LOW ENERGY KOMBUCHA FERMENTED MILK-BASED BEVERAGES

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This paper investigates manufacturing of fermented beverages from two types of milk (1 % w/w and 2.2 % w/w fat) by applying of Kombucha, which contains several yeasts and bacterial strains. The starter was the inoculum produced from previous Kombucha fermentation. The applied starter concentrations were: 10 % v/v, 15 % v/v and 20 % v/v. Also, the traditional yoghurt starter was used to produce the control samples. All fermentations were performed at 42°C and the changes in the pH were monitored. The fermentation process was about three times faster in the control yoghurt than in the Kombucha samples. Influence of Kombucha inoculum concentration on the rate of fermentation appeared not to be significant. All fermentations were stopped when the pH reached 4.4. After the production, the quality of the fermented milk beverages with Kombucha was determined and compared with the quality of the control yoghurt samples. It was concluded that the difference in fat contents in milks affects the difference in quantities of other components in the fermented milk beverages with Kombucha. Sensory characteristics of the beverages manufactured from the partially skimmed milk are much better than those of the fermented beverages produced from the low fat milk.

KEYWORDS: Kombucha, fermentation, milk, new products, low fat

INTRODUCTION

The role of milk and dairy products in satisfying the recommended daily intake of nutritionally important components is very well known. Especially interesting are fermented products from milk with low quantity of fat. A great number of nutritionally valuable beverages can be obtained by applying of starters with prevailing Streptococcus and Lactobacillus cultures. On the other hand, a symbiotic association of yeasts and bacteria, known as Kombucha, has been applied for fermentation of sweetened black and green tea for centuries (1, 2). As a result of its metabolic activity, a refreshing sour

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beverage appears. Beside monosaccharides and acetic acid, it contains a number of useful compounds (3-9) whose positive impact on human health has been, and still is being investigated by many authors (10-12).

The traditional carbon source for Kombucha fermentation is sucrose. A number of investigations concerning the main pathways of sucrose conversion into numerous compounds have been reported (1, 2, 13, 14). However, the application of lactose, glucose or fructose is possible. This was investigated by Reiss, in 1994, who has found that Kombucha on sugars other than sucrose produces beverages slightly different in flavour but significantly different in the contents of ethanol and lactic acid (13). For example, fermentation on lactose gave insignificant quantities of ethanol in comparison to the fermentation on sucrose. Beside Reiss’ results, there is very little evidence of investigations related to the influence of carbon source on Kombucha fermentation. In 1997, Petrović et al. investigated the biosyntheses of vitamin C on inulin-polysaccharide obtained from the tubers of the Jerusalem artichoke (15). In 2004, Malbaša reviewed some attempts in applying atypical nutrients, such as Coca-Cola, red wine, white wine, vinegar, extract of Echinacea, Mentha, etc (16). Atypical are also some agricultural and industrial by-products, including molasses from sugar beet processing (17). In 2003, Belloso-Morales and Hernández-Sánchez published the paper on manufacturing the Kombucha beverage from cheese whey (18). Our recent work (19) has suggested production of milk-based beverages with several Kombucha starters grown-up on sweetened black and green tea and topinambur. Although the investigations on fermentation with atypical starters are not extensive, various milk-based beverages have been manufactured as home-made products. As starter cultures, a variety of combinations of native microorganisms (mixtures of bacteria and sometimes yeasts) were applied.

This work considers manufacturing of milk-based beverages from two milk samples with the different fat contents by applying of Kombucha starter grown-up on the sweetened black tea, aiming to determine the level of the differences among chemical compositions as well as sensory characteristics of these beverages.

MATERIALS AND METHODS

Milks
Two kinds of homogenized and pasteurized cow’s milk (low fat and medium fat) were taken from AD Novosadska mlekar, Novi Sad, Serbia, for the production of all milk-based beverages. The first kind of milk contained 1 % w/w fat, while the second contained 2.2 % w/w fat. Physical, chemical and microbiological characteristics of both milk samples were entirely in accordance with the actual standards.

Cultures
Yoghurt was obtained by applying of standard culture B3, produced by Chr. Hansen, Denmark, containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Local Kombucha culture was applied for production of two series of milk-based beverages. Previous investigations (20) have shown that the applied fungus contained at least five yeast strains (*Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, Torulopsis sp. and *Zygosaccharomyces* sp.) and two bacterial strains of
the genera *Acetobacter*. Preparation of Kombucha, before its application as a starter culture, was performed by the following procedure; 1 L of boiled tap water with 70 g of sucrose and 1.5 g of black tea leaves (Indian tea, "Vitamin", Horgoš, Serbia) were heated at 100°C for 5 min. The leaves were removed by filtration and the obtained solution was cooled to room temperature. After cooling, the solution was inoculated with 10 % v/v fermentation broth from previous Kombucha fermentation, covered with cheesecloth and incubated (at constant temperature of 29.5±1°C) for 6 days. The system thus (whose characteristics were as follows: pH 3.21, lactic acid 0.04 % w/w and total acids 0.29 % w/w) was used as a starter for producing Kombucha containing milk-based beverages. The counts of viable yeasts and bacterial cells in the inoculums were 6.62 log cfu mL⁻¹, which corresponds to the same or higher exponential rate in similar fermentation media (21).

*Milk-based beverages*

In accordance with the plan of experiments (Table 1), two groups of fermented products were manufactured. The first group consisted of the traditional yoghurt and three fermented beverages, all manufactured from the low fat milk, inoculated at 42°C, with yoghurt starter (3 % w/w) and Kombucha starters (10 % v/v, 15 % v/v and 20 %v/v). The second series was obtained from the medium fat milk, according to the same experimental plan. The fermentations lasted 130–455 minutes to reach pH 4.4. Afterwards, the gels were cooled to 8°C, homogenized by mixing and packed in plastic containers. Each fermentation was repeated three times.

**Table 1.** Plan of experiments – the fermented beverages

<table>
<thead>
<tr>
<th>Group of products from milk with 1 % w/w fat</th>
<th>Group of products from milk with 2.2 % w/w fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt Fermented beverages</td>
<td>Yoghurt Fermented beverages</td>
</tr>
<tr>
<td>B3 starter Kombucha starter</td>
<td>B3 starter Kombucha starter</td>
</tr>
<tr>
<td>3 % w/w 10% v/v 15% v/v 20% v/v</td>
<td>3 % w/w 10% v/v 15% v/v 20% v/v</td>
</tr>
<tr>
<td>M1Y M1K10 M1K15 M1K20</td>
<td>M2Y M2K10 M2K15 M2K20</td>
</tr>
</tbody>
</table>

*Methods of analysis*

Chemical analyses of milk, yoghurt and fermented beverages were performed by standard methods (22). The following quantities were determined: dry matter content after drying at 105°C; fat according to Gerber and Van Gulik; total nitrogen according to Kjeldahl; lactose in milk by titrimetry; ash after mineralization at 550°C; total acids by the volumetric method with the standard solution of sodium hydroxide and phenolphthalein as an indicator, and L-lactic acid according to Steinholt and Calbert (23). Lactose and energy value in the fermented beverages were calculated.

The pH values were measured on an electric pH-meter (Iskra, MA 5713, Kranj, Slovenia). Sensory characterization of both categories of yoghurt and remaining fermented beverages was performed by qualified evaluators, as well as by common consumers, who assessed each particular element of quality as follows: appearance (0-1), colour (0-1), consistency (0-4), odour (0-2) and taste (0-12).
RESULTS AND DISCUSSION

Development of fermentation

The development of all fermentation processes was followed by measuring pH values in time and are presented in Fig. 1. The diagram in Fig. 1A shows the fermentation curves for two kinds of milk inoculated either with traditional yoghurt starter or with 10 % v/v Kombucha starter. It can be noticed that an almost identical metabolic activity showed the yoghurt starter in both milk samples. The fermentation rates in both yoghurts were much higher than the rates in Kombucha systems. When comparing metabolic activity of 10 % v/v Kombucha starter in two milk samples, significantly faster fermentation was found in low fat milk, particularly during the first 90 min of the process. After this first phase, both fermentations stagnated till approximately 130 min. Finally, a decrease of pH got faster in milk with higher content of fat, resulting in a shorter fermentation process. Different shapes of fermentation curves implicate the differences in metabolic activities of the applied starters. Precise explanation requires further investigations.

Similar conclusions can be drawn after the analysis of the remaining two diagrams (in Fig. 1B and Fig. 1C). Among all three diagrams in Fig. 1, there is a great level of similarity. Having in mind that the presented systems differed only in Kombucha starter concentrations (10 % v/v, 15 % v/v or 20 % v/v), it can be concluded that inoculum concentration did not affect significantly the fermentation rate. These findings are in agreement with our earlier results on sucrose fermentation (24). Namely, when investigating the effect of time, temperature and inoculum concentration on the rate of Kombucha governed fermentation, the following order of significances was found: the most significant was the process duration, less significant was temperature, while the least significant was inoculum concentration. It should be emphasized that the curves M1Y and M2Y in Fig.1A-C are related to the same fermentations of low fat and medium fat milk with the yoghurt culture.

Comparison of the Kombucha fermentations on different systems shows that the process is very fast on milk (6-7 h), as obtained in these investigations; it is slower on both the sweet whey and acidic whey (96 h), as found by Bellosi-Morales and Hernández-Sánchez, (18). The slowest fermentation is typical of black tea sweetened with 70 g/L sucrose (5-7 days), as found by many authors (11, 12, 16, 17, 21). If the required quantity of the fermented product increases, the fermentation time increases too (9-10 h), which is important to have in mind when scaling-up the fermentation process (21).

Together with measuring pH changes during fermentation, the pH values of all products were measured after 5-day storage at 4°C. It was found that Kombucha became rather inactive, contrary to the yoghurt culture that continued very slow fermentation. So, the pH in the Kombucha containing beverages remained unchanged (4.4), while the yoghurt samples became slightly sourer, reaching pH 4.37. This is important to know when thinking about commercial production of Kombucha beverage.
Figure 1. pH changes during fermentation process in both milk samples
Labels of all samples are given in Table 1

Chemical composition

Chemical compositions of all fermented beverages are presented in Table 2. Analysis of compositions of low fat products: M1Y, M1K10, M1K15 and M1K20 (see labels in Table 1) showed that Kombucha beverages had lower level of dry matter (9.1% w/w in average) than yoghurt (9.55% w/w). Also, fat content of all beverages remained unchanged (1% w/w) regardless of the applied starter. Protein content varied from 3.0 to 3.5 % w/w in Kombucha samples but reached 3.7 % w/w in yoghurt. Ash content in low-fat Kombucha systems (0.64 % w/w in average) is smaller than ash quantity in yoghurt (0.72 % w/w). The level of total acids in the same Kombucha systems was two times lower (0.5 % w/w in average) than the level in yoghurt (1 % w/w), while the calculated contents of lactose in Kombucha beverages and in yoghurt were exactly the same (4.13 % w/w). As far as energy values of products are concerned, almost negligible difference between Kombucha beverages (171 kJ/100g in average) and yoghurt (178 kJ/100g) was found.

Chemical analysis of the medium-fat products: M2Y, M2K10, M2K15 and M2K20 (see labels in Table 1) showed that Kombucha beverages contained lower level of dry matter (9.6 % w/w in average) than yoghurt (10.5 % w/w). Fat content were: 1.8 – 2.2 %
w/w for Kombucha beverages and 2.2 % w/w for yoghurt. Protein contents varied from 3.0 to 3.6 % w/w in Kombucha samples and reached 3.6 % w/w in yoghurt. Ash content in Kombucha metabolites (0.62 % w/w in average) was lower than ash quantity in yoghurt (0.67 % w/w). The level of total acids was lower in Kombucha beverages (0.59 % w/w in average) compared to yoghurt (0.8 % w/w). Also, the calculated content of lactose in Kombucha beverages (3.75 % w/w in average) was smaller than lactose in yoghurt (4.03 % w/w). As for energy values of the products, the traditional yoghurt releases 221 kJ/100g, while Kombucha beverages have lower energy values (185 - 215 kJ/100g).

Table 2. Chemical composition of the fermented beverages

<table>
<thead>
<tr>
<th>Product</th>
<th>C o m p o n e n t (% w/w)</th>
<th>EVb (kJ/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matt. a</td>
<td>Fat a</td>
</tr>
<tr>
<td>M1Y</td>
<td>(9.55±0.17)c</td>
<td>1±0.021</td>
</tr>
<tr>
<td>M1K10</td>
<td>9.20±0.14</td>
<td>1±0.025</td>
</tr>
<tr>
<td>M1K15</td>
<td>9.10±0.13</td>
<td>1±0.019</td>
</tr>
<tr>
<td>M1K20</td>
<td>8.90±0.09</td>
<td>1±0.019</td>
</tr>
<tr>
<td>M2Y</td>
<td>10.5±0.16</td>
<td>2.2±0.041</td>
</tr>
<tr>
<td>M2K10</td>
<td>10.1±0.13</td>
<td>2.2±0.042</td>
</tr>
<tr>
<td>M2K15</td>
<td>9.86±0.13</td>
<td>1.9±0.036</td>
</tr>
<tr>
<td>M2K20</td>
<td>8.94±0.09</td>
<td>1.8±0.032</td>
</tr>
</tbody>
</table>

a) measured values  
b) calculated values  
c) mean value ± standard deviation

In general, Kombucha containing beverages manufactured from one type of milk have lower content of dry matter, proteins, ash, lactose and total acids, as well as energy value, than yoghurt from the same milk. Comparison of the two groups of milk-based beverages, obtained from two types of milk, showed that low-fat products were richer in proteins and ash but poorer in dry matter. Low fat beverages differed from the medium fat beverages significantly, regarding not only fat content but quantities of all measured components, including energy value. Also, a significant difference was found between the yoghurt and Kombucha samples from the same milk.

There is very little evidence on the production of fermented beverages, as a result of metabolic activities of Kombucha starter on various substrates. When sweet (fresh and reconstituted) and sour whey were exposed to the fermentation (18), as the main metabolic products were ethanol, lactic and acetic acid. The wheys used had protein concentrations of 0.89% w/w, 1.67% w/w and 0.94% w/w, while the supernatant fluids had concentrations 0.69% w/w, 1.11% w/w and 0.67% w/w. The content of total acids was 0.07 mol/L (at the final pH of 3.3), while the lactose concentration dropped to less than 12 g/L. The final ethanol content was low (5 g/L) in all fermented whey samples. A comparison of milk-based beverages and whey-based beverages has shown that the former product is superior due to a higher content of proteins and lactose and similar content of total acids.
Lactic acid is one of the metabolites associated with nutritive and functional characteristics of fermented beverages. In this investigation, lactic acid content was determined in yoghurt and three Kombucha-based beverages, produced from the two kinds of milk. The obtained values are presented in Fig. 2. The greatest content of lactic acid in Kombucha beverages (0.5 % w/w) was found in the systems M1K10 and M2K10. This value is significantly lower than the content of lactic acid found in yoghurts (0.68 % w/w and 0.67 % w/w). The other Kombucha beverages contained even less quantity of lactic acid, which means that higher concentration of Kombucha starter caused lower production of lactic acid. Also, fat content of the applied milk did not affect level of lactic acid in the obtained product.

An analysis of Kombucha-fermented sweet whey beverages (18) showed that the lactic acid concentration reached 0.34% w/w, which is smaller than the lactic acid concentration in milk-based beverages. Also, at the end of acid whey fermentation, approximately 0.6% w/w lactic acid was found.

**Sensory characteristics**

A certain difference between sensory characteristics of yoghurt, on one the hand, and remaining investigated beverages on the other, might be expected. Therefore, sensory characterization of yoghurt and the Kombucha beverages was performed and the results are given in Fig. 3. As obvious, both yoghurts got maximal points for all assessed characteristics. Comparison of sensory characteristics between two groups of Kombucha beverages showed that M1K10 was the best in low fat category, while M2K15 was the best in higher-fat category. Among all Kombucha beverages, absolutely the best was the product M2K15. Negative observations associated with the Kombucha beverages were as follows: whey separation (samples M1K15, M1K20), foam formation (sample M2K10), grains formation (samples M1K15, M1K20), sharp odour (samples M1K10, M1K15 and M1K20), bitter taste (sample M1K10), sweet taste (sample M1K15), watery taste (sample M1K20), etc. Analysis of products consistency showed that an increase of inoculum volume (from 10 % v/v to 20 % v/v) decreased metabolites density. Fortunately, this did not cause whey separation, but did cause the appearance of some kind of inhomogeneity.
Figure 3. Sensory characteristics of beverages after production (A) and after storage (B)

The fermented whey products were tested by the authors (18) and described as strongly sour, salty and non-sparkling. Their sensorial characteristics could be improved if the whey was previously demineralized and a fruit flavour added at the end of process.

CONCLUDING REMARKS

These investigations have proven that fat content in milk significantly affects chemical and sensory characteristics of the product from this milk, which was obtained after milk inoculation with Kombucha culture. According to the results, Kombucha containing products from milk with 1% w/w fat are richer in proteins, mineral components (ash) and lactose, but poorer in dry matter than products from milk with 2.2 % w/w fat content. Sensory characteristics of low fat category products are not as good as the characteristics of higher fat category products except the beverage M1K10. This particular product, manufactured from milk with 1% w/w fat inoculated with 10% v/v Kombuha starter, can be regarded as the best, especially having in mind all advantages associated with the low fat products.

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