Evaluation of Flavor and Aroma Compounds Amounts in Kefir from Soymilk

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Abstract: Soykefir is a fermented product that obtained from inoculation of kefir grain to soymilk. The aim of this study was to evaluate the effect of kefir grain inoculation rate and incubation temperature on production of flavor and aroma compounds in kefir from soymilk. Sterilized soymilk with 2% sucrose was inoculated with 2, 3 and 4 percent of kefir grain and incubated at 22°C and 25°C. The flavor and aroma compounds (acetaldehyde, diacetyl, acetoin and ethanol) were measured by GC. Moreover, sensory evaluation (flavor, odour, appearance, texture, sourness and effervescence) was conducted by ranking method. The results indicated that amount of flavor and aroma compounds in soykefir increased with raising of kefir grain inoculation rate and incubation temperature. Soykefir sample 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. Levels of acetaldehyde, diacetyl, acetoin and ethanol decreased after two weeks of cold storage.

Key words: Flavor and aroma compounds · Incubation temperature · Kefir grain · Soykefir

INTRODUCTION

Soymilk is made by aqueous extraction from whole soybeans. It contains no cholesterol. Moreover, soymilk lacks lactose and it contains low quantities of saturated fatty acids [1, 2].

Soymilk has high quantities of protein, Iron and niacin in comparison with cow’s milk. Moreover, it is a suitable alternative for individuals with lactose intolerance. Soymilk as well as cow’s milk is a suitable medium for growth of microorganisms because of its neutral pH. The most important problem in development of soymilk consumption is decrease of its beany flavor and preparation of a product with a mild beany flavor like cow’s milk [3]. Beany flavor of soymilk can be improved by lactic acid fermentation, so production of fermented soymilks such as kefir is important.

Kefir is a fermented milk that obtained by inoculating milk with kefir grain. Microflora of kefir grain consists of lactic acid bacteria and yeasts. Microorganisms of kefir grains produce lactic acid, carbon dioxide, ethanol, acetaldehyde, diacetyl and acetoin. These compounds have important roles in flavor and aroma [4].

Soykefir is a fermented product that obtained from inoculation of kefir grain to soymilk. The aim of this study was to evaluate the effect of kefir grain inoculation rate and incubation temperature on flavor and aroma compounds amounts in kefir from soymilk.

MATERIALS AND METHODS

Kefir Grains: Iranian native kefir grains were used in this study. These grains were isolated by Motaghi et al. [5].

Production of Kefir from Soymilk: Sterilized soymilk with 2% sucrose was inoculated with 2, 3 and 4 percent of kefir grains and incubated at 22°C and 25°C. All treatments were shown in Table 1.

When the pH dropped to 4.5-4.6, kefir grains were filtered. All experiments were performed in triplicate.

Determination of pH and Acidity: pH was measured by direct measurement with a pH-meter (120 Corling, USA) and Titratable acidity was measured by AOAC method [6].
Determination of Flavor and Aroma Compounds:
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 [7]. Again, samples were passed through XAD-2 column (10cm*1cm) to remove sugars and non polar compounds [8]. Passed solution was extracted with 15 ml diethyl ether two times and cooled at 0°C.

Flavor and aroma compounds were measured by GC (Varian 60). For the volatile component analysis, 8 mL was transferred into GC vials, injected into a 3 m Propac Q column (CBP5-F25,1.6 inch diameter, Shimadzo, Japan) maintained 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 [9].

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.

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 [10]. 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

pH and acidity of soykefir samples were shown in Table 2. There was no significant difference in pH and acidity of samples. Fermentation time in tested samples was different. It was 24 h, 23 h, 18 h, 22 h, 21 h and 16 h for P1T1, P2T1, P3T1, P1T2, P2T2 and P3T2, respectively. Lactic acid is very important for production of high quality fermented milk. Previous studies showed that yogurt culture cannot produce adequate lactic acid in soymilk [11, 12]. Liu and Lin found that addition of 1% glucose or lactose to soymilk resulted in lactic acid concentrations similar to those of milk kefir, indicating that the addition of these carbohydrates improves the ability of microorganisms in kefir grains to produce lactic acid in soymilk [13].

The amounts of flavor and aroma compounds in tested samples were shown in Table 3.

There was a significant difference (p<0.01) in ethanol contents of samples. Sample P2T2 had the most amount of ethanol. The least amount of ethanol was related to sample P1T1. Some factors such as fermentation time, fermentation temperature and starter culture can affect on amount of ethanol in kefir [14]. Bakhshandeh et al. found that amount of ethanol in milk kefir produced by commercial starter culture increased by raising of incubation temperature [15]. Liu and Lin demonstrated that the ethanol level for soymilk kefir was lower than for milk kefir. Moreover, they found that addition of 1% glucose or lactose to soymilk increases the amount of ethanol [13].

There was a significant difference (p<0.01) in acetaldehyde content of samples. Sample P3T2 had the most amount of acetaldehyde. The least amount of acetaldehyde was related to sample P1T1. Liu et al. investigated the amount of acetaldehyde in milk kefir and soymilk kefir. Acetaldehyde content of their samples was higher compared to our samples. Moreover they found that acetaldehyde level for soymilk kefir was higher than for milk kefir [16].

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Table 1: Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Inoculation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1T1</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>P2T1</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>P3T1</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>P1T2</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>P2T2</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>P3T2</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2: pH and acidity of soykefir samples (values are means ±SD for n=3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Acidity (°D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1T1</td>
<td>4.52±0.02</td>
<td>65.7±2.38</td>
</tr>
<tr>
<td>P2T1</td>
<td>4.53±0.01</td>
<td>64.8±0.9</td>
</tr>
<tr>
<td>P3T1</td>
<td>4.53±0.02</td>
<td>64.5±2.38</td>
</tr>
<tr>
<td>P1T2</td>
<td>4.54±0.02</td>
<td>64.2±2.59</td>
</tr>
<tr>
<td>P2T2</td>
<td>4.54±0.02</td>
<td>63.9±2.26</td>
</tr>
<tr>
<td>P3T2</td>
<td>4.54±0.02</td>
<td>63.9±2.26</td>
</tr>
<tr>
<td>Treatment</td>
<td>Ethanol (ppm)</td>
<td>Acetaldehyde (ppm)</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>P1T1</td>
<td>720±5.28</td>
<td>2.8±0.2</td>
</tr>
<tr>
<td>P2T1</td>
<td>954±4.37</td>
<td>3.3±0.08</td>
</tr>
<tr>
<td>P3T1</td>
<td>1020±5.97</td>
<td>3.5±0.07</td>
</tr>
<tr>
<td>P1T2</td>
<td>995±5.7</td>
<td>3.7±0.05</td>
</tr>
<tr>
<td>P2T2</td>
<td>1105±6.98</td>
<td>3.8±0.01</td>
</tr>
<tr>
<td>P3T2</td>
<td>1089±4.3</td>
<td>4.2±0.02</td>
</tr>
</tbody>
</table>

There was no significant difference in diacetyl content between samples P2T1 and P2T2. However, these two samples had significant difference (p<0.01) compared to other samples. Sample P3T1 had the most amount of diacetyl. The least amount of diacetyl was related to sample P1T1. Liu et al. evaluated the diacetyl level in soymilk kefir. Our findings are similar to results reported by Liu et al. Moreover, they found that diacetyl level for soymilk kefir was lower than for milk kefir [16].

There was a significant difference (p<0.01) in acetoacetin content among samples P1T1, P2T1, P3T1 and P3T2. However, there was no significant difference in acetoacetin content among samples P3T1, P2T1, P2T2 and P2T2.

Granata and Morr reported that acetaldehyde, diacetyl, acetoacetin and ethanol are the most important flavor and aroma compounds in fermented soymilk. The acetaldehyde level for soymilk kefir was slightly higher than for milk kefir, but its diacetyl, acetoacetin and ethanol concentrations were lower [2].

Sensory properties of the samples were shown in Table 4. There was a significant difference (p<0.01) in texture between sample P3T1 and other samples and this sample had the best texture. There was no significant difference in other sensory characteristics between samples.

The higher quality sample (P3T1) was stored at 4°C for two weeks. Acidity, pH and flavor and aroma compounds were determined during cold storage (Tables 5 and 6).

Acidity of this sample increased significantly (p<0.01) during cold storage. pH decreased but this decrease was not significant (Table 5). Bakhshandeh et al. showed that pH of milk kefir decreased significantly (p<0.05) and acidity increased significantly (p<0.05) after two weeks of cold storage [15].

Ethanol content of sample P3T1 differed significantly (p<0.01) during cold storage (Table 6). Ethanol level increased on 7th day and decreased on 14th day. Bakhshandeh et al. reported that ethanol level of milk kefir decreased after two weeks of cold storage [15].

Acetaldehyde content of sample P3T1 differed significantly (p<0.01) during cold storage (Table 6). Acetaldehyde level increased on 7th day and decreased on 14th day. Diacetyl content decreased significantly (p<0.01) during cold storage (Table 6). Acetoin content differed significantly (p<0.01) during cold storage (Table 6).
Acetoin level was constant on 7th day and decreased on 14th day. Beshkova et al. reported that the highest amount of flavor compound in milk kefir produced during first day and decreased until 7th day of cold storage [15]. Bakhshandeh et al. demonstrated that acetaldehyde, diacetyl and acetoin levels in milk kefir decreased significantly (p<0.05) during cold storage [15].

CONCLUSION

Amounts of flavor and aroma compounds in soymilk kefir are affected by kefir grain inoculation rate and incubation temperature. Production of acetaldehyde, diacetyl, acetoin and ethanol increase with raising of kefir grain inoculation rate and incubation temperature. Soykefir produced from 4 percent of kefir grain incubated at 22°C was 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.

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REFERENCES