

Evaluation of a Functional Soy Product with Addition of Soy Fiber and Fermented With Probiotic Kefir Culture

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ABSTRACT

*The objective of this study was to evaluate the chemical, sensory properties and stability of a functional soy product with soy fiber and fermented with probiotic kefir culture. The product was characterized by the chemical composition, color and sensory analysis. The stability of the product was evaluated by pH, acidity, viscosity, firmness, syneresis measurements and cells counts. The functional soy product presented better chemical composition and difference in color compared to the fermented product without fiber. Sensory analysis showed that the functional soy product had good acceptance and had better firmness and reduced syneresis compared to fermented product without fiber. The lactic acid bacteria counts decreased slightly during 28 days at 4°C of the storage and the product showed good microbiological stability. The functional soy product due to high *Lactococcus lactis* counts could be considered as a probiotic for the entire storage period.*

Key words: Soy fiber, Kefir culture, Functional fermented soy product, Storage, Probiotic product

INTRODUCTION

The development of non-dairy probiotic products is a challenge to the food industry in its effort to use the abundant natural resources by producing high quality functional products (Charalampopoulos et al. 2002). Most probiotic foods at the markets worldwide are milk-based and very few attempts are made to the development of probiotic foods using other fermentation substrates such as cereals (Angelov et al. 2006).

Soybean and its derivatives have good potential for application in the functional food industry, because they contain a large quantity of components that are beneficial to health, such as proteins, isoflavones, fiber, essential fatty acids, oligosaccharides, etc (Liu 1997). Despite its excellent nutritive value, soybean grains have not been accepted in many western countries due to its

undesirable flavors and characteristics tastes (Silva et al. 2010). However, soy fermentation can improve its acceptability. Overall, functional foods, or beverages are fortified through the addition of exogenous functional compounds, or using the microorganisms that produce biogenic compounds, or having probiotic features (Servili et al. 2011). Fermented soy products may be supplemented with the compounds claiming to have functional properties, such as fibers and probiotics.

Kefir is a complex mixture of bacteria and yeast (Urdaneta et al. 2007) that co-exist in a symbiotic association and can be used for acid and alcohol fermentation. Kefir production using kefir grains is difficult to put into practice. Attempts have been made toward standardizing kefir production using the defined cultures. Because of its microbial complexity and the benefits derived from its use, kefir may be considered an adequate

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source of potential probiotic microorganisms (Romanin et al. 2010). To confer health benefits, probiotic products should provide a minimum count of 10^6 CFU/g in the fermented product (Shah 2007; Ramchandran and Shah 2010). During the storage of fermented soy products, some studies indicate that there is a reduction in the growth and number of microorganisms. According to Liong (2011), the challenge of these products is to ensure probiotic stability. Many studies have indicated that soymilk fermented with kefir could be beneficial to the human health (Kwon et al. 2006; Apostolidis et al. 2007).

Fermentation approaches have been attempted extensively to develop various fermented products and thus overcome the limitations in the consumption of soy products. However, studies about the fermented soymilk were concerned with the bacterial growth, or the taste of the product, but not with the totality of characteristics evaluation as a probiotic food with soy fiber. Still, the effect of using fibers from alternative sources in fermented milk products has been widely investigated. However, there are no published studies about the functional soy product with the addition of soy fiber and fermented with probiotic kefir culture. The objective of this study was to evaluate the chemical and sensory properties and stability of a functional soy product with the addition of soy fiber and fermented with probiotic kefir culture.

MATERIALS AND METHODS

Materials and Starter Culture

Soymilk was prepared with lipoxigenase-free BRS 257 soybean. For the formulation of the fermented soy product, the following commercial ingredients were used: soy fiber, sucrose, antifoaming and artificial milk and vanilla flavoring. For fermentation, lyophilized kefir starter culture (Sacco®-Lyofast TM 036 LV) composed of a mixed stock of *Lactococcus lactis* spp *lactis*, *Lactococcus lactis* spp *lactis* *diacetylactis*, *Lactobacillus brevis*, *Leuconostoc* spp and *Saccharomyces cerevisiae* was used.

Soymilk and Fermented Soy Product Preparation

Soymilk was prepared after soybean screening and washing. The soybeans in a ratio of 1:10 (w:v; soybean grains:water) were soaked for 14 h,

triturated and filtered to obtain the soymilk. The residue was discarded.

Formulations containing 87.7% soymilk (w/w), 3.0% soy fiber (w/w), 9.0% sucrose (w/w) and 0.1% antifoam (w/w) were subjected to heat treatment at 95°C for 15 min according to Ferragut et al. (2009). After cooling to 25°C, 0.2% milk and vanilla flavorings (w/w) were added and the mixture was dispensed into 600 mL glass vials. The mixture was fermented at 25°C with kefir culture (0.01 UC/L) until a pH of 4.5 ± 0.1 was attained. The vials were cooled to 4°C, homogenized for 6 min at constant speed (Homogenizer Contrac, Mod 1000) and stored for at least 12 h before the analysis.

From optimization studies on the formulation of fermented soy products with kefir and soy, oat and wheat fibers, the optimal formulation (KF) containing 3.0% soy fiber (w/v) was established. The formulated product was stored for 28 days at 4°C. At 7 days intervals, the pH, acidity, viscosity, firmness and syneresis characteristics were evaluated and kefir microorganisms counts. The product without soy fiber (KC) was prepared for comparison purposes and soymilk volume was adjusted to 90.7%.

Chemical Characterization and Sensory Analysis

Protein, fat, ash, moisture and total dietary fiber contents were determined in triplicate in fermented products (AOAC, 2006), and the results expressed in dry basis (d.b.). Color (10 replicates) was measured with a Minolta CR-400 colorimeter (Konica Minolta Sensing, Incorporation), with lighting D65, and the results were expressed in the CIELAB system (L^* , a^* and b^*).

For sensory analysis, the study was approved by the Ethics Committee of institution (Opinion No. 0163.0.268.000-10) and samples were analyzed for coliform at 45°C, *Bacillus cereus* and *Salmonella* spp counts, according to Brasil (2003). The sensory analysis was performed by the acceptance test with 68 untrained consumers. The consumers received 30 g of the product at 10°C in plastic drinking cups coded with three-digit random numbers. The formulations were evaluated for color, aroma, texture, flavor and overall acceptability attributes. The panelists used a 9-point hedonic scale, ranging from "dislike extremely (1)" to "like extremely (9)" (Stone and Sidel 2004).

Evaluation and Stability During Storage

Fermented and stored products were evaluated for pH, lactic acid content, viscosity, firmness, syneresis and kefir microbial counts every 7 days until day 28. The pH of the fermented products was determined with a digital potentiometer (Hanna, HI 223). The lactic acid content was measured by titration with NaOH (0.1 mol/L) and expressed in g lactic acid in 100 g of sample. Viscosity was determined using a digital Brookfield viscometer with a plus *spindle* 4, speed of 1.26 rad/s (12 rpm) and a 600 mL sample at $4 \pm 1^\circ\text{C}$; the results were expressed in centipoise (cP). Centipoise corresponds at 10^{-3} Pa s (SI Unit). Syneresis was measured (five replicates) according to a modification of Guirguis et al. (1984) methodology and was used with the fabric tunnel overlapped under a bolter for drainage. Syneresis was expressed as ml exudate in 100 g of sample. The firmness was evaluated by the measurements carried out in a TA-XT2i texturometer (*Stable Micro Systems*), with a cylindrical acrylic probe acrylic P 25/L, 10 mm compression depth, sensor compression speed 2 mm/s, trigger force of 0.05 N and time of 0.5 s. Firmness was expressed in Newtons (N). Cell counts for *Lactococcus lactis* (Irigoyen et al. 2005), *Leuconostoc* spp and yeast (Fontán et al. 2006; Zajsek and Gorsek 2010) were carried out and the results were expressed as log CFU/g of the fermented product.

Data Analysis

Data regarding the chemical composition, color and sensory analysis were subjected to a t-test for comparison of the KC and KF products. The storage stability data of the fermented products was also subjected to a t-test for comparison of the KC and KF products at the same storage period. The analysis of variance (ANOVA) and the Tukey test ($p < 0.05$) were performed to compare the changes in pH, acidity, viscosity, firmness, syneresis and microbial kefir counts during the storage of KC or KF products.

RESULTS AND DISCUSSION

Chemical Composition and Sensory Acceptance

The chemical composition on dry basis (Table 1) of fermented soy products with kefir and with addition of soy fibers (KF) presented protein,

dietary fiber, carbohydrates and ash contents higher than the product without soy fibers (KC). These increases in the components content were due the 3% soy fiber addition and the chemical composition of soy fiber. The lipid content did not differ in the KC and KF product (Table 1). Color parameters (Table 1) in soy products fermented with kefir and with the addition of soy fibers (KF) and without soy fibers (KC) showed significant differences, and judges preferred the color of the product without fibers (Fig. 1). The higher L^* parameter in the KC product indicated a lighter color than in the KF product. The a^* parameter (red-green component) was higher in the KC product. The a^* negative values were also obtained by Cruz et al. (2007) for soymilk. The b^* parameter (yellow-blue component) was lower in the KC product; the addition of 3% soy fiber conferred a yellowish KF product.

Table 1 - Chemical composition and color of fermented soy products with kefir and with addition of soy fibers (KF) and without soy fibers (KC).

	Products*	
	KC	KF
Chemical composition		
d.b. (g/100g)		
Protein	15.65 ± 0.26^b	20.18 ± 0.14^a
Fat	7.02 ± 0.59^a	7.03 ± 0.62^a
Total fiber	0.70 ± 0.05^b	7.53 ± 0.08^a
Carbohydrate	74.91 ± 0.00^a	62.95 ± 0.00^b
Ash	1.69 ± 0.14^b	2.30 ± 0.03^a
Color		
L^*	73.74 ± 2.45^a	70.34 ± 1.52^b
a^*	-2.93 ± 0.13^a	-1.15 ± 0.07^b
b^*	8.20 ± 0.39^b	9.57 ± 0.23^a

The mean \pm standard deviation values in the same row that do not have a common superscript are significantly different ($p \leq 0.05$) by the t-test.

*Products: KC (fermented soy product without fiber) and KF (fermented soy product with 3 % soy fiber)

Before performing the sensory analysis of the fermented product, the microbiological assays were carried out for coliform at 45°C , *Bacillus cereus*, or *Salmonella* spp to ensure the safety of the product. The acceptance test consisted of 68 judges, 23 men and 45 women, aged 15-50 years and 93% of the judges reported consuming soy products and fermented products. In the sensory analysis (Fig. 1), in which color, aroma, texture, taste and overall acceptance attributes were evaluated, the KC product showed a greater acceptance and differed significantly from the KF

product. The KC product presented values above 7 and the KF product showed values between 6.3 and 6.9. On the hedonic scale, point 6 indicated that the judges "liked regularly" and point 7 stated that product was "liked slightly". The addition of functional components such as fibers, changed the characteristics of the product and, therefore, might lead to lower acceptance. Most often, these products are consumed due to the numerous health benefits they can offer to the consumer, even if there is little loss in sensory acceptance. These results were better to those described in other sensory analyses of fermented soy products with soluble fiber (Rinaldoni et al. 2012) that presented maximum acceptance of 40% in soymilk fermented with inulin. Umbelino et al. (2001) found values 5.0 a 6.2 for the acceptance of soy yogurt enriched with calcium salts.

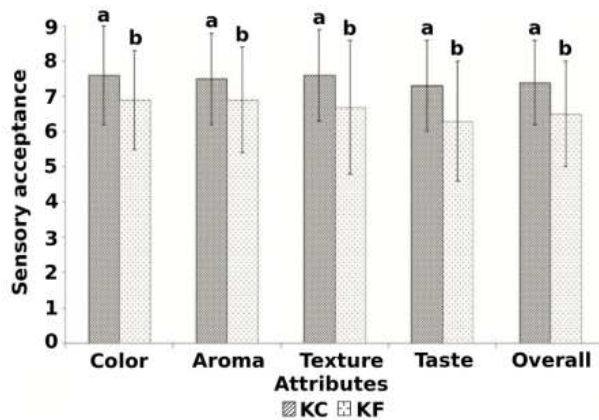


Figure 1 - Sensory acceptance of soy products fermented with kefir and with addition of soy fibers (KF) and without soy fibers (KC).

Evaluation of the Stability

pH and Acidity

During the storage at 4°C, the pH of the fermented soy products with kefir and with addition of soy fibers (KF) and without soy fibers (KC) decreased until day 28 (Fig. 2). The pH was approximately 4.4 and decreased to 4.1-4.2 during the KC and KF storage. This pH range was considered optimal for soymilk gel formation. The decrease of pH during the storage is common in fermented foods (Lucey 2004) and can be attributed to the growth of bacteria and lactic acid production. In other study (Rinaldoni et al. 2012), the pH of soymilk fermented decreased during the storage until 4.8-4.6. According to Svensson (1999), the

formulations with higher protein content could have greater buffering capacity and decrease the pH fall of product, as observed in the KF product (Table 1 and Fig. 2). The decrease in the pH with decreased buffering capacity of the medium also was observed by Mall et al. (2010).

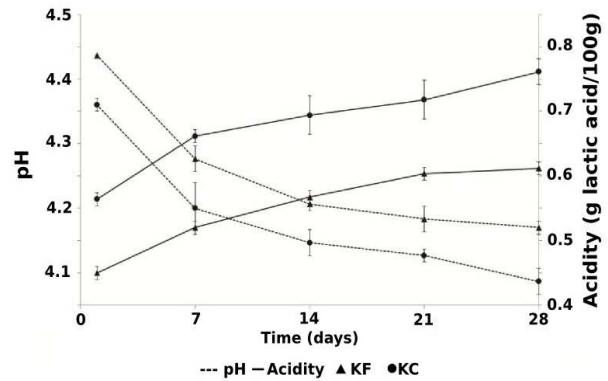


Figure 2 - pH and acidity of a soy products fermented with kefir and with addition of soy fibers (KF) and without soy fibers (KC) during storage at 4°C.

For the same storage period (Fig. 2), the formulation containing 3% soy fiber (KF) showed higher acidity than the formulation without fibers (KC). According to Fernandez-Garcia and McGregor (1997), some fibers may provide nutrients, or factors that stimulate the starter culture, which promotes higher acidity. The ability of some fibers to increase the acidity of fermented products has also been described using the orange, soy, rice, maize, oats and sugar beet fibers (Fernandez-Garcia and McGregor 1997; Lario et al. 2004; Garcia-Perez et al. 2006). This is a positive feature, as it indicates that some fibers may stimulate the metabolism of starter culture.

Viscosity, Firmness and Syneresis

During the storage, the functional soy product with the addition of soy fiber (KF) and without soy fibers (KC) and fermented with probiotic kefir culture showed significant differences in viscosity, firmness and syneresis measurements (Table 2). The initial viscosity of the products was quite different due to the addition of soy fiber. Table 1 showed that the fermented soy fiber had higher protein and total fiber content, which greatly increase the viscosity of the product. Soluble fibers contribute to the formation of viscous

systems, and in low amounts, they also modify the product characteristics. The addition of different fiber sources, such as bamboo, apples, wheat, or inulin fibers, affects the viscosity in yogurt (Dello Staffolo et al. 2004). The soluble polymers of high molecular weight, such as proteins, increase viscosity in low concentrations and viscosity follows an exponential relationship with the protein concentration (Damodaran et al. 2007).

The KC product viscosity remained constant during the storage, while the viscosity and firmness of the KF product increased positively with gel formation due to the addition of 3% soy fiber to the formulation. In milk yogurt, results similar to those observed in this study have been described by García-Pérez et al. (2006) and McCann et al. (2011) who used carrot cell wall particles and orange fiber, respectively. The KF product had a viscosity 5.5 times higher than that of the KC product after 28 days of storage. According to Fernandez-Garcia and McGregor (1997), the high viscosity of KF product may be associated with fiber components, such as hydrocolloids that interact with the proteins of the fermented product and increase the viscosity. The amount of protein (Table 1) could also be responsible for different viscosities and firmness of the formulations. In a soy yogurt formulation, Kovalenko and Briggs (2002) found that the soy protein viscosity and gel strength were dependent on protein concentration. Dello Staffolo et al. (2004) and Sendra et al. (2010) have also reported increased firmness and viscosity in fermented milk products with the addition of different fibers. Syneresis is the spontaneous separation of the whey from the fermented product and is a very important characteristic during the storage (Peng et al. 2009; Zare et al. 2011). The syneresis of the KC and KF product (Table 2) decreased significantly during the storage and KF product, did not show syneresis from day 14 to day 28. Ferragut et al. (2009) observed that soy yogurts without fibers have a gradual decrease in syneresis. In general, in the fermented soy products, the syneresis rate was low and similar to the rates obtained by Kovalenko and Briggs (2002) for soy yogurt. According to Jaros et al. (2002) and Ünal et al. (2003), formulations with a lower total solids content have a higher syneresis value, as noted in the product without fiber (KC). Coggins et al. (2010) and Liong (2011) observed that an increase in the viscosity and firmness and a reduction in syneresis might be due to pH

reduction during the storage of the fermented products, allowing for gel contraction. Furthermore, the exopolysaccharides produced by lactic acid bacteria may decrease the syneresis and increase the viscosity and firmness during the product storage (Jolly et al. 2002). The results of this study indicated that the addition of 3% soy fiber produced higher firmness and reduced syneresis of the KF fermented product.

Table 2 - Viscosity, firmness and syneresis values of fermented soy products with kefir and with addition of soy fibers (KF) and without soy fibers (KC).

Time (days)	Products*	
	KC	KF
Viscosity (cP)		
1	235.2 ± 12.6 ^{A,b}	389.5 ± 31.6 ^{J,a}
7	249.5 ± 19.7 ^{A,b}	639.3 ± 4.5 ^{L,a}
14	259.2 ± 16.3 ^{A,b}	922.8 ± 51.9 ^{H,a}
21	257.5 ± 14.0 ^{A,b}	1114.3 ± 95.8 ^{G,a}
28	252.0 ± 17.6 ^{A,b}	1400.7 ± 52.6 ^{F,a}
Firmness (N)		
1	0.17 ± 0.01 ^{A,b}	0.26 ± 0.01 ^{G,a}
7	0.17 ± 0.01 ^{A,b}	0.26 ± 0.01 ^{G,a}
14	0.18 ± 0.01 ^{A,b}	0.28 ± 0.01 ^{G,a}
21	0.16 ± 0.01 ^{A,B,b}	0.33 ± 0.01 ^{F,a}
28	0.15 ± 0.01 ^{B,b}	0.34 ± 0.01 ^{F,a}
Syneresis (mLexudate/100g)		
1	14.3 ± 0.9 ^{A,a}	5.1 ± 0.4 ^{F,b}
7	11.5 ± 0.5 ^{B,a}	1.5 ± 0.3 ^{G,b}
14	8.4 ± 0.4 ^{C,a}	0.0 ± 0.0 ^{H,b}
21	5.8 ± 1.0 ^{D,a}	0.0 ± 0.0 ^{H,b}
28	4.5 ± 0.6 ^{D,a}	0.0 ± 0.0 ^{H,b}

The mean ± standard deviation values in the same column that do not have common superscript capital letters are significantly different ($p \leq 0.05$) by the Tukey test.

The mean ± standard deviation values in the same row that do not have common superscript lowercase letters are significantly different ($p \leq 0.05$) by the t-test.

*Products: KC (fermented soy product without fiber) e KF (fermented soy product with 3% soy fiber).

Kefir Culture Microorganism Counts in the Fermented Product

Lactococcus lactis, *Leuconostoc* spp and yeast counts in the fermented soy products with kefir and with addition of soy fibers (KF) and without soy fibers (KC) decreased significantly (Fig. 3) until day 28 during the storage at 4°C. The pH reduction and acidity increase in the fermented product during the storage inhibited the lactic acid

bacteria growth (McCann et al. 2011). According to Panesar et al. (2010), the hydrogen ion concentration of medium has the maximum influence on the microbes. Other factors, such as the presence of promoters, or growth inhibitors, presence of hydrogen peroxide and oxygen, concentrations of metabolite and nutrients, and buffering capacity of the environment can also affect the probiotic survival during the storage (Donkor et al. 2006). According to Brazilian legislation, *Lactococcus lactis* is a probiotic and must possess a minimum count of 10^8 CFU in the product (Brasil 2007), which corresponds to 6 log

CFU/g in a 100 g portion. Thus, the KF and KC products could be considered to be probiotic foods because a 7.9 log CFU/g minimum count was provided during the entire storage period. The control formulation (KC) from the seventh day of storage had lower lactic acid bacteria and *Lactococcus lactis* counts than those of the product with the addition of soy fiber (KF). The addition of fibers reinforced the growth and survival of bacteria during the storage, an observation, which was also reported by Sendra et al. (2008).

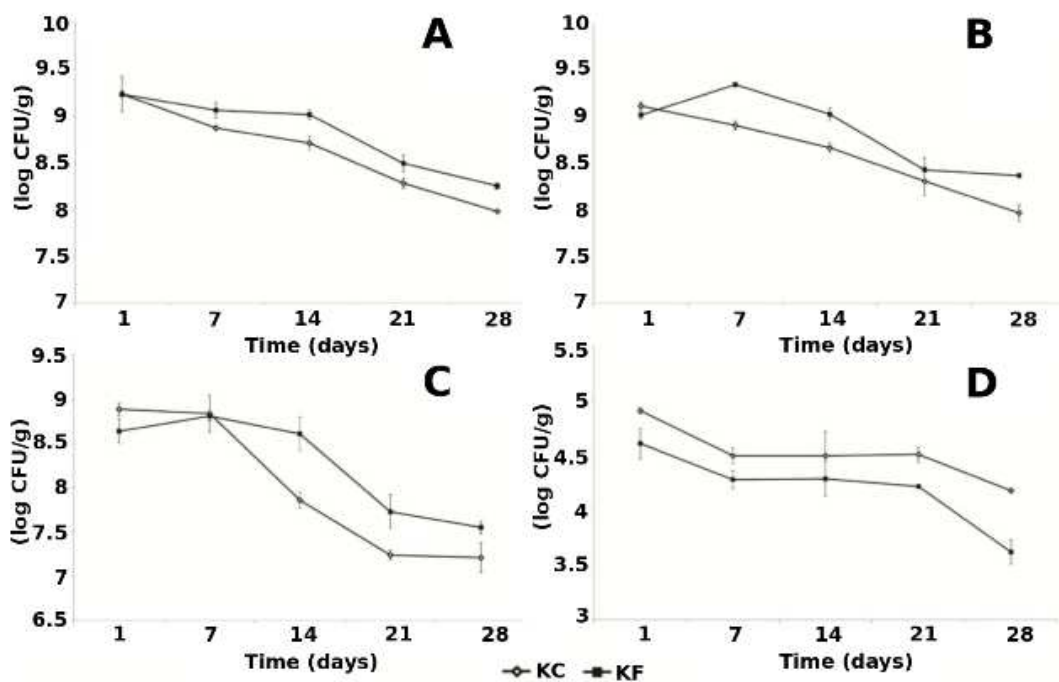


Figure 3 - Growth and survival of Lactic acid bacteria (A), *Lactococcus lactis* (B), *Leuconostoc* spp (C) and yeast (D) in soy products fermented with kefir and with addition of soy fibers (KF) and without soy fibers (KC) during storage at 4°C.

According to Saarela et al. (2006), the fibers may protect probiotic cultures under stress conditions such as lyophilization, drying and storage. Borderías et al. (2005) found that the fibers could alter the fermentation capacity of products. Svensson (1999) observed that the formulations with increased protein content could enhance the survival of probiotic microorganisms during the storage. This study also observed increase in the protein content of the soy fiber (Table 1) and high microbiological counts during the storage (Fig. 3).

CONCLUSIONS

The functional soy product with soy fiber addition and fermented with probiotic kefir culture presented better chemical composition and difference in color compared to the fermented product without fiber. Sensory analysis showed that the fermented product had good acceptance. The functional soy product produced higher firmness and reduced syneresis compared to the fermented soy product without fiber. There was a

small decrease in the of lactic acid bacteria counts during the storage period and, therefore, the developed product showed good microbiological stability. The functional soy product due to high *Lactococcus lactis* counts could be considered as a probiotic product during the entire storage period.

ACKNOWLEDGMENTS

This work was partially funded by CNPq/Fundação Araucária-Pronex. Thanks also go to CNPq for a graduate scholarship to TRB. EII is CNPq Research Fellows.

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Received: January 24, 2013;
Accepted: October 16, 2013.