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Chemical, Microbiological and Sensory Properties of Soymilk Kefir during Cold Storage

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Abstract: Soymilk kefir is a fermented product that obtained by inoculating soymilk with kefir grain. The aim of this study was to evaluate the changes of chemical, microbiological and sensory properties of soymilk kefir during cold storage. Sterilized soymilk with 2% sucrose was inoculated with 2, 3 and 4 percent of kefir grain and incubated at 22 °C. Sensory evaluation (flavor, odour, appearance, texture, sourness and effervescence) was conducted by hedonic method. Soymilk kefir produced from 4 percent of kefir grain incubated at 22 °C was considered as the best sample with regard to sensory properties. This sample stored at 4°C for two weeks. pH, acidity, acetaldehyde, diacetyl, acetoin and ethanol contents, yeasts and bacterial counts of this sample were determined during cold storage. Acidity increased after 14 days of cold storage but decrease of pH was not significant. Levels of acetaldehyde, diacetyl, acetoin and ethanol decreased after two weeks of cold storage. Population of yeasts, lactococci and lactobacilli decreased during cold storage. The results of sensory evaluation showed that score of sourness increased but score of effervescence decreased after two weeks of cold storage. There were no significant changes in other sensory characteristics.

Key words: chemical characteristics, cold storage, microbial counts,sensory evaluation, soymilk kefir.

INTRODUCTION

Kefir is a fermented milk that obtained by inoculating milk with kefir grain. Kefir grains contain lactic acid bacteria, yeasts and sometimes acetic acid bacteria. The microflora is held together in a slimy polysaccharide matrix (Marshall *et al*., 1984). The flora of kefir grains produce lactic acid, carbon dioxide, ethanol, acetaldehyde, diacetyl and acetoin. These compounds have a cinsiderable influence on flavor and aroma of kefir (Beshkova *et al*., 2003).

Soymilk is made by aqueous extraction from whole soybeans. It has nutritional and health benefits. Soymilk lacks cholesterol and lactose and it contains small quantities of saturated fatty acids. Soymilk has a beany flavor and it can be improved by lactic acid fermentation (Murti *et al*., 1993; Granata and Morr, 1996). Therefore production of fermented soymilks such as soymilk kefir is important. Soymilk kefir is a fermented product that obtained from inoculation of kefir grain to soymilk (Liu and Lin, 2000).

The aim of this study was to evaluate the changes of chemical, microbiological and sensory properties of soymilk kefir during cold storage.

MATERIALS AND METHODS

Kefir Grains:

Iranian native kefir grains were used in this study. These grains including yeasts, lactobacilli and lactococci were isolated by Motaghi *et al*.(2007).

Production of Soymilk Kefir:

Sterilized soymilk with 2% sucrose was inoculated with 2, 3 and 4 percent of kefir grains and incubated at 22 °C. When the pH dropped to 4.5-4.6, kefir grains were filtered. All experiments were performed in triplicate.

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Chemical Analyses:

-pH and Acidity:

pH was measured by direct measurement with a pH-meter (120 Corling, USA) and titratable acidity was measured by AOAC method (2002).

-Acetaldehyde, Diacetyl, Acetoin and Ethanol:

Twenty grams of each sample was diluted with 30 mL distilled deionized water, HCl was added until the pH decreased to 2.5, vortexed for 1 h at 25 °C. Samples were left for 2 h to coagulate, centrifuged for 10 min (4000 rpm) to separate coagulants, collected upper solutions and defatted with normal hexane. They were passed through Sephadex column (G 75-15*1 cm) to remove proteins and other polymers (Dean, 1974). Again, samples were passed through XAD-2 column (10cm*1cm) to remove sugars and non polar compounds (Grabarczyk and Korolczuk, 2010). Passed solution was extracted with 15 ml diethyl ether two times and cooled at 0°C.

For the volatile component analysis, 8 mL was transferred into GC vials, injected into a 3 m Propac Q column (1.6 inch diameter) maintened at 100°C. The column temperature programmed at 150°C and temperature of FID detector was 250°C. Argon (flow of 20 mL/min) was used as the carrier gas (Determann, 1972).

Standard solutions of acetaldehyde, diacetyl, acetoin and ethanol were prepared with distilled deionized water. To remove error, standard addition was done for all samples and analysis was repeated. Qualification of the volatile components in the experimental samples was accomplished by comparison between retention time of samples and standard solutions.

Microbial Analyses:

Soymilk kefir samples for counts of lactobacilli and lactococci were plated on MRS agar and Azid agar (Merck Co.), respectively (Atlas, 2006). Both cultures were incubated for 3 days at 30 °C. Malt extract agar (Merck Co.) was used for counts of yeasts. Samples were incubated for 3 days at 25 °C (Atlas, 2006). Microbiology count data were expressed as log of colony forming units per ml of soymilk kefir (cfu/ml).

Sensory Evaluation:

Sensory evaluation of soykefir samples was carried out by a panel of 12 trained members. Organoleptic characteristics included smell, appearance, flavor, texture, sourness and effervescence. Hedonic method was used (Law, 1997). In this method numbers 5, 4, 3, 2 and 1 equal to very good, good, moderate, bad and very bad degree of acceptability, respectively.

Statistical Analysis:

Statistical analysis was performed by using the SPSS Software (SPSS 18). One-way Analysis of Variance (ANOVA) and Duncan Test were used for statistical comparison.

RESULTS AND DISCUSSION

Sensory properties of soymilk kefir samples including SK1, SK2 and SK3 were shown in Table 1. These samples obtained from inoculation of 2, 3 and 4 percent of kefir grain to soymilk, respectively. There was a significant difference ($p<0.01$) in texture between sample SK3 and other samples and this sample had the best texture. There was no significant difference in other sensory characteristics between samples.

Table 1: Sensory characteristics of soymilk kefir samples (values are means ±SD for n=3).

Treatment	Smell	Appearance	Flavor	Texture	Sourness	Effervescence
SK1	3.4±0.84	3.5±0.48	3.2±0.78	4.1±0.73	3.5±1.08	3.5±1.08
SK2	3.6±0.84	3.7±1.26	3.2±1.03	$3.6±0.51^{b}$	3±1.05	3±1.05
SK3	3.9±0.73	4.1±0.31	3.9±0.56	4.3±0.48	4±0.94	4±0.94

The higher quality sample (SK3) was stored at 4 °C for two weeks. pH, acidity, and flavor and aroma compounds were determined during cold storage (Tables 2 and 3).

Table 2: pH and acidity of sample SK3 during storage at 4°C (values are means ±SD for n=3).

Chemical properties	Fresh product	7 d	14 d
pH	4.54±0.02	4.53±0.02	4.5±0.03
Acidity (°D)	64.5±2.07	65.4±1.03	67.2±1.87

Acidity of this sample increased significantly ($p<0.01$) during cold storage. pH decreased but this decrease was not significant (Table 2).

Bakhshandeh *et al*. (2011) showed that pH of milk kefir decreased significantly ($p<0.05$) and acidity increased significantly ($p<0.05$) after two weeks of cold storage.

Table 3: Flavor and aroma compounds of sample SK3 during storage at 4°C (values are means ±SD for n=3).

Flavor and aroma compounds	Fresh product	7 d	14 d
Ethanol (ppm)	1020±5.97	1047±4.83	983±6.65
Acetaldehyde (ppm)	3.5±0.07	4.1±0.04	3.8±0.01
Diacetyl (ppm)	58±1.65	56±1.58	49±1.37
Acetoin (ppm)	15±0.90	15±1.37	12±1.68

Ethanol content of sample SK3 differed significantly ($p<0.01$) during cold storage (Table 3). Ethanol level increased on 7th day and decreased on 14th day.

Bakhshandeh *et al*. (2011) reported that ethanol level of milk kefir decreased after two weeks of cold storage.

Acetaldehyde content of sample SK3 differed significantly ($p<0.01$) during cold storage (Table 3). Acetaldehyde level increased on 7th day and decreased on 14th day. Diacetyl content decreased significantly ($p<0.01$) during cold storage (Table 3). Acetoin content differed significantly ($p<0.01$) during cold storage (Table 3). Acetoin level was costant on 7th day and decreased on 14th day.

Beshkova *et al*. (2003) reported that the highest amount of flavor compound in milk kefir produced during first day and decreased until 7th day of cold storage.

Bakhshandeh *et al*. (2011) demonstrated that acetaldehyde, diacetyl and acetoin levels in milk kefir decreased significantly ($p<0.05$) during cold storage.

The results of microbial counts of sample SK3 was indicated in Table 4.

Table 4: Microbial population (log cfu/ml) of sample SK3 during storage at 4°C (values are means ±SD for n=3).

Microorganism	Fresh product	7 d	14 d
Yeasts	9.23±0.07	9.11±0.17	8.72±0.24
Lactococci	8±0.02	7.9±0.01	7±0.18
Lactobacilli	6.64±0.02	7.53±0.03	8.5±0.03

Population of yeasts differed significantly ($p<0.01$). Yeasts counts in fresh product were 9.23 log cfu/ml. They were similar to fresh product on 7 day and decreased one logarithmic cycle on 14 day.

Population of yeasts in this study was higher than that in another study conducted by Liu and Lin (2000).

Population of lactococci differed significantly ($p<0.01$). Lactococci counts in fresh product were 8 log cfu/ml. They were nearly the same on 7 day but decreased one logarithmic cycle on 14 day.

Population of lactococci in fresh soymilk kefir were similar to results of Irigoyen *et al*. (2005) for milk kefir.

Population of lactobacilli decreased significantly ($p<0.01$) during cold storage. Lactobacilli counts decreased one and two logarithmic cycles on 7 day and 14 day, respectively.

Irigoyen *et al*. (2005) reported similar results for milk kefir. Population of lactobacilli was approximately similar to findings of Liu and Lin (2000) for soymilk kefir without adding of carbohydrate.

The results of sensory evaluation of sample SK3 was shown in Table 5. The score of sourness increased significantly ($p<0.01$) and score of effervescence decreased significantly ($p<0.05$) after two weeks of cold storage.

Table 5: Sensory characteristics of sample SK3 during storage at 4°C (values are means ±SD for n=3).

Sensory characteristics	Fresh product	7 d	14 d
Smell	4±0.56	4.2±0.63	4.5±0.52
Appearance	4.1±0.31	4.1±0.56	3.9±0.56
Flavor	3.9±0.56	4.6±0.51	4±0.47
Texture	4.3±0.48	4.7±0.48	4.4±0.69
Sourness	3.9±0.73	4±0.66	4.6±0.63
Effervescence	3.7±0.42	3.6±1.07	3.3±0.67

Kilic *et al*. (1999) found that the score of sensory characteristics of milk kefir decreased significantly after 3 days.

Bakhshandeh *et al*. (2011) reported that the score of flavor of milk kefir decreased significantly ($p<0.05$) and score of sourness increased significantly ($p<0.05$) after two weeks of cold storage.

Conclusion:

Soykefir produced from 4 percent of kefir grain incubated at 22 °C is considered as the best sample with regard to sensory quality. Levels of flavor and aroma compounds in this sample decrease after two weeks of cold storage. Yeasts, lactococci and lactobacilli counts decrease during cold storage and it corresponds with decreasing of flavor and aroma compounds amounts in this sample. The score of sourness increases after two weeks of cold storage and it corresponds with increasing of acidity in this sample. The score of effervescence decreases after 14 days of cold storage and it corresponds with decreasing of yeasts counts in this sample.

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