Original

Effect of starter culture and inulin addition on microbial viability, texture, and chemical characteristics of whole or skim milk Kefir

Efeito do tipo de cultura starter e da adição de inulina na viabilidade microbiana, textura e características químicas de Kefir de leite integral ou desnatado

Flávia Daiana MONTANUCI[1], Tatiana Colombo PIMENTEL[1], Sandra GARCIA[1], Sandra Helena PRUDENCIO[1*]

Abstract

The effect of inulin addition and starters (Kefir grains or commercial starter culture) on the microbial viability, texture, and chemical characteristics of Kefir beverages prepared with whole or skim milk was evaluated during refrigerated storage. The type of starter did not influence microbial viability during the storage of the beverages, but the chemical and textural changes (decreases in pH, lactose concentration, and inulin and increased acidity, firmness, and syneresis) were more pronounced in the formulations fermented with grains than those fermented with the starter culture. The addition of inulin did not influence acidity or viability of lactic acid bacteria, but in general, its effect on the survival of acetic acid bacteria, *Lactococcus* and yeasts, firmness, and syneresis depended on the type of milk and starter culture used. Generally, the yeast, acetic acid bacteria, and *Leuconostoc* counts increased or remained unchanged, while the total population of lactic acid bacteria and *Lactococcus* were either reduced by 1 to 2 logs or remained unchanged during storage.

Keywords: *fermented milk; fat replacer; storage stability.*

Resumo

O efeito da adição de inulina e do tipo de iniciador (grãos de Kefir ou cultura *starter* comercial) da fermentação sobre a viabilidade microbiana, textura e características químicas de bebidas Kefir, formuladas com leite integral ou desnatado, foi avaliado durante o armazenamento refrigerado. O tipo de iniciador não teve influência sobre a viabilidade microbiana ao longo da estocagem das bebidas, mas as alterações químicas e de textura (redução do pH, teores de lactose e inulina e aumento da acidez, firmeza e sinérese) foram mais acentuadas nas formulações fermentadas com grãos do que com cultura *starter.* A adição de inulina não influenciou a acidez ou a viabilidade de bactérias ácido-láticas, mas, em geral, seu efeito sobre a sobrevivência das bactérias ácido-acéticas, *Lactococcus* e leveduras, firmeza e sinérese foi dependente do tipo de leite e da cultura de fermentação utilizados. De modo geral, a contagem de leveduras, bactérias ácido-acéticas e *Leuconostoc* aumentou ou permaneceu inalterada, enquanto que a população total de bactérias ácido-lácticas e de *Lactococcus* reduziu de 1 a 2 log ou se manteve durante o armazenamento das bebidas.

Palavras-chave: *leite fermentado; substituto de gordura; estabilidade à estocagem.*

1 Introduction

Kefir is a sour milk produced by incubating milk with kefir grains (GARROTE; ABRAHAM; DE ANTONI, 2001). Kefir beverage has a thick, creamy consistency, mild acid flavor, and mild aroma of fresh yeast; it has a natural carbonated effervescence and may contain between 0.08 to 2% alcohol (IRIGOYEN et al., 2005; FARNWORTH; MAINVILLE, 2008; HOZER; KIRMACI, 2010).

Kefir grains are irregularly shaped, gelatinous masses varying from 1 to 6 mm in diameter and are usually white or lightly yellow and resemble small cauliflower florets (OTLES; CAGINDI, 2003). The grains are composed of a polysaccharide matrix of glucose and galactose, called Kefiran, in which a complex microbiota coexists in a symbiotic relationship (ORDÓÑEZ, 2005). This microbiota is composed of lactic acid bacteria - LAB (*Lactobacillus*, *Lactococcus*, *Leuconostoc,* and *Streptococcus* spp.), acetic acid bacteria - AAB (*Acetobacter)* and yeasts; some are lactose-fermenting yeasts (*Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Torula Kefir*) and other are non-lactose fermenting yeasts (*Saccharomyces cerevisiae)* (IRIGOYEN et al., 2005; PIERMARIA; CANAL; ABRAHAM, 2007).

There are complex interactions between yeasts and lactic acid bacteria in the Kefir grains, but the activity of each microorganism and how they contribute to the symbiosis equilibrium have not been studied in depth. One theory is that yeasts can assimilate galactose, which is a product of some lactic acid bacteria, and that yeast may produce vitamins which enhance the growth of the lactic acid bacteria. Lactic acid bacteria also produce lactate, which the yeast may assimilate, resulting in a slight increase in pH, which allows further growth and lactate production by the bacteria (LOPITZ-OTSOA et al., 2006). The microorganisms of the Kefir grains are able to

Received 19/3/2012
Accepted 25/9/2012 (005640)
[1] *Programa de Pós-graduação em Ciência de Alimentos, Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciências Agrárias, Universidade Estadual de Londrina – UEL, CP 6001, CEP 86051-990, Londrina, PR, Brasil, e-mail: sandrah@uel.br*
**Corresponding author*

DOI: http://dx.doi.org/10.1590/S0101-20612012005000119

produce extracellular polysaccharides (EPS), mainly the Kefiran (FARNWORTH, 2005), which can increase viscosity, water retention, and interaction with other components of the milk, resulting in increased rigidity of the casein matrix in the final product and, consequently, less syneresis (DUBOC; MOLLET, 2001). Therefore, EPS may contribute significantly to the texture of Kefir (CHEN et al., 2009), and Kefiran can positively affect viscosity and viscoelastic properties of acidic gels (PIERMARIA; CANAL; ABRAHAM, 2007).

The industrial manufacture of Kefir using grains as the starter culture is very difficult due to the complexity of their microbiological composition, which varies widely depending on the origin of the grains and conditions of storage and handling (GARCIA FONTÁN et al., 2006). Therefore, currently, there are commercial lyophilized starter cultures that mimic the microbial composition of the grains (SACCO, 2010; WILDERNESS..., 2010).

Prebiotics, such as inulin, are non-viable food components that confers a health benefit on the host associated with modulation of the intestinal microbiota (FOOD…, 2007). Inulin promotes the selective growth of bifidobacteria and lactobacilli in the colon, and it inhibits the growth of potentially harmful bacteria (MADRIGAL; SANGRONIS, 2007). In order to achieve the prebiotic effect, no less than 4 grams of inulin must be consumed daily from foods (MANNING; GIBSON, 2004). Under Brazilian law, the claim of the functional property of inulin is allowed if the daily portion of the product ready for consumption provides at least 1.5 grams, in the case of liquid food (AGÊNCIA…, 2007).

In foods, inulin can favor the development and viability of probiotic bacteria during fermentation or refrigerated storage (EL-NAGAR et al., 2002; AKIN; AKIN; KIRMACI, 2007). Inulin concentrations of 1.5% (w/v) were found to be sufficient to stimulate growth and retain the viability of probiotic cultures in fermented milk (AKALIN et al., 2007; ARYANA; McGREW, 2007; ARYANA et al., 2007).

Milk fat plays an important role in the texture development of dairy products (GUVEN et al., 2005) and reducing its concentration may cause lack of consistency or texture (EL-NAGAR et al., 2002; MAGRA; ANTONIOU; PSOMAS, 2012). Fat globules dispersed within the casein micelles interfere with protein matrix formation and resulting in the formation of a softer gel (PASEEPHOL; SMALL; SHERKAT, 2008). Inulin is also used as a fat replacer in the dairy industry and has positive effects on the rheology and stability of reduced-fat, low fat, or fat-free products (EL-NAGAR et al., 2002). However, the addition of inulin may result in changes in dairy quality attributes due to interactions between the functional ingredient and food matrix components (CRUZ et al., 2010).

There are no data available in the literature for comparison of Kefirs fermented with Kefir grains or with starter culture (combined or not with inulin) during refrigerated storage of products. For that reason, the objective of this study was to evaluate the effect of inulin addition and fermentation of milk by kefir grains or starter culture on microbial viability, texture, and chemical characteristics of Kefir made from whole or skim milk during refrigerated storage.

2 Materials and methods

2.1 Materials

Whole or skim UHT milk (Líder®), skimmed milk powder (Molico, Nestlé®), and inulin (Rafitiline® HP, Orafti, degree of polymerization of 23) were used in this experiment. Freeze-dried Kefir grains (Dominic N Anfiteatro, Australia) or Kefir starter culture (Lyofast MT 036 LV; Clerici-Sacco, Brasil) were used to produce the Kefir beverages. The kefir starter culture was composed of: *Lactococcus lactis* ssp., *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis*, *Lactobacillus brevis*, *Leuconostoc,* and *Saccharomyces cerevisiae* according to the manufacturer. The microbiological composition of the kefir grains was not thoroughly known, but the following microbial groups were identified: lactic acid bacteria, acetic acid bacteria, yeasts, and *Lactococcus* spp *and Leuconostoc* spp genera.

2.2 Methods

Kefir beverages

Eight formulations were prepared: WG (whole milk and Kefir grains), WGI (whole milk, Kefir grains, and inulin), WC (whole milk and Kefir starter culture), WCI (whole milk, Kefir starter culture, and inulin), SG (skim milk and Kefir grains), SGI (skim milk, Kefir grains, and inulin), SC (skim milk and Kefir starter culture), and SCI (skim milk, Kefir starter culture, and inulin).

The freeze-dried Kefir grains (1 g per 200 mL milk) were activated in skim milk at 25 °C in Bio Oxygen Demand (BOD) for one month and were propagated daily (24 hours) until fermentation and production of the beverage.

The freeze-dried Kefir starter culture was activated in skim milk (1 g per 100 mL milk) and divided into 10 mL flasks, which were subsequently frozen (–18 °C) until use, in accordance with the manufacturer's recommendations.

Skim milk powder was added to either 5 L of UHT whole milk or UHT skim milk to increase the solid contents and improve fermented milk consistency. Formulations with inulin (WGI, WCI, SGI, and SCI) were added with 15 $g.L^{-1}$ skim milk powder and 20 $g.L^{-1}$ inulin, while the formulations without inulin (WG, WC, SG, and SC) were added with 35 $g.L^{-1}$ skim milk powder.

After heat treatment in a water bath at 90 °C for 2 to 3 minutes and subsequent cooling to 25 °C in an ice bath, 1% (w/v) activated Kefir grains or Kefir starter culture were inoculated, and the formulations were immediately incubated in a Bio Oxygen Demand (BOD) at 25 °C for 24 hours. After fermentation, the formulations were kept in a Bio Oxygen Demand (BOD) at 4 °C for up to 28 days. With regard to the beverages fermented using grains, the coagulum was broken by stirring with a spoon, and the Kefir grains were removed – prior to refrigeration - by filtration using a sieving cloth. All containers were sterilized. The chemical composition of the fermented milks is shown in Table 1.

Table 1. Chemical composition of Kefir*.

Formulations**	Moisture	Protein	Lipids	Ash	Carbohydrates
WG	82.6	5.3	3.33	1.1	7.7
WGI	82.7	4.4	3.25	0.9	8.6
WC	83.8	5.3	3.33	1.1	6.4
WCI	83.2	4.5	3.33	0.8	8.2
SG	86.7	5.3	0.48	1.1	6.4
SGI	86.7	4.5	0.48	0.9	7.3
SC	86.1	5.4	0.48	1.1	6.8
SCI	86.9	4.5	0.48	1.1	6.9

*Results are expressed as g.100 g^{-1}. **WG = whole + grains; WGI = whole + grains + inulin; WC = whole + culture; WCI = whole + culture + inulin; SG = skim + grains; SGI = skim + grains + inulin; SC = skim + culture; SCI = skim + culture + inulin.

Physical and chemical evaluations

The pH, titrable acidity, and the lactose content (Lane-Eynon method) were determined by the official AOAC methods (ASSOCIATION…, 1995). The concentration of inulin was quantified using a Fructan HK enzymatic kit (MEGAZYME, 2009).

Firmness (N) was determined using a TA-XT2i Texture Analyser (Stable Micro Systems, Godalming, Surrey, England). In their original containers, the formulations, (DELLO STAFFOLO et al., 2004) were compressed at a depth of 20 mm using a type AB/E 35 mm acrylic cylinder probe with compression rate of 2 mm/s and force of 0.05 N for 0.5 seconds.

The syneresis in the formulations was measured according to Aryana (2003) by inverting 100 g of Kefir on a fine mesh screen (14 μm) placed on top of a funnel. The amount of whey collected after 2 hours of drainage at room temperature was used as an index to indicate the water-holding capacity of the formulation (whey volume per 100 g of sample).

Microbiological evaluations

Lactococcus spp. counts were determined in M17-lactose agar (Difco®), followed by incubation under aerobiosis at 30 °C for 48 hours (GARCIA FONTÁN et al., 2006). *Leuconostoc* spp counts were determined on APT agar (Merck®) supplemented with sucrose (100 $g.L^{-1}$) and 0.005% of sodium azide, and it was incubated under aerobiosis at 22 °C for 4 days (MAYEUX; COLMER, 1961). Total lactic acid bacteria (LAB) were enumerated on MRS agar (Merck®) and incubated under aerobiosis at 30 °C for 48 hours (GARROTE; ABRAHAM; DE ANTONI, 2001) using the Pour Plate technique.

AAB were enumerated in a selective culture medium prepared with 5% glucose, 1% yeast extract, and 2% agar (IRIGOYEN et al., 2005) using the Pour Plate technique. After sterilization at 121 °C for 15 minutes, 100 $mg.L^{-1}$ of cycloheximide were added to inhibit the growth of yeasts and 100 $mg.L^{-1}$ of chloramphenicol to inhibit the development of LAB. Incubation was carried out under aerobiosis at 25 °C for 4 days.

Yeast count was determined on YGC agar (yeast extract glucose chloramphenicol agar) using the Pour Plate technique. After sterilization at 121 °C for 15 minutes, the medium was acidified to pH 3.5 by adding a sterile (Millipore 0.45 μm membrane filter) 10% tartaric acid (w/v) solution. Incubation was performed aerobically at 25 °C for 5 days (GARCIA FONTÁN et al., 2006).

Analyses of total coliforms and coliforms at 45 °C were performed on the 1st and 28th days of storage using the most probable number technique (MPN) (HAUSLER, 1977).

Experimental design and statistical analysis

Two experiments (formulations made with whole milk and skimmed milk) were conducted three times using a completely randomized design. The analyses were conducted using a split-plot design, in which the main treatment was the formulation, and the secondary treatment was storage time. The results were evaluated by analysis of variance (ANOVA) and the significant differences among means were determined using the "t" test ($p \leq 0.05$) (SAS 9.1.3). In each repetition of the experiment, microbiological analysis (using duplicate samples) and chemical and texture analysis (using triplicate samples) were carried out.

3 Results and discussion

3.1 pH, acidity, lactose, and inulin content

The end-point of the fermentation process for the different Kefir formulations was set at 24 hours. At this point (time 0, Figures 1a, b), the pH of the whole and skim milk formulations ranged between 4.83 and 4.52. The Kefirs fermented with grains (WG, WGI, SG and SGI) had higher pH values ($p \leq 0.05$) than those made from Kefir starter culture (WC, WCI, SC and SCI). It is likely that the microorganisms present in the grains needed time to transfer from the polysaccharide matrix to the milk and also to grow in the milk during the fermentation process, and therefore, Kefirs fermented with grains had higher pH values at the end- point of fermentation (GARROTE; ABRAHAM; DE ANTONI, 1997; WITTHUHN; SCHOEMAN; BRITZ, 2005). There was no difference ($p > 0.05$) in titratable acidity levels between Kefir formulations produced with grains or starter culture at day 1 (Figure 2).

During the storage period investigated, the pH values of the Kefir formulations fermented with grains (WG, WGI, SG, and SGI) were reduced, whereas the pH of the formulations fermented with starter culture (WC, WCI, SC, and SCI) initially dropped but then returned to the initial values. The increase in the pH values could be attributed to microbial cell proteolysis (GUZEL-SEYDIM et al., 2005) or to the fact that several yeasts isolated on fermented milk products, including *Saccharomyces cerevisiae*, may assimilate lactate when in co-culture with LAB slightly increasing the pH values of products (LOPITZ-OTSOA et al., 2006). On the 28th day, the pH of the grain fermented formulations was much lower than that of the formulations fermented with starter culture (Figure 1).

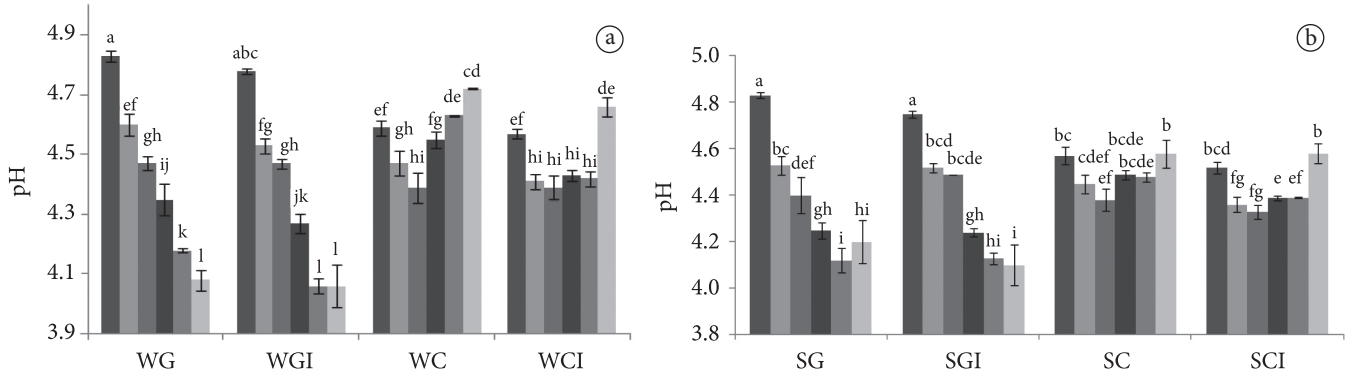

Figure 1. pH of Kefir formulations: WG (whole + grains), WGI (whole + grains + inulin), WC (whole + culture), WCI (whole + culture + inulin), SG (skim + grains), SGI (skim + grains + inulin), SC (skim + culture), SCI (skim + culture + inulin) during refrigerated storage (4 °C). Storage time (days): 0 (■), 1 (■), 7 (■) 14 (■), 21 (■), and 28 (■). (a) Whole milk formulations; (b) Skim milk formulations. Error bars represent standard deviation (n = 9).

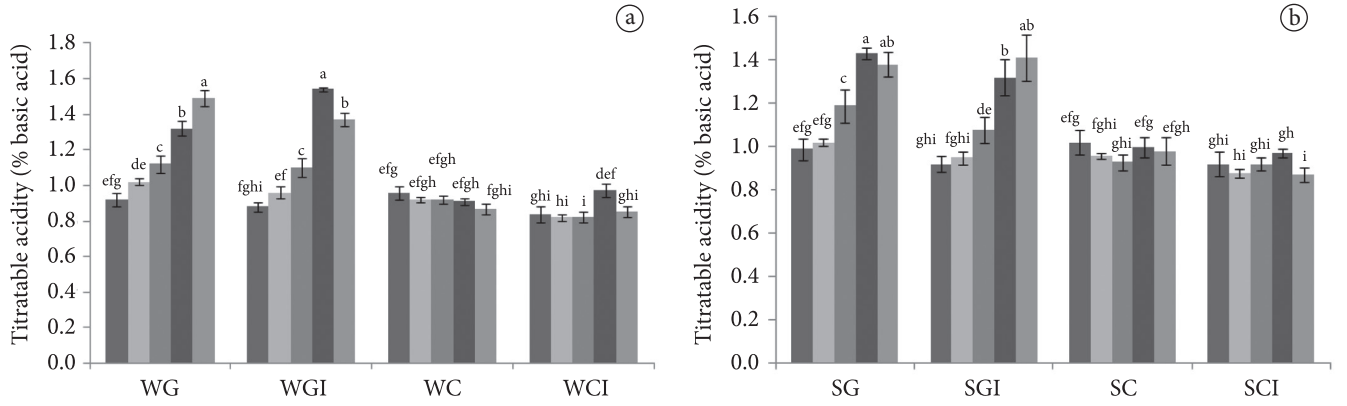

Figure 2. Titratable acidity (% lactic acid) of Kefir formulations: WG (whole + grains), WGI (whole + grains + inulin), WC (whole + culture), WCI (whole + culture + inulin), SG (skim + grains), SGI (skim + grains + inulin), SC (skim + culture), SCI (skim + culture + inulin) during refrigerated storage (4 °C). Storage time (days): 1 (■), 7 (■), 14 (■), 21 (■) and 28 (■). (a) Whole milk formulations; (b) Skim milk formulations. Error bars represent standard deviation (n = 9).

Öner, Karahan and Çakmakçi (2010) also observed more rapid decrease in the pH values of Kefir produced with grains when compared with those of Kefir produced with different starter cultures.

The same behavior was observed for titratable acidity. In the course of storage, the acidity of the Kefirs fermented with grains (WG, WGI, SG, and SGI) increased, whereas no change in acidity was observed in the beverages fermented with starter culture (WC, WCI, SC, and SCI). On day 28, the Kefirs fermented with grains had higher acidity than those fermented with starter culture (Figure 2).

The differences in acidity between the Kefir with grains and the Kefir with starter culture may be due to the differences in the microbiological profile of the two inocula or to the symbiosis between the microorganism population of the Kefir cultures, which is probably more limited in the grains than in the starter cultures (CHEN et al., 2009). Although symbiosis was present in the Kefir with grains, the fact that the LAB and yeast were embedded in the grains might have interfered with the interaction between them (CHEN et al., 2009). Consequently, yeasts present in the grain fermented Kefirs were not able to assimilate the lactate produced by lactic acid bacteria, and the acidity of the products increased. The lower acidification (higher pH values and lower titratable acidity values) of the starter culture fermented products compared to that of the grain fermented products during storage can increase the shelf life of Kefir beverages since the shelf life of fermented milk is often limited due to excessive acidification during storage (AKALIN et al., 2007).

The pH and the titratable acidity of the Kefir beverages were not affected by inulin addition ($p > 0.05$) throughout the storage period investigated, as shown by the similar results obtained for these parameters in the formulations with inulin (WGI, WCI, SGI, and SCI) and without inulin (WG, WC, SG, and SC). Ertekin and Guzel-Seydim (2010) and Glibowski and Kowalska (2012) did not find any effect of inulin on the acidity of Kefir either.

The drop in pH and the increase in acidity of Kefirs during refrigerated storage result from post-acidification and are related to the ongoing metabolization of lactose by the microbiota contained in the product (APORTELA-PALACIOS; SOSA-MORALES; VÉLEZ-RUIZ, 2005). This fact was confirmed by the reduction of the lactose content in the whole and skim milk formulations during storage (Table 2).

On the 28th day of storage, all skim milk formulations showed identical lactose contents ($p > 0.05$). Whole milk Kefirs fermented with starter culture (WC and WCI) had lower lactose levels compared to those of the beverages fermented with Kefir grains containing inulin (WGI). Farnworth and Mainville (2008) and Garcia Fontán et al. (2006) observed metabolization of lactose during the fermentation of the Kefir beverages and also during refrigerated storage of the products.

The concentration of inulin after 1 day storage was identical to the level originally added to the formulations (2%), indicating that inulin was not hydrolyzed during the fermentation of the milk (Figure 3). At the end of storage period, the inulin content was reduced by an average of 8% (whole milk formulations) and 9% (skim milk formulations); the formulations fermented with grains (WGI and SGI) had lower inulin content ($p \leq 0.05$) than the formulations fermented with starter culture (WCI and SCI). In acidic environments, treatments at elevated temperatures or prolonged storage at ambient conditions, inulin added to a food may be hydrolyzed, which results in the loss of nutritional, physicochemical and functional properties (VORAGEN, 1998; ORAFTI, 1999). Since grain-fermented Kefirs were more acidic during storage (Figure 1 and 2), the hydrolysis of inulin in these formulations was more pronounced. Cardarelli et al. (2008) and Pimentel, Garcia and Prudencio (2012) observed a reduction by 2.7% and 2.4% in inulin content in probiotic petit-suisse cheese and yoghurt, respectively, both stored for 28 days. Furthermore, according to Cruz-Guerrero et al. (2006), some strains of *Kluyveromyces* can produce inulinase; and Atputharajah, Widanapathirana and Samarajeewa (1986) suggested that some yeasts (e.g *Candida tropicalis)* are able to assimilate inulin. Therefore, it can be said that inulin could have been partially hydrolyzed by yeasts, mainly in the Kefir formulations fermented with grains during the storage time.

3.2 Firmness and syneresis

The formulations fermented with Kefir starter culture (WC, WCI, SC, and SCI) had higher firmness values ($p \leq 0.05$) compared to those of the formulations fermented with Kefir grains (WG, WGI, SG, and SGI) (Table 2). In the formulations fermented with kefir grains, the separation of the grains using a sieving cloth after the fermentation process caused

Table 2. Lactose content, firmness and syneresis of whole and skim Kefir during storage at 4 °C*.

Whole Formulations**					
	Time (days)	WG	WGI	WC	WCI
Lactose content	1	3.05 ± 0.102^{bA}	3.02 ± 0.023^{bA}	3.38 ± 0.091^{aA}	3.15 ± 0.021^{abA}
	14	2.54 ± 0.137^{aB}	2.48 ± 0.102^{aB}	2.35 ± 0.108^{abB}	2.27 ± 0.075^{bB}
	28	$2.02 \pm .006^{abC}$	2.13 ± 0.054^{aC}	1.92 ± 0.049^{bC}	1.86 ± 0.041^{bC}
Firmness	1	0.48 ± 0.005^{bB}	0.42 ± 0.001^{bB}	1.82 ± 0.027^{aB}	1.79 ± 0.020^{aB}
	14	0.84 ± 0.026^{bA}	0.67 ± 0.070^{cA}	2.02 ± 0.023^{aA}	1.93 ± 0.005^{aA}
	28	0.57 ± 0.001^{bB}	0.60 ± 0.001^{bA}	2.12 ± 0.057^{aA}	2.03 ± 0.023^{aA}
Syneresis	1	26.52 ± 0.417^{aB}	26.87 ± 0.260^{aA}	23.92 ± 0.417^{bA}	26.45 ± 0.385^{aA}
	14	27.33 ± 0.333^{aB}	27.27 ± 0.430^{aA}	23.00 ± 0.350^{bA}	22.67 ± 0.494^{bB}
	28	31.67 ± 1.430^{aA}	26.48 ± 1.365^{bA}	22.23 ± 0.498^{cA}	23.75 ± 0.443^{cB}
Skim Formulations**					
	Time (days)	SG	SGI	SC	SCI
Lactose content	1	2.93 ± 0.057^{aA}	2.86 ± 0.073^{aA}	3.02 ± 0.083^{aA}	2.81 ± 0.101^{aA}
	14	2.29 ± 0.089^{aB}	2.37 ± 0.057^{aB}	2.10 ± 0.062^{aB}	2.25 ± 0.094^{aB}
	28	1.96 ± 0.047^{aC}	2.08 ± 0.051^{aC}	1.83 ± 0.072^{aC}	1.90 ± 0.091^{aC}
Firmness	1	0.33 ± 0.032^{cB}	0.37 ± 0.017^{cC}	1.46 ± 0.002^{aB}	1.33 ± 0.052^{bB}
	14	0.77 ± 0.001^{bA}	0.49 ± 0.010^{cB}	1.69 ± 0.047^{aA}	1.60 ± 0.035^{aA}
	28	0.71 ± 0.043^{cA}	0.78 ± 0.010^{cA}	1.70 ± 0.006^{aA}	1.54 ± 0.044^{bA}
Syneresis	1	32.25 ± 0.281^{aB}	31.57 ± 0.392^{aC}	32.78 ± 0.239^{aA}	30.67 ± 0.511^{aB}
	14	30.67 ± 0.422^{bB}	36.75 ± 1.153^{aB}	25.00 ± 0.258^{cC}	32.25 ± 1.320^{bAB}
	28	39.83 ± 1.014^{bA}	46.58 ± 1.987^{aA}	29.70 ± 0.998^{dB}	33.70 ± 0.792^{cA}

*Results are expressed as g.100 g^{-1} (lactose). N (firmness). mL.100 g^{-1} (syneresis). Means ± standard deviation in the same row with different small letters superscripts indicate significant differences at $p \leq 0.05$ among Kefir formulations for the same day of storage. Means ± standard deviation in the same column with different capital letters superscripts indicate difference at $p \leq 0.05$ for each formulation affected by storage. (n = 9). **WG = whole + grains; WGI = whole + grains + inulin; WC = whole + culture; WCI = whole + culture + inulin; SG = skim + grains; SGI = skim + grains + inulin; SC = skim + culture; SCI = skim + culture + inulin.

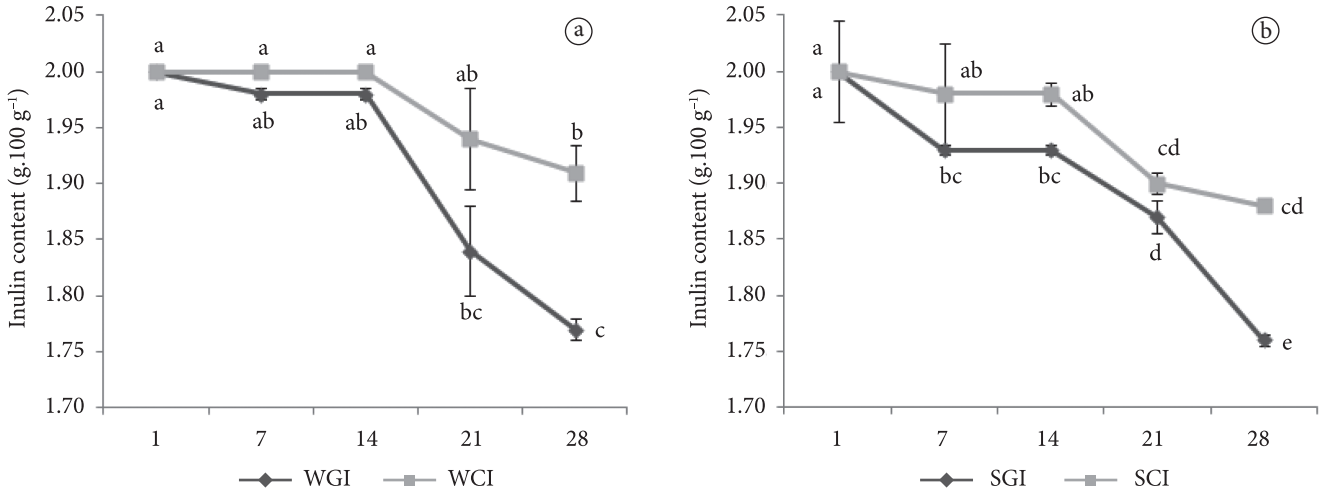

Figure 3. Inulin content (g.100 g^{-1}) of Kefir formulations: WGI (whole + grains + inulin), WCI (whole + culture + inulin), SGI (skim + grains + inulin), SCI (skim + culture + inulin) during refrigerated storage (4 °C). (a) Whole milk formulations; (b) Skim milk formulations. Error bars represent standard deviation (n = 9).

the disruption of the gel structure formed and, thus, these formulations were less firm than those prepared with starter culture.

Inulin had no influence on the firmness of whole milk Kefirs fermented with starter culture (WC and WCI) ($p > 0.05$) during refrigerated storage. For whole and skim milk Kefirs fermented with grains (WG and SG), the addition of inulin (WGI and SGI) caused a reduction in firmness on the 14th day of storage, but on the 28th day, the formulations with or without inulin had similar ($p > 0.05$) firmness values. For skim milk Kefirs fermented with starter culture (SC), the addition of inulin (SCI) resulted in a less firm product ($p \leq 0.05$) on the 1st and 28th day of storage. The long polysaccharide chain of inulin may have remained dispersed among the casein micelles, interfering in the formation of the protein matrix and being responsible for a softer gel (PASEEPHOL; SMALL; SHERKAT, 2008).

During storage, whole milk Kefirs (WGI, WC and WCI) and skim milk Kefirs (SG, SGI, SC, and SCI) showed an increase in firmness ranging from 13 to 115%, and the greatest variations were found among the skim formulations fermented with Kefir grains (SG and SGI). Whole milk formulation with grains (WG) showed an increase in firmness up to the 14th day, followed by a decrease until it returned to its initial firmness. Post-acidification in fermented milk causes a reduction of the pH, which leads to the contraction of the casein micelle, which, in turn, results in a firmer and more cohesive structure (KAILASAPATHY, 2006; ACHANTA; ARYANA; BOENEKE, 2007).

With regard to the syneresis of the Kefirs during storage, WGI and WC did not show any variation, while WG, SG, SGI, SCI showed an increase and WCI, and SC showed a decrease (Table 2). On the 28th day, the formulations fermented with grains exhibited a greater loss of liquid than that of the formulations fermented with starter culture. Serum separation occurs in fermented milk products due to the aggregation of protein particles during storage and sedimentation under gravity (KESENKAS et al., 2011), and some other factors such as stabilizers, acidity, total solids, and milk and culture type can affect the syneresis of fermented milk beverages (LUCEY et al., 1998). The Kefirs fermented with the starter culture were firmer and had lower acidity (Table 2). According to Brennan and Tudorica (2008), higher firmness makes fermented milk less susceptible to structure rearrangements and, therefore, less susceptible to serum separation.

In the skim milk formulations (SG and SC), the addition of inulin resulted in products (SGI and SCI) with higher values of syneresis ($p \leq 0.05$) on the 14th and 28th days of storage; and in whole milk Kefir fermented with culture (WC) an increased syneresis was observed only on the 1st day of storage due to inulin addition (WCI). In the whole milk formulations fermented with grains (WG), a reduction of serum separation was observed on the 28th day of storage due to inulin addition (WGI). Fibres, such as inulin, have been reported to reduce syneresis in fermented milks during storage because of their high water holding capacity (APORTELA-PALACIOS; SOSA-MORALES; VÉLEZ-RUIZ, 2005; GUVEN et al., 2005). However, the presence of a long-chain inulin could affect the development of a 3-dimensional structure of casein resulting in a weak gel incapable of retaining water (LUCEY et al., 1998). The results indicate that the influence of inulin on syneresis is related to the type of milk (whole or skim) and starter (grain or culture) used.

3.3 Microbiological evaluations

The inulin addition did not change the total LAB counts in Kefir formulations ($p > 0.05$) throughout the storage period (Figures 4a and 5a). The growth and viability of cultures in the presence of inulin vary with the degree of polymerization and are more efficient in short chains (MAKRAS; VAN ACKER; DE VUYST, 2005). Furthermore, the microorganisms in the Kefir grains or starter cultures are mesophilic, and the use of milk powder creates an environment rich in lactose, the preferred substrate of lactic acid bacteria (MAKRAS; VAN ACKER; DE VUYST, 2005; AKALIN et al., 2007). Thus, the degree of polymerization of inulin (average of 23), the low storage temperature (4 °C), and the use of powdered milk

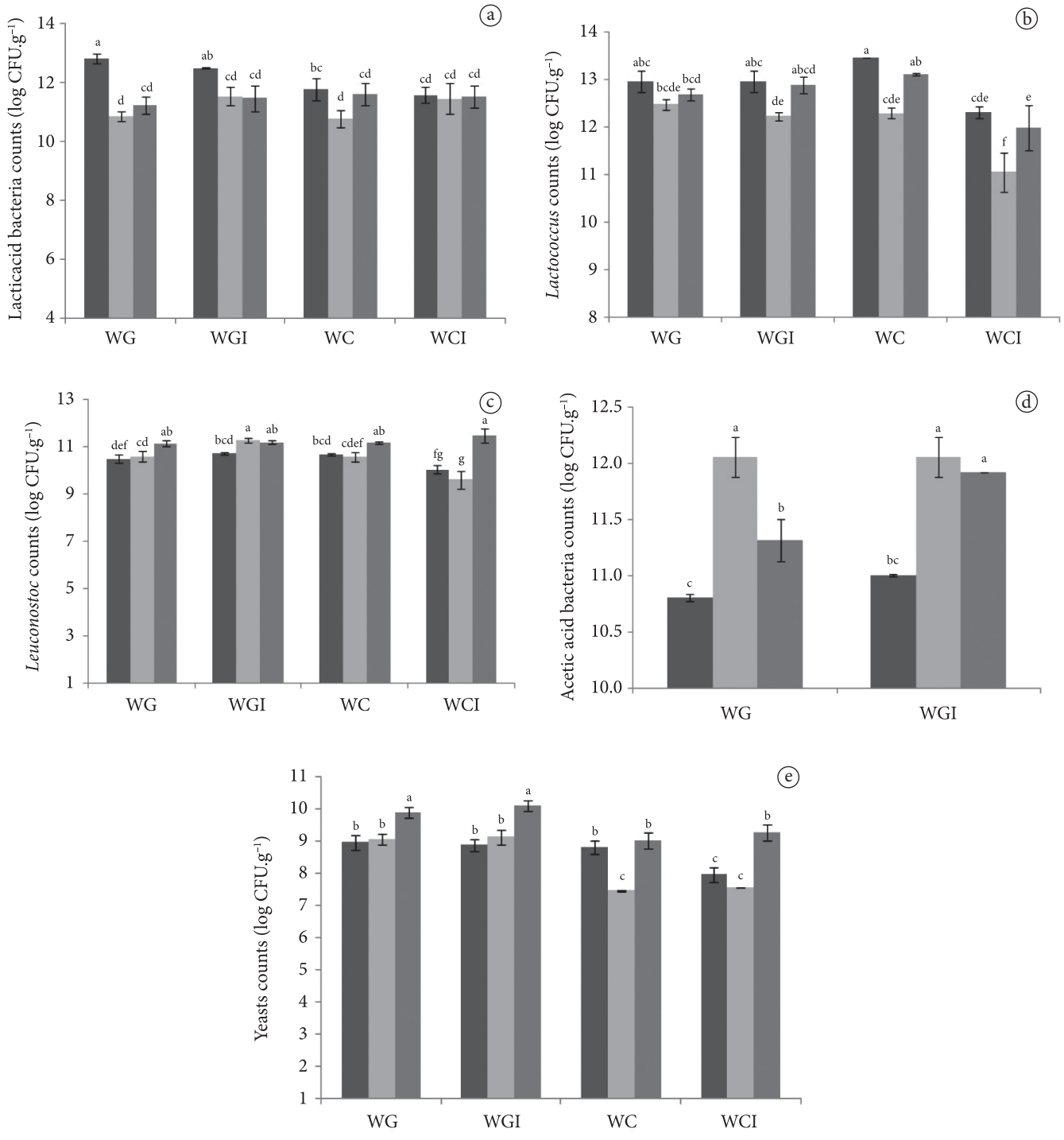

Figure 4. Microbial viability (log CFU.g^{-1}) in whole Kefir formulations: WG (whole + grains), WGI (whole + grains + inulin), WC (whole + culture), WCI (whole + culture + inulin) during refrigerated storage (4 °C). Storage time (days): 1 (■), 14 (■) and 28 (■). (a) Counts of Total Lactic acid bacteria; (b) Counts of *Lactococcus;* (c) Counts of *Leuconostoc;* (d) Counts of Acetic acid bacteria, (e) Counts of Yeasts. Error bars represent standard deviation (n = 6).

probably contributed to the lack of effect of inulin on the viability of LAB in this study.

The refrigerated storage resulted in the reduction by 1 log in the viability ($p \leq 0.05$) of LAB in the whole milk formulations fermented with grains (WG and WGI), whereas the whole milk formulations fermented with Kefir starter culture (WC and WCI) and the skim milk formulations (SG, SGI, SC, and SCI) did not show any variation ($p > 0.05$). Decreases in the counts of LAB in WG and WGI formulations may be related to the decrease in pH during storage. The lactic acid bacteria

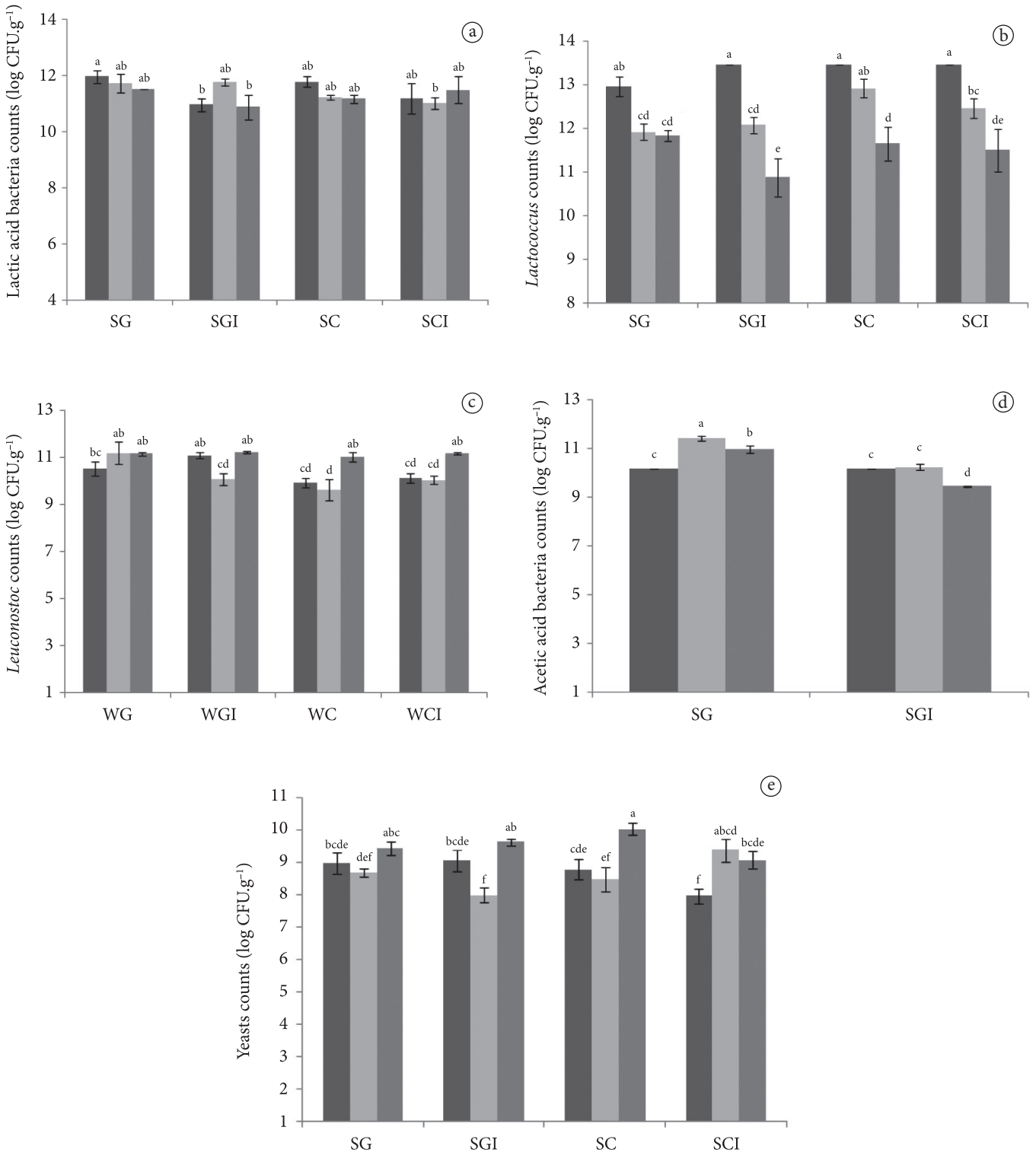

Figure 5. Microbial viability (log CFU.g^{-1}) in skim Kefir formulations: SG (skim + grains), SGI (skim + grains + inulin), SC (skim + culture), SCI (skim + culture + inulin) during refrigerated storage (4 °C). Storage time (days): 1 (■), 14 (■) and 28 (■). (a) Counts of Total Lactic acid bacteria; (b) Counts of *Lactococcus;* (c) Counts of *Leuconostoc;* (d) Counts of Acetic acid bacteria, (e) Counts of Yeasts. Error bars represent standard deviation (n = 6).

are neutrophilic; their optimum pH for growth lies between 5 and 9 and show growth inhibition at pH values lower than 4.5 (CRUZ et al., 2011). The major contributing factors to the loss of viability of some bacteria genus in fermented milks are the decrease in pH during storage (post-acidification) and the accumulation of organic acids (SHAH, 2000). This inhibition is related to the reduction of intracellular bacterial pH caused by an undissociated form of lactic acid, which causes a collapse

in the electrochemical gradient of protons in sensitive cells (CRUZ et al., 2011).

Despite the changes in the viability of LAB, on the 28th day of storage all formulations presented similar LAB counts, demonstrating that the use of grain or culture as starter culture did not influence survival of LAB. Total LAB counts were between 10.78 and 12.84 log CFU.mL^{-1} during the storage time, which is in agreement with the findings of Garrote, Abraham and De Antoni (1997) (10.52 log CFU.mL^{-1}) and Magalhães et al. (2011a) (12.41 log CFU.mL^{-1}), and are higher than the levels reported by Ertekin and Guzel-Seydim (2010), around 9.3 and 9.9 log CFU.mL^{-1}.

As for the *Lactococcus* population (Figures 4b and 5b), there was a reduction in the counts between the 1st and 14th day of storage in the whole milk Kefirs (WG, WGI, WC, and WCI), followed by an increase, so that by the 28th day, the number of CFU was identical to the initial population. In the skim milk formulations (SG, SGI, SC, and SCI), a 2-log reduction was observed during storage. The changes in *Lactococcus* counts during storage occurred regardless of the use of grains or culture as starter culture. Irigoyen et al. (2005) also observed a reduction in lactococci counts during refrigerated storage of Kefir. The decreases in the counts in the present study could be related to the increased acidity of the products during refrigerated storage (Figures 1 and 2). In fact, Garrote, Abraham and De Antoni (1998) and Magra, Antoniou and Psomas (2012) reported that the lactococci in Kefir were sensitive to low pH. It is likely that the whole milk formulations had a less inhibitory environment for the survival of this microorganism than the skim milk formulations. Vinderola, Bailo and Reinheimer (2000) reported that the rate of loss of bacterial cell viability depended on the type of fermented milk used (100% fat or fat free).

SG and WC formulations had higher counts ($p \leq 0.05$) than those of SGI and WCI formulations on the 28th day, indicating that the inulin addition resulted in products with lower counts of *Lactococcus*. No effect ($p > 0.05$) of inulin addition (WGI and SCI) on *Lactococcus* counts was observed for WG and SC formulations. During refrigerated storage, the *Lactococcus* population was between 10.88 and 13.47 log CFU.mL^{-1}, which is in agreement with the findings of Thamer and Penna (2005) (8.90 to 13.38 log CFU.mL^{-1}), and it was higher than the that found by Wszolek et al. (2001) (8.34 to 9.14 log CFU.mL^{-1}) and Garrote, Abraham and De Antoni (1997) (9.78 log CFU.mL^{-1}).

Leuconostoc survival was not affected ($p > 0.05$) by inulin addition or use of grains or culture as starter culture (Figures 4c and 5c) since all formulations presented similar counts on the 28th day of storage. In the whole milk formulations (WG and WCI) and skim milk formulations fermented with starter culture (SC and SCI), the counts of *Leuconostoc* increased during storage. In the whole milk formulations (WGI and WC) and in the skim milk formulations fermented with Kefir grains (SG and SGI), the count at the end of storage was similar to the initial count. The significantly higher numbers of *Leuconostoc* during refrigerated storage indicate that part of the lactose was used by the metabolism of *Leuconostoc* (Table 2). *Leuconostoc* counts in the Kefir formulations were between 9.63 and 11.51 log CFU.mL^{-1}, which is in agreement with Magalhães et al. (2011a) (10.41 log CFU.mL^{-1}).

The counts of AAB in the whole milk formulations fermented with Kefir grains (WG and WGI) increased during storage (Figures 4d and 5d). Inulin addition resulted in an improved survival of AAB in whole milk formulations, since on the 28th day, WG exhibited a lower number ($p \leq 0.05$) of AAB than that of WGI; whereas in the skim milk Kefir formulations, the inulin addition decreased ($p \leq 0.05$) AAB survival since the count of AAB in SGI was 1 log lower than that in SG on the 28th day of storage.

Li and Macrae (1991) found that acetic acid bacteria isolated from sugarcane roots were able to assimilate galactose, glucose, fructose, and sucrose but not inulin. Therefore, the survival of AAB in Kefir formulations in the presence of inulin could be related to the type of milk used. There is a close relationship between the viability of a particular strain of bacteria and the characteristics of the products, including their fat content (VINDEROLA; BAILO; REINHEIMER, 2000). The AAB population level during refrigerated storage was between 9.47 and 12.06 log CFU.mL^{-1}, higher levels than those recorded by Magalhães et al. (2011a) (7.72 log CFU.mL^{-1}) and similar to those of Montanuci, Garcia and Prudencio (2011) (10.18 to 11.01 log CFU.mL^{-1}).

With regard to yeast counts (Figures 4e and 5e), the whole milk formulations (WG, WGI, and WCI) and the skimmed formulations fermented with starter culture (SC and SCI) showed increased yeast viability during storage, whereas in SG, SGI, and WC, the final count was similar to the initial yeast population. Guzel-Seydim et al. (2005) also observed increases in the counts of yeasts during the cold storage of Kefir.

At the end of the storage period, the whole milk formulations fermented with starter culture (WC and WCI) exhibited lower yeast values than those of the whole formulations fermented with Kefir grains (WG and WGI), demonstrating that the increase in the counts of yeasts was less pronounced in culture fermented products than in grain fermented products. The same behavior was not ($p > 0.05$) observed for the skim milk formulations.

Inulin had no influence in yeast counts in skim milk formulations fermented with grains (SG and SGI) and in whole milk formulations (WG, WGI, WC, and WCI). SCI formulation count was 1-log lower than that of SC on the 28th day of storage, indicating that the inulin addition resulted in products with lower yeast counts in this formulation. The yeast population during refrigerated storage was between 7.47 and 10.12 log CFU.mL^{-1}, in accordance with the levels recorded by Garrote, Abraham and De Antoni (1998) (7.30 log CFU.mL^{-1}) and Magalhães et al. (2011a) (8.11 log CFU.mL^{-1}).

Prebiotics, such as inulin, are capable of increasing the viability of probiotic and starter culture microorganisms during fermentation and storage of yoghurts and other dairy products (ARYANA, 2003; HOZER; KIRMACI, 2010; MADRIGAL; SANGRONIS, 2007). In the present study, it was not possible to determine whether or not this effect is real since the addition of inulin had no influence on total LAB and *Leuconostoc* viabilities,

and the influence of inulin on AAB, *Lactococcus* and yeast survival depended on the milk type (whole or skim) and starter (grains or culture) used.

Total coliforms and coliforms at 45 °C were not found in the formulations during storage, according to the microbiological standards for sanitary food production. The increase in substrate acidity inhibits the development of undesirable or pathogenic microorganisms (MAGALHÃES et al., 2011b).

4 Conclusions

The fermentation of milk by grains or starter culture to produce Kefir beverages results in products with different storage stability. The post-acidification effects are more pronounced in the formulations fermented with Kefir grains than in those fermented with starter culture. Therefore, it can be said that the use of starter culture results in a product with more chemically and physically stable characteristics during storage. The viability of the microorganisms does not depend on the starter of fermentation (kefir grains or culture), demonstrating that the commercial starter culture mimics the microbial composition of the grains.

The addition of inulin has no effect on the total LAB and *Leuconostoc* viability and chemical characteristics, whereas the influence of inulin on AAB, *Lactococcus* and yeast survival, firmness, and syneresis in Kefir formulations depended on the milk type used (whole or skimmed) and use of grain or culture as starter culture. During the storage period, inulin degradation was less than 10%, demonstrating that inulin has a satisfactory stability in acidic environment. It can be concluded that the use of inulin has no adverse effect on the chemical, microbiological, and textural characteristics of Kefir.

Acknowledgments

The authors are grateful for the financial support provided by Capes for the Masters scholarship offered to the first author, to Clariant-Brazil for the donation of inulin, and to Clerici-Sacco Brazil for the donation of the starter culture.

References

ACHANTA, L. R.; ARYANA, K. J.; BOENEKE, C. A fat free plain set yogurt fortified with various minerals. **LWT-Food Science and Technology**, v. 40, p. 424-429, 2007. http://dx.doi.org/10.1016/j.lwt.2006.01.001

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA - ANVISA. **Alimentos com alegações de propriedades funcionais e ou de saúde, novos alimentos/ingredientes, substâncias bioativas e probióticos**. ANVISA, 2007. Atualizado em agosto de 2007. IX - Lista das alegações de propriedades funcionais aprovadas.

AKALIN, A. S. et al. Effects of fructooligosaccharide and whey protein concentrate on the viability of lactic culture in reduced-fat probiotic yogurt during storage. **Journal of Food Science**, v. 72, p. 222-227, 2007. PMid:17995644. http://dx.doi.org/10.1111/j.1750-3841.2007.00436.x

AKIN, M. B.; AKIN, M. S.; KIRMACI, Z. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. **Food Chemistry**, v. 104, p. 93-99, 2007. http://dx.doi.org/10.1016/j.foodchem.2006.11.030

APORTELA-PALACIOS, A.; SOSA-MORALES, M. E.; VÉLEZ-RUIZ, J. F. Rheological and physicochemical behavior of fortified yogurt, with fiber and calcium. **Journal of Texture Studies**, v. 36, p. 333-349, 2005. http://dx.doi.org/10.1111/j.1745-4603.2005.00020.x

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis of AOAC**. 16th ed. Arlington: AOAC, 1995. v. 2.

ARYANA, K. Folic acid fortified fat-free plain set yoghurt. **International Journal of Dairy Technology**, v. 56, p. 219-222, 2003. http://dx.doi.org/10.1046/j.1471-0307.2003.00105.x

ARYANA, K. J.; McGREW. P. Quality attributes of yogurt with Lactobacillus casei and various prebiotics. **LWT-Food Science and Technology**, v. 40, p. 1808-1814, 2007. http://dx.doi.org/10.1016/j.lwt.2007.01.008

ARYANA, K. J. et al. Fat-free plain yogurt manufactured with inulins of various chain lengths and Lactobacillus acidophilus. **Journal of Food Science**, v. 72, p. 79-84, 2007. PMid:17995804. http://dx.doi.org/10.1111/j.1750-3841.2007.00302.x

ATPUTHARAJAH, J. D.; WIDANAPATHIRANA, S.; SAMARAJEEWA, U. Microbiology and biochemistry of natural fermentation of coconut palm sap. **Food Microbiology**, v. 3, p. 273-280, 1986. http://dx.doi.org/10.1016/0740-0020(86)90009-2

BRENNAN, C. S.; TUDORICA, C. M. Carbohydrate-based fat replaces in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilization of barley beta-glucan, guar gum and inulin. **International Journal of Food Science and Technology**, v. 43, p. 824-833, 2008. http://dx.doi.org/10.1111/j.1365-2621.2007.01522.x

CARDARELLI, H. R. et al. Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially symbiotic petit-suisse cheese. **LWT-Food Science and Technology**, v. 41, p. 1037-1046, 2008. http://dx.doi.org/10.1016/j.lwt.2007.07.001

CHEN, T. H. et al. Microbiological and chemical properties of kefir manufactured by entrapped microorganisms isolated from kefir grains. **Journal of Dairy Science**, v. 92, p. 3002-3013, 2009. PMid:19528577. http://dx.doi.org/10.3168/jds.2008-1669

CRUZ, A. G. et al. Sensory analysis: relevance for prebiotic, probiotic, and synbiotic product development. **Comprehensive Reviews in Food Science and Food Safety**, v. 9, p. 358-373, 2010. http://dx.doi.org/10.1111/j.1541-4337.2010.00115.x

CRUZ, A. G. et al. Leites fermentados e iogurtes probióticos e prebióticos. In: SAAD, S. M. I.; CRUZ, A. G.; FARIA, J. A. F. (Eds.). **Probióticos e prebióticos em alimentos**. São Paulo: Livraria Varela, 2011.

CRUZ-GUERRERO, A. E. et al. Inulinase hyperproducing strains of *Kluyveromyces* sp. isolated from aguamiel (agave sap) and pulque. **World Journal of Microbiology and Biotechnology**, v. 22, p. 115-117, 2006. http://dx.doi.org/10.1007/s11274-005-9005-4

DELLO STAFFOLO, M. et al. Influence of dietary fiber addition on sensory and rheological frozen yogurt characteristics. **International Dairy Journal**, v. 14, p. 263-268, 2004. http://dx.doi.org/10.1016/j.idairyj.2003.08.004

DUBOC, P.; MOLLET, B. Applications of exopolysaccharides in the dairy industry. **International Dairy Journal**, v. 11, p. 759-768, 2001. http://dx.doi.org/10.1016/S0958-6946(01)00119-4

EL-NAGAR, C. G. et al. Rheological quality and stability of yog-ice cream with added inulin. **International Journal of Dairy**

Technology, v. 55, p. 89-93, 2002. http://dx.doi.org/10.1046/j.1471-0307.2002.00042.x

ERTEKIN, B.; GUZEL-SEYDIM, Z. Effect of fat replaces on kefir quality. **Journal of the Science of Food and Agriculture**, v. 90, p. 543-548, 2010.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO. **FAO Technical meeting report on prebiotics**. FAO, 2007. Disponível em: <http://www.fao.org/ag/agn/agns/files/Prebiotics_Tech_Meeting_Report.pdf>. Acesso em: 15 nov. 2011.

FARNWORTH, E. R. Kefir - a complex probiotic. **Food Science and Technology Bulletin**: Functional Foods, v. 2, p. 1-17, 2005. http://dx.doi.org/10.1616/1476-2137.13938

FARNWORTH, E. D.; MAINVILLE, A. **Kefir-A fermented milk product**. 2nd ed. Taylor & Francis, 2008. p. 89-128. (Handbook of fermented Functional Foods and Nutraceuticals Series).

GARCIA FONTÁN, M. et al. Microbiological and chemical changes during the manufacture of Kefir made from cows' milk, using a commercial starter culture. **International Dairy Journal**, v. 16, p. 762-767, 2006.

GARROTE, G. L.; ABRAHAM, A. G.; DE ANTONI, G. L. Preservation of Kefir grains, a comparative study. **LWT-Food Science and Technology**, v. 30, p. 77-84, 1997. http://dx.doi.org/10.1006/fstl.1996.0135

GARROTE, G. L.; ABRAHAM, A. G.; DE ANTONI, G. L. Characteristics of kefir prepared with different grain:milk ratios. **Journal of Dairy Research**; *v.* 65, p. 149-154, 1998. http://dx.doi.org/10.1017/S0022029997002677

GARROTE, G. L.; ABRAHAM, A. G.; DE ANTONI, G. L. Chemical and microbiological characterization of Kefir grains. **Journal of Dairy Research**, v. 68, p. 639- 652, 2001. http://dx.doi.org/10.1017/S0022029901005210

GLIBOWSKI, P.; KOWALSKA, A. Rheological, texture and sensory properties of kefir with high performance and native inulin. **Journal of Food Engineering**, v. 111, p. 299-304, 2012. http://dx.doi.org/10.1016/j.jfoodeng.2012.02.019

GUVEN, M. et al. The effect of inulin as fat replacer on the quality of set-type low-fat yogurt manufacture. **International Journal of Dairy Technology**, v. 58, p. 180-184, 2005. http://dx.doi.org/10.1111/j.1471-0307.2005.00210.x

GUZEL-SEYDIM, Z. et al. Turkish kefir and kefir grains: microbial enumeration and electron microscopic observation. **International Journal of Dairy Technology**, v. 58, p. 25-29, 2005. http://dx.doi.org/10.1111/j.1471-0307.2005.00177.x

HAUSLER, W. J. **Standard methods for the examination of dairy products**. 13th ed. Washington: American Health Association, 1977.

HOZER, B.; KIRMACI, H. A. Functional milks and dairy beverages. **International Journal of Dairy Technology**, v. 63, p. 1-15, 2010. http://dx.doi.org/10.1111/j.1471-0307.2009.00547.x

IRIGOYEN, A. et al. Microbiological, physicochemical, and sensory characteristics of kefir during storage. **Food Chemistry**, v. 90, p. 613-620, 2005. http://dx.doi.org/10.1016/j.foodchem.2004.04.021

KAILASAPATHY, K. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. **LWT-Food Science and Technology**, v. 39, p. 1221-1227, 2006. http://dx.doi.org/10.1016/j.lwt.2005.07.013

KESENKAS, H. et al. Physicochemical, microbiological and sensory characteristics of soymilk kefir. **African Journal of Microbiology Research**, v. 5, p. 3737-3746, 2011.

LI, R. P.; MACRAE, I. C. Specific association of diazotrophic acetobacters with sugarcane. **Soil Biology and Biochemistry**, v. 23, p. 999-1002, 1991. http://dx.doi.org/10.1016/0038-0717(91)90181-I

LOPITZ-OTSOA, F. et al. Kefir: a symbiotic yeasts-bacteria community with alleged healthy capabilities. **Revista Iberoamericada de Micologia**, v. 23, p. 67-74, 2006. http://dx.doi.org/10.1016/S1130-1406(06)70016-X

LUCEY, J. A. et al. A comparison of formation, rheological properties and microstructure of acid skim milk gels made with a bacterial culture or glucono-δ-lactone. **Food Research International**, v. 31, p. 147-155, 1998. http://dx.doi.org/10.1016/S0963-9969(98)00075-1

MADRIGAL, L. Y.; SANGRONIS, E. La inulina y derivados como ingredientes claves en alimentos funcionales. **Archivos Latinoamericanos de Nutrición**, v. 57, p. 387-396, 2007. PMid:18524324.

MAGRA, T. I.; ANTONIOU, K. D.; PSOMAS, E. I. Effect of milk fat, kéfir grain inoculum and storage time on the flow properties and microbiological characteristics of kefir. **Journal of Texture Studies**, v. 43, p. 299-308, 2012. http://dx.doi.org/10.1111/j.1745-4603.2011.00343.x

MAKRAS, L.; VAN ACKER, G.; DE VUYST, L. Lactobacillus casei subsp. casei 8700:2 degrades inulin-type fructans exbiting different degrees of polymerization. **Applied and Environmental Microbiology**, v. 71, p. 6531-6537, 2005. PMid:16269678. PMCid:1287650. http://dx.doi.org/10.1128/AEM.71.11.6531-6537.2005

MANNING, T. S.; GIBSON, G. R. Prebiotics. **Best Practice & Research Clinical Gastroenterology**, v. 18, p. 287-298, 2004. http://dx.doi.org/10.1016/j.bpg.2003.10.008

MAGALHÃES, K. T. et al. Brazilian Kefir: structure, microbial communities and chemical composition. **Brazilian Journal of Microbiology**, v. 42, p. 693-702, 2011a. http://dx.doi.org/10.1590/S1517-83822011000200034

MAGALHÃES, K. T. et al. Chemical composition and sensory analysis of cheese whey-based beverages using kefir grains as starter culture. **International Journal of Food Science and Tecnology**, v. 46, p. 871-878, 2011b. http://dx.doi.org/10.1111/j.1365-2621.2011.02570.x

MAYEUX, J. V.; COLMER, C. A. Selective medium for Leuconostoc detection. **Journal of Bacteriology**, v. 81, n. 6, p. 1009-1011, 1961. PMid:13768594. PMCid:314774.

MEGAZYME. **Fructan HK Procedure for the measurement of fructo-oligosaccharides (FOS) and fructan polysaccharide**. Megazyme, 2009. Disponível em: <http://www.megazyme.com>. Acesso em: 03 maio 2010.

MONTANUCI, F. D.; GARCIA, S.; PRUDENCIO, S. H. Caracterização sensorial e aceitação de Kefir adoçado integral e desnatado com inulina. **Brazilian Journal of Food Technology**, 6º Sensiber, de 19-21 de agosto de 2010, p. 79-90, 2011. Edição especial. http://dx.doi.org/10.4260/BJFT201114E000110

ÖNER, Z.; KARAHAN, A. G.; ÇAKMAKÇI, M. L. Effects of different milk types and starter cultures on kefir. **GIDA**, v. 35, p. 177-182, 2010.

ORAFTI. **Application File**: Fermented Dairy Products Doc A89-90*01/99. Belgium,1999. 10 p. Active Food Ingredients.

OTLES, S.; CAGINDI, O. Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. **Food Engineering Department**, v. 2, p. 54-59, 2003.

ORDÓÑEZ, J. A. **Tecnologia de Alimentos** - **Alimentos de Origem Animal**. Artmed, 2005. v. 2, 279 p.

PASEEPHOL, T.; SMALL, D. M.; SHERKAT, F. Rheology and texture of set yogurt as affected by inulin addition. **Journal of Texture**

Studies, v. 39, p. 617-634, 2008. http://dx.doi.org/10.1111/j.1745-4603.2008.00161.x

PIERMARIA, J. A.; CANAL, M. L.; ABRAHAM, A. G. Gelling properties of Kefiran, a food-grade polysaccharide obtained from kefir grain. **Food Hydrocolloids**, v. 22, p. 1-8, 2007.

PIMENTEL, T. C.; GARCIA, S.; PRUDENCIO, S. H. Effect of long-chain inulin on the texture profile and survival of Lactobacillus paracasei ssp. paracasei in set yoghurts during refrigerated storage. **International Journal of Dairy Technology**, v. 65, p. 104-110, 2012. http://dx.doi.org/10.1111/j.1471-0307.2011.00739.x

SACCO. **Lyofast MT 036 LV**: technical sheet. Sacco, 2010. Disponível em: <http://www.saccosrl.it>. Acesso em: 20 mar. 2010.

SHAH, N. P. Probiotic bacteria: selective enumeration and survival in dairy foods. **Journal of Dairy Science**, v. 83, p. 894-907, 2000. http://dx.doi.org/10.3168/jds.S0022-0302(00)74953-8

THAMER, K. G.; PENNA, A. L. B. Efeito do teor de soro, açúcar e de frutooligossacarídeos sobre a população de bactérias lácticas probióticas em bebidas fermentadas. **Brazilian Journal of Pharmaceutical Sciences**, v. 41, p. 393-400, 2005.

VINDEROLA, C. G.; BAILO, N.; REINHEIMER, J. A. Survival of probiotic microflora in Argentinian yogurts during refrigerated storage. **Food Research International**, v. 33, p. 97-102, 2000. http://dx.doi.org/10.1016/S0963-9969(00)00011-9

VORAGEN, A. G. J. Technological aspects of functional food-related carbohydrates. **Trends in Food Science & Technology**, v. 9, p. 328-335, 1998. PMid:21299575. http://dx.doi.org/10.1016/S0924-2244(98)00059-4

WILDERNESS FAMILY NATURALS. 2010. Disponível em: <http://www.wildernessfamilynaturals.com>. Acesso em: 03 maio 2010.

WSZOLEK, M. et al. Properties of Kefir made in Scotland and Poland using bovine, caprine and ovine milk with different starter cultures. **LWT-Food Science and Technology**, v. 34, p. 251-261, 2001. http://dx.doi.org/10.1006/fstl.2001.0773

WITTHUHN, R. C.; SCHOEMAN, T.; BRITZ, T. J. Characterization of the microbial population at different stages of Kefir production and Kefir mass cultivation. **International Dairy Journal**, v. 15, p. 383-389, 2005. http://dx.doi.org/10.1016/j.idairyj.2004.07.016