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Kefir production from soymilk

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ABSTRACT

Soymilk kefir is a fermented product that is obtained by adding grains to soymilk.In this study, the effects of kefir grain inoculation rate (2%, 3% and 4%) and incubation temperature (22 and 25℃) on physicochemical, microbiological and sensory characteristics of soymilk kefir were investigated. pH, titratable acidity, content of dry matter, protein, ash, population of lactobacilli, lactococci and yeasts of all the samples were analyzed. Inoculation rate and incubation temperature affected microbial populations of soymilk kefir samples, while these factors did not have any effect on physicochemical properties. Sensory analysis revealed maximum acceptability levels in the sample produced using 4% kefir grain and incubated at 22℃.

Key words: kefir grains, lactobacilli, soymilk kefir, yeast.

INTRODUCTION

Kefir is a fermented milk product that has its origin in the Caucasian mountains, Tibet or Mongolia, many centuries ago [1]. The starter culture used to produce this beverage is an irregularly shaped, gelatinous white/ yellow grain [2]. 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 [3-5]. In the kefir grains, lactic acid bacteria and yeasts are embedded in a slimy polysaccharide matrix named kefiran, thought to be produced by the lactobacilli in the grain [3].

Soybean is an excellent source of low-cost protein and has been an important nutritional component in the typical diets of many countries for many generations. Many nutritional and medical investigations have revealed the great potential of soy foods for lowering blood cholesterol levels and the incidence of heart disease and cancer [6-7]. Soybean is a unique dietary source of the isoflavone genistein, which is a specific inhibitor of protein tyrosine kinases and also DNA topoisomerases and other critical enzymes involved in signal transduction. In vitro, genistein suppresses the growth of a wide range of cancer cells [6]. Soymilk, obtained by aqueous extraction from whole soybean, is a well-known food product that is growing in popularity in many areas of the world [8]. Furthermore, soymilk may proffer nutritional and health benefits, because it contains no cholesterol or lactose and only small quantities of saturated fatty acids. Soymilk kefir is obtained by adding kefir grains to soymilk [9].

The aim of this study was to evaluate the effect of inoculation rate of kefir grains and incubation temperature on microbiological, physicochemical and sensory attributes of soymilk kefir.

MATERIALS AND METHODS

Kefir grains and activation of grains

The kefir grains in this study were obtained from Iranian Research Organization for Science and Technology [10]. They were washed with distilled water and inoculated in low fat UHT cow's milk (1.5% fat). After incubation at 25 °C for 20 h, the grains were separated from the fermented milk by filtering them through a sieve. Then they were washed for later use and kept at 4 °C.

Soymilk

The UHT soymilk fortified by calcium and sucrose was used in this study. The soymilk composition was including 1 g Total fat, 40 mg Sodium, 3.5 g Carbohydrate, 40 mg Calcium, 2 g Sucrose and 2.5 g Protein in 100 g soymilk. The soymilk was supplied by Maxsoy Company (Karaj, Iran).

Production of milk kefir and soymilk kefir

Low fat UHT cow's milk (1.5% fat content) was inoculated with 5% (W/V) kefir grains. The sample was incubated at 25 °C for 24 h. At the end of fermentation, kefir grains were filtered through a sieve.

The samples of soymilk kefir were made by adding an inoculum consisting of 2%, 3% and 4% (W/V) kefir grains to sterilized soymilk. In order to increase the lactobacilli and lactococci populations, 5 ml the milk kefir sample was added to 100 ml the soymilk. All inoculated soymilk samples were incubated at 22 °C and 25 °C. At the end of fermentation, when pH value reached 4.5-4.6, kefir grains were filtered through a plastic sieve. The samples were taken into glassy bottles and were kept at 4 °C.

Physicochemical analysis

pH values of the samples were measured at room temperature using a pH meter (MA235, HANNA, Milan, Italy). Titratable acidity, dry matter (DM), protein and ash content were determined by AOAC method [11].

Microbiological analysis

Lactobacilli counts were performed on MRS agar (Difco) at 30 °C under anaerobic conditions for 3 days. Lactococci counts were carried out on M17 agar (Difco) at 30 °C under anaerobic conditions for 2 days. Cycloheximide (200 mg/ l) and Nistatin (50 µg/ ml) were added to the two above-mentioned media to inhibit yeast growth [12]. Yeast counts were performed on Malt extract agar (Difco) at 25 °C under aerobic conditions for 3 days [13].

Sensory evaluation

All samples were evaluated 1 day after production by 10 trained panelists. The sensory attributes were aroma, taste, appearance, texture, effervescence and sourness. The acceptability values were scored on 5 (very good), 4 (good), 3 (moderate), 2 (bad) and 1 (very bad).

Statistical analysis

Experiments were performed in triplicate. The data were analyzed using One-way Analysis of Variance (ANOVA) and Duncan test by SPSS 18.0.

RESULTS AND DISCUSSION

Physicochemical analysis

Table 1 presents the values of the main physicochemical parameters in the soymilk kefir samples made using the 2%, 3% and 4% added kefir grains.

Kefir grain inoculation rate and incubation temperature did not significantly affect the pH values as well as titratable acidity. Fermentation was stopped at pH 4.5-4.6 and the final acidity was 64-65 °D in all the samples. But the samples reached the mentioned pH (4.5-4.6) in different periods. The samples contained the highest added kefir grains (4%) and incubated at 25 °C reached pH 4.5-4.6 in the shortest fermentation time (16 h), while the samples inoculated with 2% kefir grains and incubated at 22 °C had the longest fermentation period (24 h). It shows that incubation at higher temperature with more inoculation rate of kefir grains lead to decline in fermentation time.

Fermentation resulted in decreasing the pH value, whereas the titratable acidity increased. The acid production in kefir depends on the growing of microorganisms and their ability for fermentation of the carbohydrates in milk and soymilk. The major carbohydrates present in soymilk are sucrose, raffinose and stachyose, whereas in milk it is lactose [14]. Liu and Lin found that adding 1% glucose or lactose to soymilk resulted in lactic acid concentrations similar to those of milk kefir, showing that the addition of these carbohydrates improves the ability of microorganisms in kefir grains to produce lactic acid in soymilk [9].

No significant differences in the dry matter contents of the samples were found ($p>0.05$). Kefir grain inoculation rate as well as incubation temperature did not have significant effect on the dry matter content of the samples. The dry matter content of the source soymilk was about 9.5% which was including 2% sucrose. It seems that microorganisms of kefir grains consume sucrose in the soymilk in amount of their need, which resulted in decreasing the dry matter content of the samples compare to soymilk. Donkor showed *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* reduce raffinose in soymilk significantly [15]. The levels of breakdown of raffinose and stachyose depend on the α-galactosidase activity of these microorganisms. Mital and Stainkraus reported that fermentation of soymilk with lactic cultures possessing α-galactosidase activity reduced raffinose and stachyose contents [16]. Irigoyen *et al.* recorded that dry matter values of the kefir made using the 1% and 5% inoculation rate were not significantly different from the dry matter content of milk. Accordingly fermentation did not affect the dry matter content of milk. Also they reported that kefir grain inoculation rate did not affect the dry matter in the samples [12]. Ottogalli *et al.* found that the dry matter in recently manufactured kefir varied according to the geographic origin of the granules, with variations in dry matter of between 9.4% and 11.1% [4].

There were no significant differences (p>0.05) in protein content of the samples (2.59-2.61%). The amount of protein in this study differed from results of Motaghi *et al.* for milk kefir. They reported the mean of protein content of milk kefir is 3.16 %. This difference was due to various protein contents of milk and soymilk [10]. Kefir grain inoculation rate significantly affected on protein content of the samples (p<0.05), while incubation temperature had no significant effect.

Table 1: Chemical properties of soymilk kefir samples (values are means ± SD)

Treatments		Chemical Properties				
T (°C)	Kefir grain%	pH	Titratable acidity(°D)	DM (%)	Protein (%)	Ash (%)
22	2	4.52±0.02[a]	65.7±2.38[a]	8.5±0.1[a]	2.61±0.00[a]	0.53±0.11[a]
22	3	4.53±0.01[a]	64.8±0.9[a]	8.46±0.11[a]	2.61±0.01[a]	0.53±0.11[a]
22	4	4.53±0.02[a]	64.8±0.9[a]	8.26±0.11[a]	2.60±0.01[a]	0.60±0.00[a]
25	2	4.54±0.02[a]	64.5±2.07[a]	8.46±0.15[a]	2.61±0.0[a]	0.60±0.00[a]
25	3	4.54±0.02[a]	64.5±2.07[a]	8.33±0.05[a]	2.59±0.01[a]	0.53±0.11[a]
25	4	4.54±0.02[a]	64.5±2.07[a]	8.3±0.1[a]	2.59±0.01[a]	0.53±0.11[a]

Values in the same column shown with similar letters are not significantly different (p>0.05).

With respect to Table 1, the ash contents of the samples were not significantly different (p>0.05). The amount of ash in all samples was in range of 0.53-0.6%. Kefir grain inoculation rate and incubation temperature did not significantly influence ash content of the samples. The amount of ash in this study differed from results reported by Assadi *et al.* for milk kefir. They reported the ash content of milk kefir in range of 0.75-0.84% [17]. This difference was due to various ash contents of milk and soymilk.

Microbiological analysis

Table 2 depicts the counts of the different microbial groups of the treatments. The lactobacilli population of the samples were different significantly (p<0.05). The findings showed that the samples produced using 3% kefir grains and incubated at 25 °C had the highest lactobacilli levels (9.64±0.03 log CFU/ml). In contrast, the lowest lactobacilli counts were observed in the samples made using 4% kefir grains and incubated at 22 °C (8.14±0.03 log CFU/ml). Kefir grain inoculation rate as well as incubation temperature significantly affected the lactobacilli counts (p<0.01). Liu and Lin showed that the lactic acid bacteria increased from 7.4±0.2 to 9.0±0.1 log CFU/ml in soymilk inoculated with 5% kefir grain, after incubation at 20 °C for 32 h. They found that lactic acid bacteria from kefir grains grew well in soymilk, even when no extra carbohydrate was added. It means that these organisms can utilize soymilk carbohydrates for growth. They also reported that these microorganisms grew more slowly in soymilk, even with the addition of carbohydrates [9].

The significant differences in lactococci levels of the samples were found (p<0.05). Kefir grain inoculation rate and incubation temperature had significant effects on the lactococci population (p<0.01). The samples made using 2% added kefir grains and incubated at 25 °C were observed the highest lactococci levels (9.49±0.07 log CFU/ml), while the lowest lactococci counts were found in the sample produced using 4% added kefir grains and incubated at 22 °C (8.01±0.17 log CFU/ml). There was no evidence of lactococci population in soymilk kefir by other researchers.

Our results were similar with another research for milk kefir that lactococci levels relate to kefir grain inoculation rate inversely [12]. Garrote *et al.* observed a rapid increase in acidity with a sharp drop in lactococci for 100 g/l inoculation rate, much higher than the level used in this study. This was probably due to the sensitivity of these bacterial strains to low pH levels [18].

The counts of yeasts in the samples were different significantly ($p<0.01$). The results demonstrated that the samples produced using 4% kefir grains and incubated at 25 °C had the highest yeasts counts (9.48±0.04 log CFU/ml). Whereas, the lowest yeasts levels were recorded in the samples made using 2% kefir grains and incubated at 22 °C (8.21±0.05 log CFU/ml). Kefir grain inoculation rate as well as incubation temperature had the significant effect on the yeasts levels ($p<0.01$). Liu and Lin recorded that the initial counts of yeasts in milk and soymilk (inoculated with 5% kefir grain, after incubation at 20 °C for 32 h) without added carbohydrate were 5.8±0.1 log CFU/ml and 4.7±0.2 log CFU/ ml, respectively. Soymilk kefir with 1% glucose had the highest yeast counts (6.4±0.1 log CFU/ml) at the end of fermentation, while soymilk without added carbohydrate had the lowest level. The yeast count did not differ significantly between milk and 1%-glucose soymilk kefir at the end of fermentation. They concluded that the addition of 1% glucose greatly enhances growth of both lactic acid bacteria and yeast in soymilk [9]. Yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide, which give the kefir drink its unique taste.

Table 2: Microbial counts of soymilk kefir samples (values are means ± SD

Treatments		Microbial populations (log CFU/ml)		
T (°C)	Kefir grain(%)	* lactobacilli	* lactococci	**Yeast
22	2	$8.5±0.21^{b}$	$8.44±0.01^{c}$	$8.21±0.05^{d}$
22	3	$8.22±0.03^{c}$	$8.24±0.02^{d}$	$8.22±0.15^{c}$
22	4	$8.14±0.03^{c}$	$8.01±0.17^{e}$	$8.89±0.6^{b}$
25	2	$9.59±0.07^{a}$	$9.49±0.07^{a}$	$9.02±0.14^{c}$
25	3	$9.64±0.03^{a}$	$9.48±0.08^{a}$	$9.31±0.19^{ab}$
25	4	$9.58±0.02^{a}$	$9.11±0.22^{b}$	$9.48±0.04^{a}$

****Values in the same column shown with different letters are significantly different (p<0.05).*
***Values in the same column shown with different letters are significantly different (p<0.01).*

Kefir grains usually contain lactose-fermenting yeasts such as *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Torula kefir*, as well as nonlactose-fermenting yeasts such as *Saccharomyces cerevisiae* and *Pichia fermentans* [19-20]. Because the type of fermentable carbohydrates present in soymilk and milk are different, the growth characteristics of microorganisms in kefir grains may change, resulting in dissimilarity of the microflora present in kefir grains. Lactic acid is extremely important for producing high quality fermented milk, and appropriate concentrations are needed to ensure proper flavor with minimum syneresis during storage [21-22].

Sensory analysis

Table 3 gives the sensory analysis results for the samples. The score of aroma, appearance, taste and effervescence of the samples were not different significantly (p>0.05), while acceptability of sourness of the samples had significant difference (p<0.01). Our findings showed that the highest score of sourness observed in the samples made using the 4% kefir grain and incubated at 22 °C, but the lowest value was related to the sample made using the 3% kefir grain and incubated at 22 °C. The score of texture in the samples was significantly different (p<0.01). The samples made using 4% kefir grains and incubated at 22 °C had the highest texture score, while the lowest values were remarked in the sample containing 3% kefir grains and incubated at 22 °C, similarly the sample made using 2% kefir grains and incubated at 25 °C. The incubation temperature did not significantly affect the sensory properties (p>0.05). The results for certain attributes showed that the highest overall acceptability was related to the sample made using the 4% kefir grain and incubated at 22 °C. Motaghi *et al.* studied sensory properties of milk kefir samples and concluded that milk kefir produced by 5% kefir grain and incubated at 25 °C for 24 h has the best organoleptic quality [10].

Table 3: Table 3: Sensory properties of soymilk kefir samples (values are means ± SD)

Treatments	Sensory attributes					
T (°C) kefir grain%	Effervescence	sourness	Texture	Taste	Appearance	Aroma
222	3.6 ± 1.03^{a}	3.5 ± 1.08^{ab}	4.1 ± 0.73^{ab}	3.2 ± 0.78^{a}	3.5 ± 0.48^{a}	$3.\ 4\pm0.84^{a}$
223	3.2 ± 0.96^{a}	3.0 ± 1.05^{b}	3.6 ± 0.51^{b}	3.2 ± 1.03^{a}	3.7 ± 1.26^{a}	3.6 ± 0.84^{a}
224	3.7 ± 0.48^{a}	4 ± 0.94^{a}	4.3 ± 0.48^{a}	3.9 ± 0.56^{a}	4.1 ± 0.31^{a}	3.9 ± 0.73^{a}
252	3.8 ± 0.84^{a}	3.6 ± 0.51^{ab}	3.6 ± 0.69^{b}	3.4 ± 0.51^{a}	4.1 ± 0.47^{a}	3.4 ± 0.51^{a}
253	4 ± 0.66^{a}	3.5 ± 0.51^{ab}	4.1 ± 0.48^{ab}	3.9 ± 0.56^{a}	3.8 ± 0.63^{a}	3.8 ± 0.78^{a}
254	$.6\pm0.91^{a}$	4 ± 0.66^{a}	4.1 ± 0.56^{ab}	3.7 ± 0.67^{a}	4 ± 0.31^{a}	3.9 ± 0.73^{a}

Values in the same column shown with different letters are significantly different (p<0.01).

CONCLUSION

Kefir grain inoculation rate and incubation temperatures did not significantly influence pH, titratable acidity, dry matter, protein and ash contents, although they had significant effect on microbial population. Lactobacilli counts increased in the samples incubated at higher temperature, whereas lactococci counts rose in the sample containing lower kefir grains and higher incubation temperature. Furthermore yeast population grew in the samples made using the higher kefir grains and incubated at higher temperature. Sensory analysis relieved the best acceptability level in the sample made using 4% kefir grains and incubated at 22 °C.

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