactic acid per 100 mL of sample) by titration with NaOH (0.1 M) (IAL, 2008). The pH, soluble solids and acidity measurements were performed every hour during the fermentation period, totaling 20 points (the first point was at time zero, as soon as the ingredients were mixed, and the last point was at 19 h).

Concentrations of sugars (lactose, raffinose, glucose, galactose), ethanol, organic acids (acetic acid and lactic acid) and glycerol were determined by High Performance Liquid Chromatography (HPLC) according to the methodology adapted from López and Gómez (1996) and Schwan, Mendonça, Silva Jr., Rodrigues, & Wheals (2001). The HPLC equipment was an Acella LC System (Thermo-Scientific, Waltham/MA) equipped with refractive index (RI) detector, automatic injection and HyperREX XP column, maintained at 30 °C. Results were integrated and quantified using the Chromquest software. The mobile phase consisted of ultrapure water acidified with sulfuric acid (pH 2.6) and flow rate of 0.7 mL/min. Limit of detection (LOD) for all compounds was 0.01 g/L and retention times were 2.045 min for raffinose, 2.270 min for lactose, 2.618 min for galactose, 2.822 min for glucose, 3.565 min for acetic acid, 3.782 min for lactic acid, 4.457 min for glycerol, and 6.053 min for ethanol. Standard curves were prepared using a mixed solution of all components analyzed and with concentrations ranging from 0.5 to 20 g/L. All the reagents were purchased from Sigma Aldrich (St. Louis/MO).

Sample preparation for the HPLC analysis included protein precipitation using 6% potassium ferrocyanide solution and 12% zinc acetate solution (IAL, 2008). After protein precipitation, samples were centrifuged at 12,000 rpm for 15 min (model Mikro 200, Hettich, Tuttlingen, Germany) and the supernatant was diluted with ultrapure water and filtered in polyvinylidene membrane with pore size of 0.22 µm and 13 mm of diameter (Durapore®, Merck Millipore, Darmstadt, Alemanha). Filtered samples were stored in 2 mL vials at −20 °C until HPLC analysis.

2.3. Microbiological analysis

LAB counting was conducted according to Hall, Ledenbach, and Flowers (2001). Appropriate dilutions were pour plated in MRS agar (de Man, Rogosa and Sharpe, Merck, Darmstadt/Germany) supplemented with natamycin (50 mg/mL) and acidified with acetic acid (1 N) to pH 5.5 ± 0.1 (Botes, Todorov, von Mollendorff, Botha, & Dicks, 2007; Alvarenga, 2008), following incubation at 37 °C for 48 h. Yeasts were counted following the methodology of Beuchat and Cousin (2001), by surface plating on yeast malt extract agar (MEA) formulated with malt extract, glucose, agar (Merck, Darmstadt/Germany) and peptone (Himedia, Mumbai/India) and supplemented with chloramphenicol (100 mg/L) and tetracycline (100 mg/L) with the pH adjusted to 6.7. Incubation was performed at 15 °C for five days.

Brazilian legislation (ANVISA, 2001) for fermented dairy products includes the determination of coliforms and *Salmonella*. Coliforms determination was conducted by the Most Probable Number (MPN) method according to Manafi and Kneifel (1990). Samples were inoculated at concentrations of 1:10, 1:100 and 1:1000 in Fluorocult LMX broth (Merck, Darmstadt/Germany) and incubated at 37 °C for 24 h. After this period, growth was evaluated in dark chamber with ultraviolet light and ambient light. *Salmonella* detection was done by *Salmonella Express* System (3M Food Safety - St. Paul/MN). Samples were inoculated in supplemented *Salmonella* Enrichment Base for 18 h at 41 °C. After pre-enrichment, one mL aliquots were transferred to selective enrichment Rappaport-Vassiliadis Broth and incubated at 41.5 °C for eight hours. Samples were streaked in *Salmonella* Petrifilm plates, incubated at 41 °C for 25 h, and then evaluated to check the presence of characteristic colonies. All microbiological analyses were performed in duplicate. For microbial growth curves were fitted data using the DMFit 3.0 Excel add-in software (Institute of Food Research, Norwich, UK). Growth parameters for LAB and yeast [lag time (λ), specific growth rate (μ), maximum population (yEnd)] were calculated using the model of Baranyi and Roberts (1994) after fitting the model to the data. R^2 was also verified.

2.4. Sensory evaluation

The sensory analysis covered discriminative tests (multiple comparison tests) and affective tests (hedonic test with 50 consumers, aged 18–45 years). The multiple comparison test was performed between the three formulations containing increased levels of water-soluble soybean extract (0, 50 and 100% v/v) on the kefir formulations to checking if there would be a difference among these different levels. Samples (10 mL) were presented at 5 °C in a complete balanced block design (Drake, 2007).

2.5. Statistical analysis

The association between sugars, lactic and acetic acids and formulations was examined by Principal Component Analysis (PCA), using the package *FactoMine* built to *R* (version 3.3.1) (*The R Foundation for Statistical Computing*, Viena, Austria). Differences in growth rate, maximum population and lag time calculated for LAB and yeast in the different formulations were compared by an analysis of variance (ANOVA, *post hoc* analysis Tukey test with a critical value $p = 0.05$; and *t*-test $p = 0.05$) using *R*.

3. Results and discussion

3.1. Formulations

At the beginning of the experiments, the addition of low percentages of kefir grains (0.2% w/v), low fermentation temperature (25 °C) and high incubation time (65 h) were tested, but the fermented beverage presented higher syneresis and the formulation 100%M was more gelatinous than the formulations containing soybean extract (data not are shown). These findings indicate that fermented soybean extract beverages have lower viscosity due to lower production of kefiran (Liu & Lin, 2000). According to Tomazi (2007), syneresis can occur because of gel contraction caused by high incubation temperature, heat treatment of milk for a long time or low total solids. The formulations containing soybean extract were then added to 1.5% of soybean extract powder to increase the total solids content to avoid this phenomenon. Besides, the change in the amount of the kefir grains (7.7%) and the incubation at 37 °C for 19 h was employed in all formulations.

3.2. Physicochemical analysis

The pH, soluble solids content (°Brix) and acidity (expressed as lactic acid) for the three formulations tested are at Fig. 1. For all formulations, an increase in the amount of lactic acid due to the carbohydrates consumption by the microorganisms of kefir grains, which consequently caused a decrease in soluble solids content, can be noted. The final acidity values obtained for formulations 100%M, 50%S50%M and 100%S were, respectively, 0.765 g of lactic acid/100 mL, 0.738 g of lactic acid/100 mL and 0.600 g of lactic acid/100 mL of beverage.

Pereira et al. (2009) explained that the pH of soybean extract beverages has a direct influence on the stability, flavor, aroma, and texture of these products. By reducing the pH to below 4.0, proteins precipitate because of the acidity caused by compounds such as lactic acid, acetaldehyde, diacetyl, among others, which are formed during the carbohydrates fermentation by the LAB. These compounds can mask the volatile compounds from soybean, such as n-hexanal, giving a refreshing taste to the beverage and inhibiting the characteristic flavor of soybean extract, classified as “beany flavor and taste” (Moraes, Haj-Isa, Almeida, & Moretti, 2006; Silva, Prudêncio, Felberg, Deliza, & Carrão-Panizzi, 2007).

After 10 h of fermentation, the initiation of soybean extract proteins denaturation in the formulations was observed, increasing viscosity. At that time, formulations presented pH between 5.5 and 5.7. The same occurred in experiments conducted by Pereira et al. (2009), who

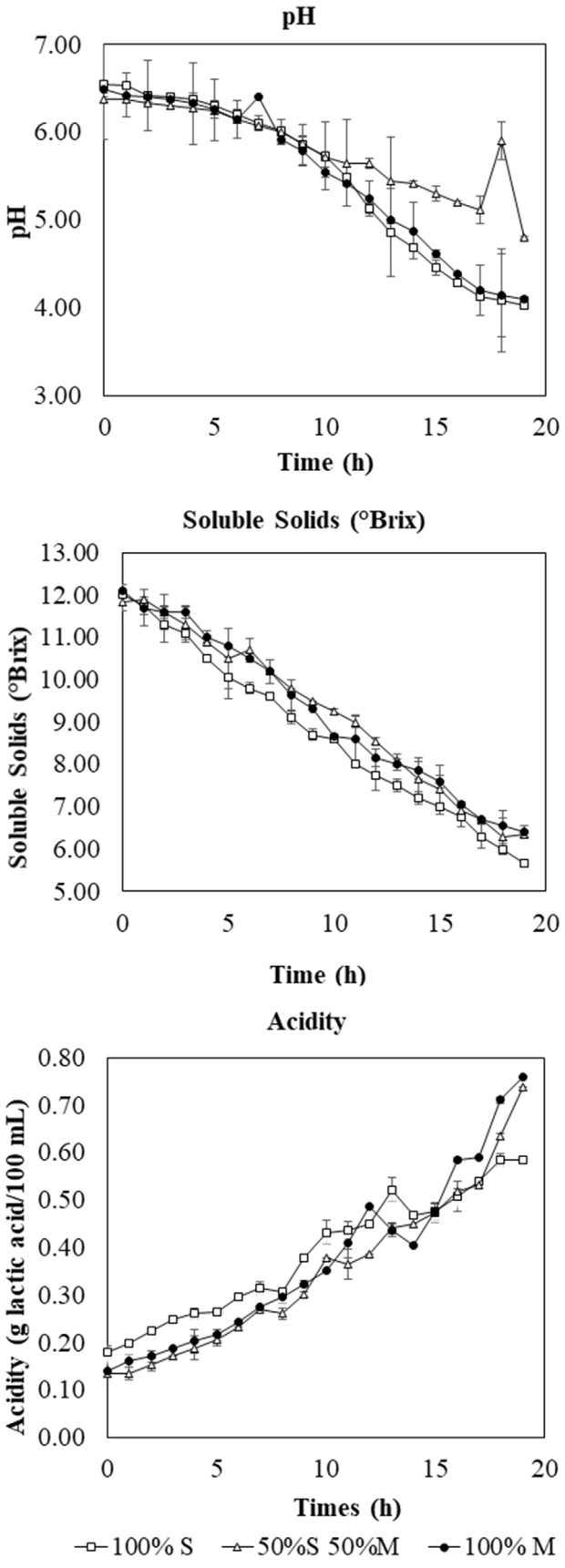

Fig. 1. pH, soluble solids content (°Brix) and acidity (expressed as lactic acid) during kefir fermentation process (19 h) for all formulations tested (S – soybean extract, M – milk).

observed that the denaturation of proteins in fermented beverage prepared with soybean extract and fruit led to changes in the consistency due to curd formation. The authors credited this phenomenon to pH lowering (pH of samples was between 5.3 and 5.7). Kesenkas et al. (2011b) observed a pH of 4.6 after the first day of storage of different formulations produced using soybean extract and milk at different proportions and fermented with kefir grains or commercial kefir culture. Dadkhah, Pourahmad, Assadi, and Moghimi (2011) concluded that the inoculation rate of kefir grains and the temperature influenced the fermentation time to reach the pH 4.5–4.6, while formulation with the highest amount of kefir grains (4%) achieved this pH in 16 h at 25 °C, samples inoculated with 2% kefir grains at 22 °C reached after 24 h.

Cheirsilp, Shimizu, and Shioya (2001) and Cheirsilp, Shoji, Shimizu, and Shioya (2003), by mathematical modeling, observed that the optimum pH for maximum production of kefiran during the exponential growth phase is 5.0. *Lactobacillus kefiranofaceins* can convert lactose in kefiran, lactic acid, and galactose. Also, a higher quantity of kefiran is produced by a mixed culture under aerobic conditions than under anaerobic conditions. Thus, in this study, it can be considered that the presence of lactose and the pH close to the optimum during the exponential phase are inherent factors of higher lactic acid production in the formulations containing milk (50%S50%M and 100%M).

Results for concentrations of organic acids, sugars and alcohol in formulations 100%S, 50%S50%M and 100%M are in Figs. 2 and 3. According to Qureshi, Lolas, and Blaschek (2001) and Yamaguishi (2008), raffinose is a non-reducing oligosaccharide and its hydrolysis results in fructose, glucose, and galactose, and this reaction is required because microorganisms consume the resulting sugars. The same occurs for lactose, which, according to Damodaran, Parkin, and Fennema (2010), should be hydrolyzed in the monosaccharides D-glucose and D-galactose to be used as an energy source. However, during the hydrolysis process, some microorganisms may become non-viable due to difficulties in adapting to the new environment. Thus, it was observed for formulation 100%S that the amount of raffinose decreased over time is correlated with the increase in amounts of glucose and galactose, and a further decrease in glucose concentration occurred probably because of consumption of this monosaccharide by microorganisms. For formulation 50%S50%M, it was considered the sum of raffinose and lactose content since the retention times of these sugars were very close. This resulted in difficulties in separating the chromatographic peaks. The raffinose/lactose content of this formulation was reduced throughout kefir fermentation. An increase and decrease of glucose and galactose amounts in formulation 100%S was observed due to consumption of sugars by microorganisms. Raffinose is commonly found in beans, some vegetables and whole grains, which explains the lack of this sugar in formulation 100%M. On the other hand, lactose is only found in milk derivatives and its reduction through fermentation makes the consumption of dairy products more accessible to people with lactose intolerance. Lactose is converted into lactic acid by homofermentative fermentation (Jay, 2005), which explains the increase in lactic acid amount for formulations 50%S50%M and 100%M. Liu and Lin (2000) observed that at the end of fermentation, lactic acid concentration in milk kefir was significantly higher than in soybean extract kefir (1.6% and 0.9%, respectively), however, when lactose or glucose was added at 1% to soybean extract, lactic acid production by the microorganisms in kefir grains were improved to similar concentrations of milk kefir.

According to Jay (2005), ethanol production is performed by heterofermentative fermentation. The increase in the amount of this alcohol over time can also be observed in Fig. 4. Magalhães, Pereira, Dias, and Schwan (2010) explain that yeasts of the genera *Saccharomyces, Candida, Kluyveromyces* and *Torulaspora* are the main responsible for ethanol production in kefir, however some bacteria such as *Lactobacillus* and *Lactococcus* may also contribute with a fraction of ethanol concentration. Pourahmad, Moghimi, Dadkhah, and Assadi (2011) found

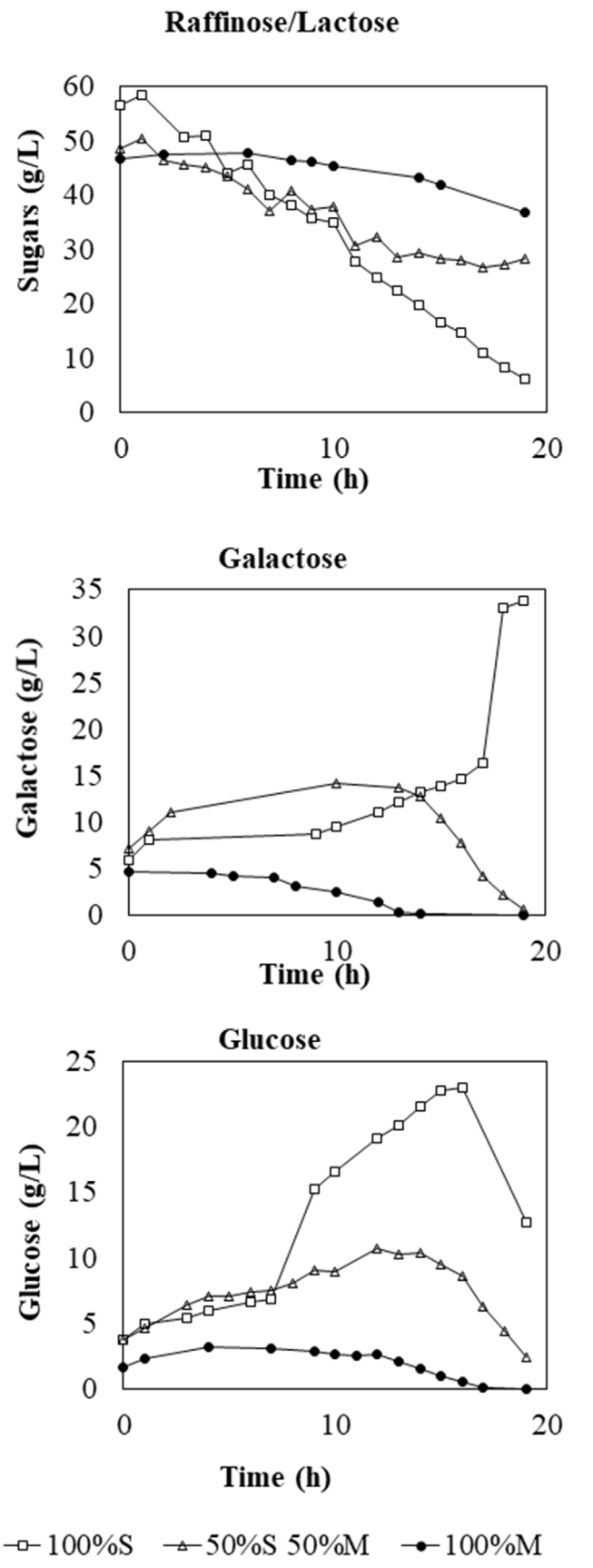

Fig. 2. Concentration of sugars during kefir fermentation process for all formulations tested (S – soybean extract, M – milk). For formulation 50%S50%M, concentrations of lactose and raffinose were expressed as a sum.

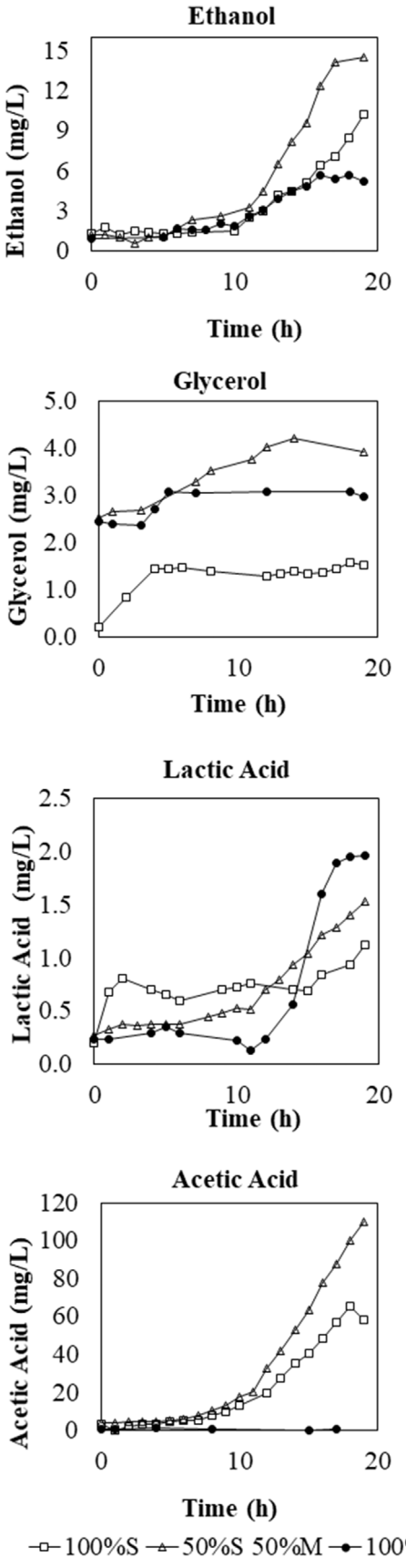

Fig. 3. Concentration of ethanol, glycerol and organic acids during the kefir fermentation process for all formulations tested (S – soybean extract, M – milk).

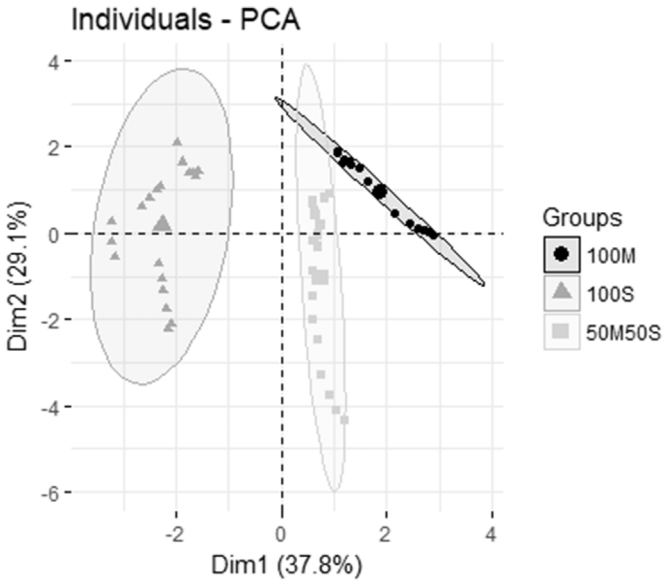

Fig. 4. Plot data in coordinated system given by the two most import component with different clusters marked by colors and ellipses.

significant differences in the ethanol contents of samples with 2, 3 or 4% addition of kefir grains and 2% sucrose and incubation at 22 or 25 °C. The sample with 3% addition of kefir grains and incubation at 25 °C presented the higher amount of ethanol (1105 g/L), while the lower concentration of ethanol was for the sample with the addition of 2% kefir grains and incubation at 22 °C (720 g/L). Damodaran et al. (2010) and Gutierrez (1991, pp. 55–69) observed that during alcoholic fermentation there is also the production of acetic acid and glycerol. The increase in the amount of these compounds over time can also be observed in our experiments. Part of ethanol content may be converted to acetic acid by heterofermentative bacteria of the genus *Acetobacter*, which have alcohol dehydrogenase activity converting ethanol to acetaldehyde (Magalhães et al., 2010). Other products formed during fermentation, such as diacetyl and acetaldehyde, are responsible for providing flavor to the beverage.

According to the Principal Component Analysis (PCA), the main components which differentiated the formulations were lactose, ethanol and acetic acid. Lactose showed a positive and significant correlation ($r = 0.88$, $p < 0.01$) with PC1 (which explains 42.2% of total variation), while ethanol and acetic acid showed a positive and significant correlation ($r_{ethanol} = 0.94$, $p < 0.002$; $r_{acetic} = 0.93$, $p < 0.01$) with PC2 (which explains 30.6% of total variation). Those components are responsible for clustering the three groups showed in Fig. 4.

3.3. Microbiological analysis

Results for the enumeration of LAB and yeasts over time for all formulations are shown in Fig. 5. Each curve was fitted to the model of Baranyi and Roberts (1994), and each line represents the model fitting to the experimental data. Parameters such as lag time (λ), specific growth rate (μ) and maximum population (yEnd) of LAB and yeasts of kefir fermented beverages are in Table 1. In all formulations, it was added the same quantity of kefir grains in a mass/volume ratio. However, it was not possible to know the real active mass of grains, which may explain the lower initial LAB count in the formulation 50%S50%M and the higher initial yeast count in formulation 100%S. Although the initial count of the LAB in formulation 100%S was similar to formulation 100%M, these microorganisms grew significantly faster in formulation 100%S. On the other hand, yeasts presented a significantly higher specific growth rate in formulation 50%S50%M ($p < 0.05$).

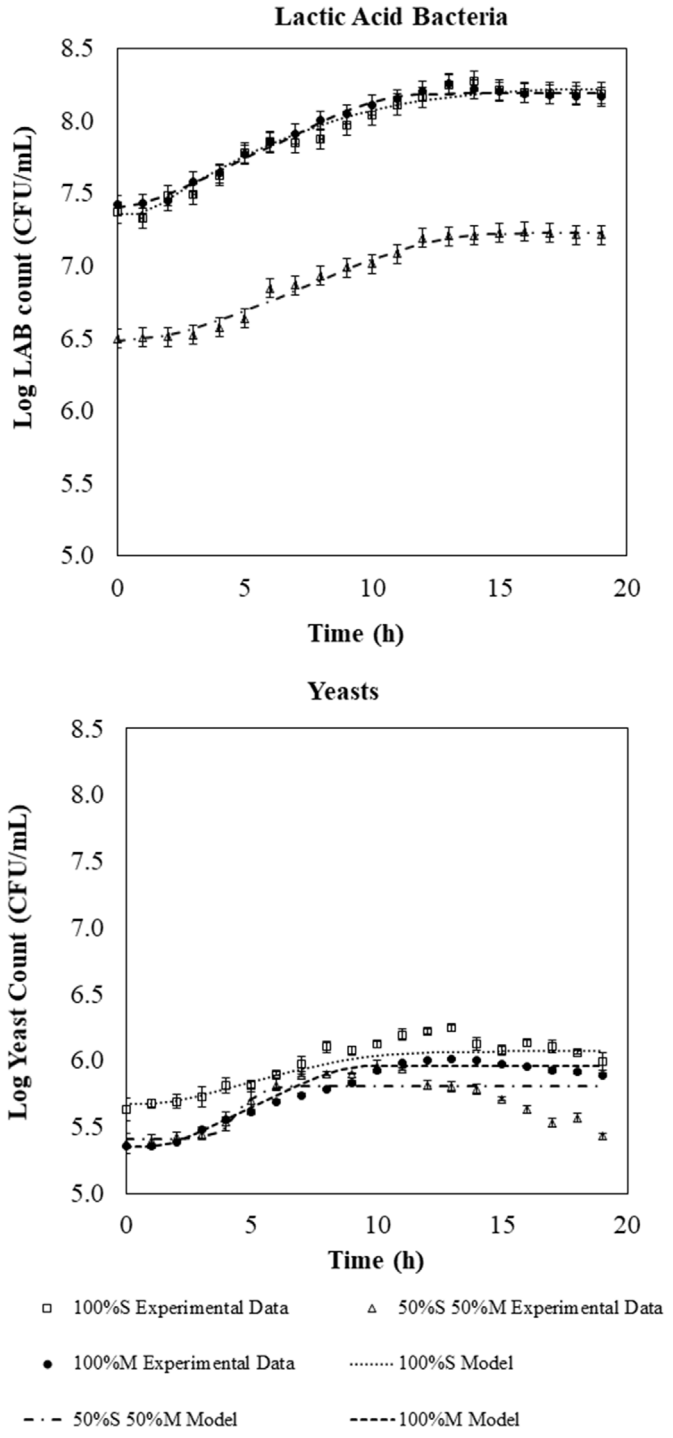

Fig. 5. Growth curves of lactic acid bacteria and yeasts fitted to Baranyi model over time for kefir fermented beverage prepared with different concentrations of soybean extract (S) and milk (M).

An increase in the LAB lag time in formulation 50%S50%M was observed (2.2 h) in comparison to lag times found in formulations 100%S and 100%M (1.0 h and 1.1 h, respectively). Fermentation parameters including water activity, temperature, pH, concentration of starter culture, among others, may affect the regulation mechanism and the lag time of LAB, as well as their effects on the properties of the final products. Changes in the food characteristics during fermentation process can be a source of stress for microorganisms, causing competition and new ways of adaptation (Serrazanetti, Gottardi, Montanari, & Gianotti, 2013). Based on these approaches, the prolonged lag time observed for the LAB in formulation 50%S50%M may be a result of their high concentration in the substrate and the reduced concentration of lactose. As a result, the competition among microorganisms may result in the stress of LAB and extension of the adaptation phase. Consequently, LAB is fastidious microorganisms which require abundant nutrients for growth and in formulation with different carbon sources, different metabolic pathways can be induced. Furthermore, yeast may secrete several metabolites, including amino acids, which permits the LAB survival in nitrogen-rich environments (Ponomarova et al., 2017).

Table 1
LAB and yeast growth parameters and correlation coefficients estimated by the model of Baranyi and Roberts (1994) for different kefir fermented beverage formulations[a,b,c].

Formulations	Specific growth rate (μ, h^{-1})	Maximum population (yEnd, $\log_{10}$ CFU/mL)	Lag time (λ, h)	R^b
LAB				
100%S	0.13 ± 0.01^A	8.2 ± 0.0^A	1.0 ± 0.1^B	0.99
50%S 50%M	0.08 ± 0.00^B	7.2 ± 0.0^B	2.2 ± 0.1^A	0.99
100%M	0.08 ± 0.00^B	8.2 ± 0.0^A	1.1 ± 0.0^B	0.99
Yeast				
100%S	0.09 ± 0.01^B	6.1 ± 0.0^A	1.5 ± 0.2^A	0.99
50%S 50%M	0.13 ± 0.00^A	5.7 ± 0.1^B	1.7 ± 0.2^A	0.99
100%M	0.09 ± 0.00^B	6.2 ± 0.2^A	1.4 ± 0.0^A	0.99

[a] Values expressed as mean ± standard deviation of duplicate samples.
[b] S = soybean extract, M = milk.
[c] Different superscript letters within a column indicate significant difference ($p < 0.05$) according to Tukey test.

After 12 h of fermentation, maximum population of LAB of 8.2 $\log_{10}$ CFU/mL was obtained in formulation 100%M. That value is lower than the maximum population found in formulation 100%S, even though it was reached after 16 h of fermentation. As previously mentioned, a high concentration of lactic acid can inhibit the growth of microorganisms. As shown in Fig. 3, formulation 100%M presented the highest concentration of lactic acid, which might have affected LAB growth after 12 h of fermentation. The formulations 100%S and 100%M presented similar maximum populations and specific growth rate of yeasts (Table 1). The maximum population values found in these formulations differed significantly from formulation 50%S50%M. Differently, from the LAB, yeast lag time did not change significantly among formulations. Liu and Lin (2000) also observed that yeast counts did not differ significantly between milk and 1%-glucose soybean kefir at the end of fermentation, although milk kefir reached this yeast concentration within 20 h while 32 h was needed for soybean kefir. According to Farnworth and Mainville (2008), yeasts are located in the interior of kefir grains and LAB on the exterior. Also, the number of yeast cells found in the final product was lower, whereas LAB was higher. This pattern was also observed in our formulations, with all formulations presenting yeast concentrations lower than LAB.

Soybean extract, as well as milk, is suitable for LAB and yeast growth because both have oligosaccharides (raffinose and stachyose for soybean extract and lactose for milk), amino acids and peptides that stimulate microbial growth. Among many microorganisms that are part of kefir grains symbiosis, *Lactobacillus* species can be highlighted because of their high probiotic importance (MAPA, 2007). However, because of soybean extract and fermentable milk carbohydrates are not the same, dissimilarities in the microflora present in kefir grains can appear as growth characteristics of microorganisms in kefir grains change (Dadkhah et al., 2011). Although LAB counts in formulations 100%S and 100%M were almost the same (Fig. 5), LAB species may vary between formulations. Abraham and de Antoni (1999) also observed that LAB and yeast growth did not differ significantly when kefir grains were inoculated in cows' milk and soybean milk. The same authors concluded that the same kefir grains that grow in milk could be replicated in soybean extract. On the contrary, Farnworth (2006)

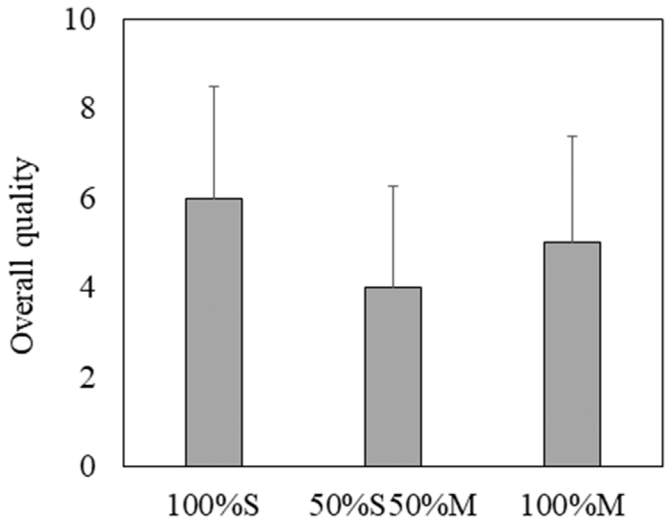

Fig. 6. Sensory evaluation of kefir formulations tested (S – soybean extract, M – milk). No statistical difference at $p < 0.05$ was observed among the treatments studied.

evaluated the growth of probiotic bacteria and bifidobacteria in a soy-based yogurt formulation and observed a probiotic *Lactobacillus* population 3 to 5 times higher than milk yogurt formulation.

Formulations 100%S and 100%M presented LAB count around 8 $\log_{10}$ CFU/mL. If a person consumes a 100-mL portion of formulations with 8 $\log_{10}$ CFU/mL, total LAB ingestion will be 10 $\log_{10}$ CFU. Even the consumption of a 100-mL portion of formulation 50%S50%M, with a smaller LAB count, would reach the requirements to be classified as a probiotic beverage. Baú et al. (2014) observed a LAB counting of 9.2 $\log_{10}$ CFU/g just after the production of soy products fermented with kefir culture with or without the addition of soy fibers. After a storage period of 28 days, the soy product containing fibers had a higher count (8.2 $\log_{10}$ CFU/g) than the product without fibers (7.9 $\log_{10}$ CFU/g). According to the authors, the addition of fibers reinforced the growth and survival of bacteria during the storage. Finally, all tested formulations showed from < 3.0 MPN/mL to 3.0 MPN/mL of coliforms. *Salmonella* was not detected in any of the three formulations tested.

3.4. Sensorial analysis

No significant differences were observed among the beverages containing with milk and soybean extract ($p > 0.05$) (Fig. 6), suggesting that water-soluble soybean extract not influence the sensory attributes of the kefir beverage. This finding is interesting, due to the recognized nutritional and functional value of soybean extract as an ingredient for improvement of consumer's health. These findings also demonstrated that is possible to produce a kefir beverage replacing milk by soybean extract as a substrate for fermentation.

4. Conclusion

This is the first study which examined the yeast and LAB growth parameters using water-soluble soybean extract fermented with kefir. Previously, Corona et al. (2016) proposed the use of vegetable extracts as a functional non-dairy beverage as an alternative to traditional milk or water kefir. The results found in this study show consistent patterns concerning microbial, physicochemical and sensory properties. Thus, these findings support the total replacement of milk by water-soluble soybean extract as an alternative substrate for kefir fermentation. The use of soybean extract brings extra nutritional and health benefits, resulting in a beverage to be consumed by vegans, vegetarians, and lactose intolerant people.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2018.03.070.

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