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EFFECT OF ADDITION OF WILD GARLIC (*ALLIUM URSINUM*) ON THE QUALITY OF KEFIRS FROM SHEEP'S MILK

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ABSTRACT

Background. Sheep's milk has a high content of total solids, which qualifies it as a very good raw material for the production of fermented milk drinks. Currently, there are commercially produced kefirs and yogurts from sheep's milk in the countries of the Mediterranean region. The growing interest in the consumption of these products is justified not only by their taste merits, but also because of their health-promoting properties. The aim of the present study was to determine the effect of the addition of 1% of lyophilized wild garlic powder on the properties of kefirs from sheep's milk.
Material and methods. Sheep's milk was pasteurized (85°C, 30 min), cooled down, enriched with 1% of freeze-dried wild garlic powder, inoculated with a Commercial VITAL kefir culture and fermented for 16 hours (26°C). The influence of wild garlic on acidity (pH, °SH), syneresis (%), texture (TPA test), colour (L*a*b*) and the sensory profile of kefirs was conducted.
Results. Wild garlic could be used as a taste and flavour modifier in the production of kefir from sheep's milk. The addition of 1% of freeze-dried wild garlic slowed down the fermentation of kefir, changed colour and reduced syneresis.
Conclusions. Wild garlic could be used as a valuable supplement and a modifier of taste and flavour in kefir from sheep's milk.

Keywords: kefir, wild garlic, sheep's milk

INTRODUCTION

Kefirs are popular fermented milk drinks in Poland. They are most frequently produced from cow's and goat's milk, rarely from sheep's milk. This is due to the low production and seasonal nature of obtaining sheep's milk. Currently, there are commercially produced kefirs and yogurts from sheep's milk in the countries of the Mediterranean area (Bonczar and Wszołek, 1997). The growing interest in the consumption of these products is justified not only by their taste qualities, but also because by their health benefits (Bonczar et al., 1995).

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Sheep's milk has a high content of total solids, which qualifies it as a very good raw material for the production of fermented milks. In the manufacture of fermented sheep's milk there is no need to regulate the solid-not-fat (SNF) level with dairy powders, which is usually performed in standard production from cow's milk (Danków and Pikul, 2011). There has been relatively little research carried out on sheep milk kefirs, especially in Poland. Bonczar and Wszołek (1997) assessed the quality and durability of kefirs from sheep's milk coming from the Małopolska region. Moreover, Cais-Sokolińska et al. (2008) wrote about changes in the physicochemical and sensory properties of sheep kefir during storage. The profile of fatty acids in kefirs from sheep's milk was also studied by Wójtowski et al. (2003). Danków et al. (2000) studied the effect of starter cultures and storage time on the quality factors of sheep milk kefirs.

Milk and milk products belong to the group of food which are often fortified with micro- and macro-elements, fibres, vitamins and other active compounds. Enrichment of milk drinks has become accepted and appreciated by consumers. The taste and aroma of kefir produced by acidic-alcoholic fermentation could produce a good combination with the addition of herbs and vegetables. Moreover, kefirs, buttermilks and other fermented milks are good components for the preparation of cold fruit or vegetable soups.

Several studies have shown that wild garlic and its bioactive compounds can be used in the prevention of many diseases, e.g. of the cardiovascular system (atherosclerosis, myocardial infarction, high blood pressure), cancers, and of bacterial, viral and fungal diseases with a strong bactericidal effect (Bat-Chen et al., 2010; Durak et al., 2004), hence the concept of enriching kefir from sheep's milk with wild garlic coming from the Podkarpackie region. Therefore, the aim of the research was to determine the effect of adding 1% of freeze-dried wild garlic powder on the properties of kefirs from sheep's milk.

MATERIAL AND METHODS

Materials

Sheep's milk was purchased in June 2015 from the farm "Owcza Zagroda" (Wyżne, Poland). A flock of 35 Olkuska breed sheep were kept on the farm.Green fodder and hay with addition of cereals (oats, barley and maize in a quantity of 0.5 kg/sheep/day) from the breeder's own farm were used for feeding animals. Milking was done by hand. Wild garlic (*Allium ursinum*) was collected in May 2015 on a farm in Dukla, Podkarpackie region, where wild garlic is cultivated on a 1 ha meadow in conditions similar to natural ones. The meadow is mowed once a year, animals are not grazed there, nor are fertilizer or pesticides used. To manufacture kefir a Kefir VITAL (Danisco-Dupont, Poland) starter culture was used. The VITAL series comprises starter cultures in sachets dedicated for home production of fermented dairy products. According to the producer's specifications, one sachet contains the correct amount of kefir grains (kefir yeast and lactic acid bacteria *Leuconostoc* subsp., *Lactococcus lactis* subsp., *Streptococcus thermophilus, Lactobacillus acidophilus*) to prepare 1–3 liters of kefir, which is also optimal for agritourism needs. The chemical composition (fat, protein, lactose, total solids) and freezing point of sheep's milk were determined using the Milk Analyzer B-150 (Bentley Instruments, USA). The total number of microorganisms and the somatic cell count were determined using BactoCount IBC-m/SCC (Bentley Instruments, USA).

Manufacture of kefirs

The leaves of the wild garlic were washed in boiled and cooled water, then dried with tissue paper and subequently frozen in a commercial freezer (–22°C). Lyophilization was processed in the Christ ALPHA 1–2 LD plus freeze-dryer (Martin Christ GmbH, Germany). Milk was pasteurized (85°C, 30 min) and cooled down to 26°C. The lyophilized leaves of garlic were ground into a powder in a mortar, then weighed and added to the milk in an amount of 1%. To determine the level of wild garlic to be added, a pre-study of preferences was made using a dose of 0.5%, 1%, 1.5%, 2% and 3%, respectively. The evaluators most preferred kefirs with a 1% garlic addition (unpublished data). One sachet of Kefir VITAL was added to 3 l of pasteurized and cooled milk supplemented with wild garlic. A control sample was made without the addition of garlic. Then the milk was poured into containers with a capacity of 100 ml with a lid and fermented for 16 hours at a temperature of 26°C. The kefirs were cooled down to 8°C and stored at this temperature for 7 days.

Analyses of the kefirs

On the seventh day of cold storage an assessment of quality was carried out. The active acidity (pH) of milk and kefir was determined with the pH-meter FiveEasy (Mettler Toledo, Switzerland). The titratable acidity of milk (°SH) was established by the Soxhlet-Henkel method, while the titratable acidity in kefir was expressed in % of lactic acid (Litwińczuk et al., 2011). Syneresis was determined by weight, as a percentage leakage of whey from 25 g of the sample after 2 h, at a temperature of 5°C (Znamirowska et al., 2016). The texturometric profile was determined by the TPA test using a CT3 Texture Analyzer (Brookfield, USA) with Texture Pro CT (Brookfield, USA) software. The sample dimensions were cylinder 66 mm × 33.86 mm and the temperature of the sample was 8°C. The test was performed using the acrylic probe TA 3/100 and the following settings: distance 15 mm, contact load 0.1 N, measurement speed 1 mm/s (Znamirowska et al., 2016). The parameters of L*, a*, b* colours were determined with the Konica Minolta Chroma Meter CR-410 colorimeter, using the CIELAB method. The image brightness was determined with the parameter L*, and chromaticity with the use of the parameters a* and b*. Before testing, the device was calibrated on a white reference standard. The assessment of kefir with the method of sensory profiling (PN-ISO 11035:1999) was carried out by tahe team of 12 persons of proven sensory sensitivity. The samples of kefir were assessed on a 9 cm linear scale, non-structured, with markings at both ends. The left end denoted the least perceptible feature and the right end denoted the most intense, the most characteristic feature. The following descriptors were studied: consistency, CO_2 saturation, colour, taste (yeasty, sour, garlic, off-flavours), aroma (of fermentation, strange, garlic). Preferences were rated with the ranking method, where position 1 denoted the most preferred yogurt, and position 3 the least preferred drink (Baryłko-Pikielna and Matuszewska, 2014).

The experiment was repeated three times, each parameter was measured five times. From the data obtained the mean and the standard deviation were calculated using Statistica v. 12 software. The significance of differences between the groups was determined by Tukey's test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the physicochemical properties of sheep's milk used for the production of kefir. The average protein, fat, lactose and total solid contents were comparable to the results reported in the literature (Bonczar et al., 2002; 2009; Cais-Sokolińska et al., 2008; Vivar-Quintana et al., 2006). The pH value determined in the tests was higher than that shown by Vivar-Quintana et al. (2006) who marked a pH in sheep's milk from 6.6 to 6.8, depending on the content of somatic cells. Sheep's milk analyzed by Bonczar et al. (2002) had a lower pH value (6.66) and a lower titratable acidity (11.2°SH) than that shown in Table 1. In other studies Bonczar and Reguła (2003) marked a pH of 6.63 and a titratable acidity of 18.8°SH in sheep's milk. Awassi ewes' milk in the studies by Şahan et al. (2005) was characterized by a pH of 6.21 to 7.29, depending on the time within 18 months of lactation. In contrast, fresh sheep's milk used by Katsiari et al. (2002) for the production of yoghurt had a lower pH (6.53) than that found in the studies conducted.

Table 1. Parameters of fresh sheep's milk used in the manufacture of kefirs

Protein, %	5.34 ±0.22
Fat, %	6.09 ±1.02
Lactose, %	5.01 ±0.12
Total solid, %	17.61 ±0.57
pH	6.85 ±0.12
Titratable acidity, °SH	12.81 ±1.02
Freezing point, °C	–0.623 ±0.07
Total bacterial counts, CFU/ml	489 290.12 ±21.31
Somatic cells count in 1 ml	55.000 ±1414.21

Values are means ±standard deviation.

The total bacterial counts (TBC) in the sheep's milk analyzed did not exceed 500 000 CFU in 1 ml. In accordance with the Regulation of the European Parliament and Council No. 853/2004, it could be used for the production of milk products even without heat treatment. Králíčková et al. (2012) also reported that the values of TBC for sheep's milk collected at different stages of lactation did not exceed 1.5×10^6 CFU per 1 ml, which is the maximal limit of TBC for raw sheep's milk. In milk of sheep from Kosovo Bytyqi et al. (2013) marked a TBC from 1211.17 CFU/ml to 6425.76 CFU/ml. Alexopoulos et al. (2011) studied the milk of sheep from the north-eastern Greece and indictaed the total bacterial counts at the level of 3×10^5 CFU/ml.

Table 2 shows the results of pH, acidity, colour and syneresis of natural kefir and kefir with the addition of wild garlic powder. In kefir with wild garlic powder there was a significantly higher pH value and a lower lactic acid content [%] than in the control kefir. The differences in the acidity of kefirs could be the result of the antimicrobial activity of wild garlic. According to the literature, *Allium ursinum* shows the antibacterial effect on the bacteria gram (+) and the bacteria gram (–) (Sapunjieva et al., 2012; Štajner et al., 2008). Sulphur compounds are mainly responsible for the biological activity of wild garlic (Dżugan et al., 2014; Radulović et al., 2015). It is estimated that cysteine sulfoxides (alliins) and non-volatile γ-glutamylcysteine peptides represent more than 82% of the total content of sulphur in wild garlic. It was also proven that allicin – one of the main components of *Allium ursinum* – has high antifungal activity (Bagiu et al., 2012; Pârvu et al., 2011). In the studies by Dżugan et al. (2014), it was found that the freeze-drying process did not cause a significant decrease in the antibacterial and antifungal activities of the wild garlic extract compared to fresh leaves. On the other hand, Bagiu et al. (2012) finds that fresh leaves contain 0.25–1.15% of alliin, while contain dried leaves 0.7–1.7% of alliin. Therefore, in the tests conducted, the addition of 1% of freeze-dried garlic slowed down the milk fermentation process, which resulted in the reported differences in pH and titratable acidity between the control kefir and kefir with wild garlic.

Wild garlic leaves contain flavonoids, chlorophylls and carotenoids, which give it its green colour (Štajner et al., 2008). The leaves of wild garlic have a high content of pigments: chlorophyll A2 2.87 ±0.03 mg/g of chlorophyll B 1.35 ± 0.01 mg/g and 9.99 ±0.01 mg/g of carotenoids (Sobolewska et al., 2015; Štajner and Szöllősi Varga, 2003). Therefore, the addition of wild garlic to kefir significantly changed the components of colours L*, a* and b* in kefir from sheep's milk (Table 2). A darkening of colour and an increase in the proportion of green and yellow colours was found in kefir with wild garlic powder compared to the control.

The addition of freeze-dried wild garlic significantly reduced syneresis of kefir from sheep's milk (Table 2). The addition of 1% of garlic powder reduces the adverse effect of syneresis by approx. 3%, which was probably the effect of rehydration of wild garlic. Vivar-Quintana et al. (2006) showed that syneresis in yogurts from sheep's milk depends on the somatic cell count (SCC) in the sheep's milk used for their production. Syneresis defined there as the volume of serum that was not retained within the structure on centrifugation was in the range from 1 ml when the SCC in milk was less than 500 000/ml and significantly increased with a higher SCC content in the milk.

Table 2. The colour (L*, a*, b*), lactic acid content, syneresis of natural sheep kefirs and with wild garlic

Properties	Control	Enriched with 1% of wild garlic
pH	4.47^a ±0.05	4.58^b ±0.04
Lactic acid, %	1.19^a ±0.01	1.06^b ±0.01
Colour		
L*	91.44^a ±1.40	57.48^b ±1.21
a*	-4.17^a ±0.10	-16.45^b ±0.19
b*	5.82^a ±0.42	12.08^b ±0.25
Syneresis, %	23.26^a ±1.20	20.14^b ±1.14

Values are means ±standard deviation.
Means with different letters within the same row (a–b) are significantly different at $p \leq 0.05$, L* represents the lightness (from 0 – black to 100 – white); a* and –a* redness and greenness, respectively; and b* and –b* yellowness and blueness, respectively.

Analyzing the texturometric profile (Table 3) of sheep's kefir with wild garlic, a significant increase was found in hardness [N], gumminess [N] and chewiness [mJ] compared to the results obtained for the control kefir ($p \leq 0.05$). The addition of wild garlic caused

Table 3. Texturometric profile (TPA test) of kefirs from sheep's milk

Texture	Control	Enriched with 1% of wild garlic
Hardness, N	0.27^a ±0.12	0.77^b ±0.32
Adhesiveness, mJ	0.14^a ±0.02	0.20^a ±0.10
Resilience	0.93^a ±0.04	0.21^b ±0.02
Stringiness length, mm	1.72^a ±0.77	2.23^a ±1.11
Cohesiveness	0.95^a ±0.05	0.55^b ±0.02
Springiness, mm	14.49^a ±0.55	15.81^a ±1.59
Gumminess, N	0.26^a ±0.09	0.42^b ±0.11
Chewiness, mJ	3.84^a ±0.34	7.08^b ±2.42

Values are means ±standard deviation.
Means with different letters within the same row (a–b) are significantly different at $p \leq 0.05$.

Table 4. Sensory properties of sheep kefirs, $n = 12$

Properties	Control	Enriched with 1% of wild garlic
Preferences	0.61^a ±0.25	0.86^a ±0.41
Consistency	2.94^a ±1.66	5.11^b ±1.03
CO_2 saturation	2.22^a ±1.96	3.17^a ±1.58
Colour	6.39^a ±0.72	4.06^b ±1.04
Yeasty taste	2.56^a ±1.72	2.50^a ±1,69
Sour taste	5.28^a ±0.27	4.11^b ±0.49
Garlic taste	0.00^a ±0.01	6.33^b ±1.68
Off-flavours	0.50^a ±0.18	0.84^a ±0.41
Sour aroma – of fermentation	3.33^a ±1.94	3.94^a ±1.80
Strange aroma	1.44^a ±0.28	1.46^a ±0.01
Aroma of garlic	0.11^a ±0.07	7.17^b ±1.26

Values are means ±standard deviation. Means with different letters within the same row (a–b) are significantly different at $p \leq 0.05$.

a decrease in the resilience and cohesiveness of kefirs. The hardness of fermented sheep's milks was studied by Bonczar et al. (2002), Bonczar and Reguła (2003), Vivar-Quintana et al. (2006). The authors classified storage time, the amount of starter cultures added and the content of somatic cells in sheep's milk to the factors influencing hardness. Glibowski and Kowalska's (2012) studies indicated that the texture of kefirs can be shaped by the addition of inulin.

Table 4 shows the results of sensory characteristics and preferences for the sheep's kefirs. The results of the ranking test showed that natural kefir was more preferred than the one with wild garlic, although some people evaluated the kefir with wild garlic as appropriate.

In the opinion of the evaluators, the addition of wild garlic significantly reduced the intensity of sour taste, which could be the result of the lower lactic acid content. Furthermore, the taste of garlic was evaluated as intense. The green colour of the kefirs was assessed as not very distinctive in comparison to the white colour of natural kefirs. Moreover, a more solid and characteristic consistency was indicated in kefirs with garlic than in the controls. The sensory analysis confirmed the results of the TPA test that the consistency of kefir with wild garlic was more solid compared to natural ones. No significant effect of the addition of wild garlic was found on saturation with CO_2, yeasty taste or off-favours and on aroma: sour-fermentation and strange. The studies by Cais-Sokolińska et al. (2008) indicate that the taste and aroma of kefirs from sheep's milk can also be modified by the selection of starter cultures.

CONCLUSIONS

Wild garlic can be used as a valuable supplement and taste and flavour modifier in the production of kefirs from sheep's milk. The addition of 1% of freeze-dried garlic slowed down the fermentation process, changed the colour and reduced the syneresis of kefirs. The addition of wild garlic to kefirs increased hardness and determined most of its sensory qualities.

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