Physicochemical and sensory characteristics of sheep kefir during storage

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Abstract. The aim of the study was physicochemical and sensory characterization of kefir manufactured from sheep milk following its production and storage at refrigerated conditions. Sheep milk inoculated with different starter cultures was incubated at the temperature of 23°C (culture DA) and 26°C (culture DC) for the period of 16-18 h until it reached pH of 4.6. The obtained kefir was assessed on the basis of: the extent of its acidification, the content of taste and smell substances and the results of sensory evaluation. The initial titratable acidity of the kefir manufactured with the assistance of the DA culture reached 48.2°SH and was by 14% higher than that of the kefir manufactured with the DC culture. After 21 days of storage, the titrable acidity of the kefir was by 6.5°SH higher in comparison with the values obtained directly after its manufacture. With the passage of storage time, the amount of free fatty acids (FFA) in the examined kefir samples increased significantly. Higher quantities of FFA were found in the kefir with the DA culture than with the DC culture. Kefir obtained with the assistance of the DA culture contained less acetaldehyde and diacetyl directly after manufacture. The quantities of these compounds underwent significant changes during storage. Kefirs manufactured using the DC cultures were more desirable from the sensory point of view than those manufactured using the DA culture. Irrespective of the applied starter culture, the highest overall acceptability scores were awarded to the kefir on the 7th day of storage.

Key words: sheep, kefir, physicochemical parameters, sensory analysis, storage

Introduction

The tradition of kefir production is one of the oldest methods of milk processing in the world. Kefir is a fermented milk product that has its origin in the Caucasian mountains, Tibet or Mongolia, many centuries ago [Duitschaever et al., 1987]. In the countries kefir until now has been produced primarily from sheep milk, whereas in Europe its production on a commercial scale is limited basically to cow milk [Wójtowski et al., 2003].
Kefir is the product of fermentation of milk with kefir grains and mother cultures prepared from grains. These grains contain lactic acid bacteria (lactobacilli, lactococci, leuconostocs), acetic acid bacteria and yeast mixture coupled together with casein. Yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide. Kefir grains usually contain lactose-fermenting yeast (*Kluyveromyces lactis* and *marxianus*, *Torula kefir*) as well as nonlactose-fermenting yeast (*Saccharomyces cerevisiae*) [Angulo et al. 1993, Simova et al. 2002, Yüksekdag et al. 2004].

The major end products of the fermentation are lactic acid, acetaldehyde, diacetyl, acetoin, ethanol, carbon dioxide and free fatty acids, for example acetic, propionic, butyric, hexanoic [Güzel-Seydim et al. 2000, Alonso and Fraga 2001, Beshkova et al. 2003]. Kefir can be made from different kind of milk (cow, goat, sheep, buffalo) and has the following characteristics: pH about 4.0; alcohol from 0.5% to 2%; fat content depends on the type of milk used; taste is acid, prickly and slightly yeasty. The sharp acid and yeasty flavour, together with the prickly sensation contributed by the carbon dioxide produced by the yeast flora can be considered as the typical kefir flavour [Irigoyen et al. 2005].

Sheep milk, due to its chemical composition and physicochemical properties is an excellent raw material for the production of some types of dairy products. This explains why especially in Europe, including the Polish market, the offer of products derived from sheep milk continues to increase. These articles include not only various types of cheeses but also different fermented beverages, including yoghurts and kefirs [Biss 1991, Giangiacomo and Messina 1991, Voutsinas et al. 1996, Wszołek et al. 2001, Katsiari et al. 2002]. These products, because of their exceptional nutritive as well as taste and smell values, are treated as delicatessen products and occupy a high position in the ranking of dairy products. The benefits of consuming kefir in the diet are numerous, as it is reported to possess the antibacterial, immunological, antitumoral and hypocholesterolemic effects [Irigoyen et al. 2005].

The objective of the research project was physicochemical and sensory characterisation of kefir manufactured from sheep milk inoculated by different starter cultures following its production and storage at refrigerated conditions.

**MATERIAL AND METHODS**

The experimental material comprised kefir from sheep milk differing with respect to the proportion of the applied starter cultures after its manufacture and further storage under refrigerated conditions. Kefir was manufactured in a small dairy plant from selected raw material of high microbiological and cytological quality. Milk for experiments was taken from sheep of a synthetic milk line of 13/15 genetic share of Friesian sheep from the Agricultural Experimental Station in Złotniki which belongs to the University of Life Sciences in Poznań, Poland.

Pooled milk was kept at the temperature of 4 ±1°C for not more than 6 h. The experimental sheep milk, after heating to the temperature of 50°C, was then subjected to a two-stage homogenisation and then pasteurised at the temperature of 95°C for 5 minutes. The milk was then cooled rapidly to the temperature of 24 ±1°C and one of two concentrated lyophilised starter cultures was applied. The cultures contained lactic fermentation bacteria in the amount of $1.0 \times 10^{10}$ cfu·g$^{-1}$ with the amount of kefir grains...
Physicochemical and sensory characteristics of sheep kefir during storage

ranging from 1.0 E + 4 to 1.0 E + 7 cfu⋅g⁻¹, known under commercial names: Kefir DA500 and Kefir DC500 derived from the Danisco-Biolacta Company collection (Olsztyn, Poland). The employed inocula contained microorganisms from the Lactococcus, Lactobacillus, Kluyveromyces fragilis, Candida kefir genera. The starter cultures differed with respect to the quantitative composition of the above-mentioned microbes. Milk inoculated with the DA culture was incubated at the temperature of 23°C and that with the DC culture – at the temperature of 26°C for the period of 16 to 18 h, i.e. to the moment when it reached pH 4.6.

The obtained curd was cooled down to the temperature of 20°C, mixed and poured into containers of 0.2 L each. The sealed containers with kefir were further cooled down to the temperature of 6 ±1°C. Six production batches of 2000 L of kefir with the DA culture and the same amount with the DC culture were manufactured and 4 kefir samples were collected from each batch for further analyses (n = 24). Assays and measurements were taken directly after the manufacture of the kefir as well as after 7, 14 and 21 days of storage at the temperature of 6 ±1°C.

Standard methods were employed to perform assays of the basic chemical composition of both kefir and milk from which it was manufactured [AOAC 2000]. The content of diacetyl in the examined kefir was determined by the Pien method [Pien 1974], the content of acetaldehyde [Lindsay and Day 1964] and free fatty acids (FFA) used Dole’s method with appropriate modifications by Doeth and Fitz-Gerald [1976]. Results of titrable acidity assays were expressed in Soxhlet-Henkle degrees [AOAC 2000]. The active acidity measurements of the obtained kefir were performed employing the CP-315 type pH meter of the Elmetron Company (Zabrze, Poland) equipped in a combined electrode type EAgP-301W Eurosensor (Gliwice, Poland) composed of a glass half-cell as well as a saturated chloride-silver half-cell.

The sensory quality of the experimental kefirs was assessed by the scaling method [ISO 4121]. Appropriately trained and prepared panel of 9 judges analysed the sensory profile of the kefir [ISO 3972, ISO 5496]. The assessment was carried out at the Sensory Analysis Workshop of the Faculty of Food and Nutrition Sciences of the University of Life Sciences in Poznań which fulfils appropriate requirements [ISO 8589], of the International Standard Organisation [ISO 6658]. In the course of the initial evaluation, the panel selected its own set of terms describing properties of the assessed product. The intensity of each of the characteristics was evaluated in a six-point score in accordance with the boundary values: 1 – absence, 6 – very clear. The following characters were assessed: bitter taste, yeasty taste, fermented taste, sour taste, fermented odour, sour odour, viscosity, astringency, serum separation and overall acceptability.

The results obtained in the course of investigations were subjected to statistical analysis using, for this purpose, Excel calculation sheet, STATISTICA (version 7.1) by StatSoft, Inc. [2005]. In addition, standard deviation (±SD), standard error (±SE) and linear regression by the method of the least squares were determined, whereas the working hypotheses were verified at the level of significance p = 0.05 [Brandt 1997].

RESULTS

Sheep milk used to manufacture kefir contained, on average, 17.12% dry matter, 5.87% protein, 6.26% fat, 4.12% lactose and 0.87% ash. The titrable acidity of the processed milk ranged from 8.1-8.2°SH, while the mean pH value was 6.83.
The active acidity of the kefir directly after manufacture, irrespective of the type of the applied starter culture was identical and complied with the assumed experimental level (pH 4.6). Significant differences in the pH value between the kefir manufacture with the assistance of the DA and DC cultures became apparent from the 14th day of storage onwards (Table 1). At this period, kefir manufactured with the addition of the DA culture was characterized by higher active acidity. These differences intensified with the passage of storage time. The final period of storage exerted a statistically significant influence on the increase of the active acidity of the kefir. After 21 days of storage, the pH value of the kefir with the DA culture decreased, on average, by 0.22 unit, whereas with the DC culture – by 0.16 pH unit.

Table 1. Changes in the value of active acidity, titratable acidity and free fatty acids of kefir during refrigerated storage

<table>
<thead>
<tr>
<th>Starter culture</th>
<th>Storage time, days</th>
<th>Standard deviation ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.59 &lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>4.53 &lt;sup&gt;Bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.61 &lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>4.52 &lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different incubation parameters, i.e. its temperature and duration time resulted in varying degrees of lactose fermentation. In the case of the kefir manufactured with the assistance of the DA culture, 3.67% lactose was determined, while in that which was obtained using the DC culture – 3.89%. The developed differences were reflected in the amount of the compounds of acid character. The titratable acidity of the DA culture kefir directly after its manufacture was 48.2°SH and was by 14% higher in comparison with the kefir produced with the aid of the DC culture (Table 1). Following the incubation of the processing milk inoculated with the DA and DC cultures, its titratable acidity increased, on average, by 5.5 times. The difference in the titratable acidity of the kefir in-
oculated with the DA and DC cultures remained unchanged throughout the storage period and amounted, on average, to 6.9°SH. After 21 days of storage the examined kefir exhibited a significantly higher titrable acidity (by 6.5°SH) in comparison with the acidity determined directly after its manufacture.

In addition, the type of the applied kefir culture exerted a significant influence with regard to the content of free fatty acids in the examined kefir (Table 1). Kefir made with the assistance of the DC culture contained significantly less FFA, both directly after its manufacture and during its further storage. It was shown that the difference in the amount of FFA between the kefir manufactured using the DA and DC cultures which amounted to 3% at the beginning of the experiment decreased with the passage of storage time. After 21 days of storage, the kefir manufactured using the DC culture contained 1.5% less FFA than that manufactured using the DA culture. Irrespective of the employed kefir culture, the quantity of FFA in kefir during the final period of storage increased over 1.5 time in comparison with the amount of FFA determined directly after manufacture.

Irrespective of the time of determination, the DC kefir was found to contain significantly more acetaldehyde than the DA kefir (Fig. 1). The largest difference (23%) in the content of this compound between the analysed kefir samples was detected directly after

![Graph showing changes in acetaldehyde and diacetyl content in kefir during storage](image-url)

**Fig. 1.** Changes in the value of acetaldehyde and diacetyl of kefir during refrigerated storage

**Rys. 1.** Zmiany zawartości aldehydu octowego i diacetylu w kefirze w czasie jego dalszego przechowywania

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the manufacture of the drink. Irrespective of the type of the applied starter culture, after 7 days of storage the analysed kefir contained significantly more acetaldehyde than directly after its manufacture. The amount of acetaldehyde in the experimental kefir decreased gradually during the further storage, so that the lowest content of the compound was determined after 21 days of storage. The determined quantities were, on average, by 40% lower than those determined in the kefir after 7 days of storage.

The type of the applied kefir culture failed to show a significant impact on the diacetyl content in the examined kefir, both directly after its manufacture and after 7 days of storage (Fig. 1). During further storage, significantly more (on average by 24.6%) diacetyl was determined in the DA than in the DC kefir. The quantities of diacetyl determined in the kefir during 21 days of its storage underwent significant variations. After 7 days of storage, the amount of diacetyl in the kefir manufactured using the DA and DC cultures declined significantly in comparison with the value determined directly after its manufacture. During further storage, the diacetyl content in kefir increased significantly. During the final stage of storage the DA kefir was characterised by statistically significantly higher diacetyl content than directly after its manufacture, contrary to the DC kefir in which no significant differences in the diacetyl content determined on day 1 and 21 of storage were determined.

Changes in the acidity and the content of taste and odour compounds in the examined kefirs were accompanied by changes in the quality characters assessed organoleptically. The type of the applied culture and the kefir storage time exerted a significant impact on the results of the sensory evaluation of its overall acceptability (Table 2). Statistically significantly higher scores for the overall acceptability were awarded to the kefir manufactured with the assistance of the DC than of the DA culture. Irrespective of the storage time, sour taste and odour were awarded higher scores in the kefir inoculated with the DA than with DC culture. On the other hand, the type of the applied starter culture failed to exert a significant influence on the evaluation results of such characters as: yeasty taste and astringency. The impact of the type of the applied starter culture on the remaining kefir quality parameters depended on the time of storage. The fermented taste was assessed higher in the kefir manufactured with the aid of the DC than DA culture beginning from day 7 of storage, fermented odour and bitter taste – from day 14 of storage, viscosity and serum separation – on day 21 of storage. Irrespective of the type of the applied starter culture, the analysed kefir was found more desirable by the panellists after 7 days of storage than directly after its manufacture. The lowest scores for the overall acceptability were given to the kefir samples evaluated after 21 days of storage.

On the basis of the calculated correlation coefficients of sensory attributes with acceptability it was found that their impact varied considerably (Fig. 2). The highest values of positive correlation coefficients with the overall acceptability were shown by such parameters as: viscosity, astringency, fermented odour, yeasty taste and fermented taste. The highest negative value of the correlation coefficient with the overall acceptability was found in the case of bitter taste, slightly lower – serum separation and the lowest – sour odour. The lowest value of the correlation coefficient with the overall acceptability was shown by the sour taste.
Table 2. Changes in the value of sensory attributes of kefir during refrigerated storage
Tabela 2. Zmiany wartości atrybutów ocenionego sensorycznie kefiru w trakcie jego dalszego przechowywania

<table>
<thead>
<tr>
<th>Sensory attributes Atrybuty sensoryczne</th>
<th>Starter culture DA Kultury starterowe DA</th>
<th>Starter culture DC Kultury starterowe DC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Storage time, days – Czas przechowywania, dni</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Bitter taste</td>
<td>Smak gorzki</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Yeasty taste</td>
<td>Smak drożdżowy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Fermented taste</td>
<td>Smak fermentacyjny</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>5.2</td>
</tr>
<tr>
<td>Sour taste</td>
<td>Smak kwaśny</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Fermented odour</td>
<td>Zapach fermentacyjny</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Sour odour</td>
<td>Zapach kwasu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Lepko</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Astringency</td>
<td>Zwięźłość</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Serum separation</td>
<td>Synereza</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ogólna pożądalność</td>
<td>4.6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Fig. 2. Correlations of sensory attributes with acceptability of kefir
Rys. 2. Zależność atrybutów ocenionych sensorycznie od ogólnej pożądalności kefiru
DISCUSSION

The quality of kefir, similarly to other fermented dairy products, depends, among other factors, on the quality and composition of the applied starter cultures. Appropriate proportions and selection of the strain types in the bacterial culture preconditions their respective development and, hence, the correct course of the coagulation process of milk proteins following the acidification of the environment leading to the formation of the casein gel of orderly network structure [Simova et al. 2002, Irigoyen et al. 2003, Cais-Sokolińska et al. 2004]. It is evident from ample literature data that the microflora used during the technological process of manufacture of fermented beverages employs different speeds and methods to utilize milk saccharides during the fermentation process leading to significant differences in the acidity of the finished product not only directly after its manufacture but also during its storage [Toba et al. 1983, Beal et al. 1999, Assadi et al. 2001, Irigoyen et al. 2005].

Changes in the acidity during the formation of curd play a significant role in the development of yoghurt viscosity and sensitivity to syneresis and affect results of its organoleptic assessment. In addition, the environmental acidity resulting from the development of microflora introduced deliberately in the course of the manufacturing process determines the degree of survivability of individual bacterial strains during further storage [Rohm and Kovac 1994, Barrantes et al. 1996]. The main metabolite developed as a result of milk fermentation, namely lactic acid, contributes to the creation of sour, refreshing taste. Its excessive concentration in fermented milk suppresses its pleasant taste. The value of about 40°SH can be accepted as the appropriate titrable acidity, also bearing in mind the structure-forming and nutritive role of the lactic acid [Cais-Sokolińska et al. 2002].

Güzel-Seydim et al. [2000], following 22 h incubation of cow milk at the temperature of 25°C inoculated with kefir culture, reported a decrease of the pH value from 6.7 to 4.5-4.6. Wszołek et al. [2001] reported a significant, 5-fold increase of titrable acidity during the process of manufacture and storage of kefir from sheep milk. The refreshing taste and odour of fermented beverages derives from acetaldehyde. Its insufficient quantities cannot smooth out the astringent diacetyl after-taste, whereas its excessive quantities result in the development of defects collectively described as “grassy” odour. Acetaldehyde develops following changes of lactose and amino acids as well as fatty acids liberated during proteolytic and lipolytic processes. Acetaldehyde is broken down to ethanol by alcohol dehydrogenase manufactured by lactic fermentation bacteria [Güzel-Seydim et al. 2000].

During the storage of fermented beverages, after the initial significant increase in the concentration of acetaldehyde observed during the first two weeks its content declines considerably later on [Ott et al. 1999]. Beshkova et al. [2003], when investigating kefir directly after its manufacture in the result of 24 h incubation of cow milk at the temperature of 22°C, reported the content of acetaldehyde at the level of 18.1 µg·g⁻¹ and that of diacetyl – at the level of 1.85 µg·g⁻¹. After 7 days, the amount of the above-mentioned compounds decreased to: 15.3 µg·g⁻¹ and 1.40 µg·g⁻¹, respectively. Güzel-Seydim et al. [2000] determined over 25 µg·g⁻¹ acetaldehyde in cow milk inoculated with a kefir culture following its 22 h incubation at the temperature of 25°C.

Diacetyl is a volatile compound with a characteristic “nutty” flavour. Its quantities depend mainly on the presence of Mn²⁺ ions and citrates in milk [Beshkova et al. 2003].
The diacetyl synthesis is initiated when the milk pH declines to the level of 5.5. The mild off-flavour of fermented products is guaranteed when the amount of this compound ranges from 0.5 to 1.0 mg·dm$^{-3}$. Excessive amounts of diacetyl in fermented beverages causes that their taste becomes harsh and pungent [Ott et al. 2000]. Lactic fermentation bacteria manufacture an enzyme, diacetyl reductase, which breaks diacetyl down irreversibly to acetoin and 2,3-butylenoglycol [Hugenholtz and Starrenburg 1992].

Another group of compounds which exert a significant impact on the taste and odour of fermented milk are free fatty acids (FFA). They derive from the milk fat which is broken down as a result of the lipolytic activity of microflora employed to inoculate milk. FFA can also develop during the transformation of lactose as well as oxidative deamination, transamination and decarboxylation of amino acids [Ott et al. 1997]. Fermented milk may contain from 5 up to 10 times more FFA than milk not subjected to fermentation. Wszołek et al. [2001] reported 4.3 times increase of FFA following the incubation of sheep milk inoculated with a kefir culture. They determined over 7 µg Eq·cm$^{-3}$ of FFA in the ready-to-serve kefir and this quantity increased significantly during subsequent storage. The rich microflora of kefir cultures contributes to the development and liberation of many compounds influencing the sensory characters of the final product. The degree of the lipolytic and proteolytic activities of lactic fermentation bacteria, i.e. the quantity of the liberated peptides and amino acids, leads to significant changes not only of the taste, odour but also of the kefir structure and consistency [Yükseldağ et al. 2004, Irigoyen et al. 2005]. These changes can either improve or aggravate the quality of the assessed product. Therefore, for instance, the elevated content of bitter peptides developing during the decomposition of β-casein will lead to the bitter taste of the obtained drink, dipeptides of glutamine and asparagine acids will intensify its sour taste [Urbach 1995]. The perceived sensations will depend not only on the concentrations of such compounds but also on their mutual proportions as well as on the product acidity.

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CHARAKTERYSTYKA CECH FIZYKOCHEMICZNYCH I SENSORYCZNYCH KEFIRU OWCZEGO PODCZAS JEGO PRZECHOWYWANIA

Streszczenie. Celem pracy była charakterystyka fizykochemiczna i sensoryczna kefiru wytworzonego z mleka owczego, po jego wyprodukowaniu i przechowywaniu w warunkach chłodniczych. Mleko owcze zaszczepione różnymi kulturami starterowymi inkubowano w temp. 23°C (kultura DA) i w temp. 26°C (kultura DC) przez okres 16-18 h do momentu uzyskania pH = 4,6. Wytworzony kefir oceniono na podstawie stopnia jego ukwaszenia, zawartości substancji smakowo-zapachowych oraz wyników ocen sensorycznej. Początkowa kwasowość miareczkowa kefiru wyтворzonego z udziałem kultury DA wyniosła 48,2°SH i była o 14% większa niż kefiru wywarzonego z kulturą DC. Po 21 dniach przechowywania kwasowość miareczkowa kefiru była o 6,5°SH większa w porównaniu z wartościami uzyskanymi bezpośrednio po jego wytworzeniu. Wraz z upływem czasu przechowywania istotnie wzrosła ilość wolnych kwasów tłuszczowych (FFA, free fatty acid) w badanych próbach kefiru. Więcej FFA oznaczało w kefirze z kulturą DA niż z kulturą DC. Kefir otrzymany na bazie kultury DA bezpośrednio po wytworzeniu zawierał mniej aldehydu octowego i diacetylu. Ilość tych związków podczas przechowywania ulegała istotnym zmianom. Bardziej pożądane sensorycznie były kefiry wytworzone z kulturą DC niż DA. Niezależnie od rodzaju użytej kultury starterowej najwyższe oceny ogólnej pożądanści uzyskał kefir w 7 dniu przechowywania.

Słowa kluczowe: mleko owcze, kefir, cechy fizykochemiczne, analiza sensoryczna, przechowywanie

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