

THE INFLUENCE OF THE DOSE OF CALCIUM BISGLYCINATE ON PHYSICOCHEMICAL PROPERTIES, SENSORY ANALYSIS AND TEXTURE PROFILE OF KEFIRS DURING 21 DAYS OF COLD STORAGE

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ABSTRACT

Background. In the process of enrichment of dairy products a priority element is the proper selection of compounds that are a mineral carrier. Calcium bisglycinate is better absorbed by the body than inorganic forms of calcium. Moreover, the lactic acid which is produced in kefir fermentation and the presence of lactose have also a positive effect on the improvement of absorption of calcium. The aim of the present study was to determine the influence of the applied dose of calcium in the form of calcium bisglycinate on the physicochemical and sensory properties and texture of kefirs during 21-day period of cold storage.

Material and methods. Processed cow milk was enriched with 0, 5, 10, 15, 20, 25 and 30 mg of calcium (for 100 g of milk), repasteurized (72°C, 1 min), cooled down (26°C), inoculated with Commercial VITAL kefir culture (Danisco, Poland) and fermented for 16 hours (26°C). The assessment of the influence of addition of calcium bisglycinate on acidity, syneresis, texture and sensory characteristics (1–9 points) of kefirs was conducted at four fixed dates (after 1 day, 7 days, 14 days and 21 days of storage).

Results. During successive weeks of cold storage in all experimental groups there was observed a tendency to decrease general acidity. On the 1st and 7th days of cold storage reduced whey leakage was observed in kefirs enriched with 25 mg and 30 mg Ca/100 g of milk. With increasing doses of enrichment with calcium both the hardness, adhesiveness and gumminess of kefirs decreased. The applied doses of calcium did not cause changes in the sensory characteristics such as colour and consistency of the fermented beverages.

Conclusions. Calcium bisglycinate may be used to enrich kefirs with calcium even with 30 mg of calcium in 100 g of milk without the modification of the product's parameters.

Key words: kefir, calcium, bisglycinate

INTRODUCTION

Kefir is a fermented milk beverage which originates from the Caucasian mountains, Tibet and Mongolia where it was primarily produced from sheep milk. Nowadays, it has spread in Europe where its production on a commercial scale is limited basically to cow milk (Cais-Sokolińska et al., 2008; Irigoyen et al., 2005).

Kefir is traditionally produced by inoculating milk with kefir grains – a complex community of around 30 species of lactic acid bacteria (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, etc.), acetic acid bacteria (*Acetobacter*) and yeasts (*Kluyveromyces*, *Saccharomyces*, *Torula*) embedded in a resilient, insoluble protein and

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polysaccharide matrix (Nielsen et al., 2014). According to Irigoyen et al. (2005) the major end products of milk fermentation are lactic acid, acetaldehyde, acetoin, diacetyl, ethanol and carbon dioxide. Moreover, during the fermentation kefir increases concentration of vitamin B₁, B₁₂, calcium, amino acids, folic acid and vitamin K. There are many health benefits of consuming kefir. Some results indicate a positive influence of kefir on the immune and alimentary systems and also on the cholesterol metabolism. This beverage reveals therapeutic activity against colorectal carcinoma and it delays the development of breast cancer. Kefir can also be consumed by persons with lactose intolerance (Glibowski and Kowalska, 2012). According to Fesnak (2000) kefir cultures, apart from synthesizing B vitamins, cause slight digestion of proteins and lactose and make them more easily digestible for the human body, thus succeeding in increasing the nutritional and dietary value of kefir. The lactic acid which is produced in kefir fermentation has also a positive influence on the improvement of digestion and absorption of calcium.

High dietary intake of calcium is recognised to prevent osteoporosis. Application areas for adding calcium to dairy products includes yoghurt, yoghurt drinks, ice cream, cottage cheese, sour cream, cream cheese preparations and desserts. Bioavailability of calcium from fermented milk beverages is higher than from milk because the acidic pH of fermented drinks ionizes calcium and thus facilitates intestinal calcium absorption (Singh and Muthukumarappan, 2008). 75% of the demand for calcium is provided by milk and its products, the remaining amount of calcium is delivered from meat products. Milk and milk products contain lactose increasing calcium absorption, moreover, they have a sufficient ratio of calcium to phosphorus – 1:1. Supporting doses of calcium from supplements, among other foods fortified with calcium, increase bone mineral resources (Kozłowska-Wojciechowska, 2007). In the process of enrichment of dairy products a priority element is the proper selection of compounds that are a mineral carrier. The used mineral compound must be well absorbed to fulfill its role.

The chemical substances used as sources of vitamins and minerals which may be added to foods should be safe and available to be used by the human body. Minerals might be added to milk fermented

beverages which is governed by the Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. However, on the list of permitted substances there is no calcium in the chemical form of calcium bisglycinate. Calcium bisglycinate is commonly used in the manufacture of food supplements and substances that may be added for specific nutritional purposes in foods for particular nutritional uses (Commission Regulation (EC) No 1170/2009 of 30 November 2009; Commission Regulation (EC) No 953/2009 of 13 October 2009). In order to keep up with scientific and technological developments, it is important to revise lists promptly.

The aim of the paper was to determine the influence of applied doses of calcium in the form of calcium bisglycinate on the physicochemical and sensory properties and texture of kefir during 21-day period of storage.

MATERIAL AND METHODS

Materials

Kefirs were produced from 2% fat pasteurized cow milk (SM Mlekovita, Poland), using commercial Vital Natural Kefir culture (Danisco, Poland) which included the microflora of kefir grains (kefir yeast and *Leuconostoc* subsp.) and *Lactococcus lactis* subsp., *Streptococcus thermophilus*, *Lactobacillus acidophilus*. For kefir enrichment calcium bisglycinate (Olimp Laboratories, Poland) was used.

Preparation of kefir

Kefirs were produced with various levels of enrichment with calcium (0 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg and 30 mg). The doses of calcium were based on molecular weight of calcium bisglycinate. Milk with addition of calcium bisglycinate was repasteurized (72°C, 1 min) and cooled down to 26°C. Then the milk was inoculated with kefir culture. Fermentation was conducted for 16 hours in disposable containers with lids (100 ml) in temperature of 26°C. Kefirs were cooled down to 5°C and stored for 21 days.

Kefirs analysis

The assessment of the influence of addition of calcium bisglycinate on acidity, syneresis, texture and sensory characteristics (1–9 points) was conducted at four fixed dates (after 1 day, 7 days, 14 days and 21 days of storage). Three production batches of kefir were manufactured and 5 kefir samples were collected from each batch for analyses ($n = 15$). Sensory quality of kefir was assessed by a 20-person panel, by the sensory profile method according to a 1–9 point scale for saturation of CO₂, consistency, sour taste, sour odour, yeasty taste, salty taste and colour. Determining potential acidity was performed by titration of 0.25N samples with standard solution of sodium hydroxide in presence of phenolphthalein as an indicator. Determining active acidity (pH) was performed with the electrometric method by measurement of activity of hydrogen ions with the use of pH/conductivity meter CPC – 505 (Elmetron) equipped with the combination electrode OSH 12–00. Susceptibility of kefir to syneresis was measured using Sahan et al. (2008) method. 25 g of fermented beverage samples were weighed on a filter paper and placed on top of a funnel. The drainage time and temperature was 120 min and 4°C respectively. Syneresis of whey was performed by gravity and the quantity of whey collected in a flask of known weight was used as a syneresis value. The texture was determined with the analyser Brookfield CT3 equipped with Brookfield Texture Pro CT software. For determination there was selected the TPA test with the following settings: sample – cylinder 66.00 mm × 33.86 mm, force 0.1 N, head speed 1 mm/s, table TA-BT-KIT, probe TA3/100. The following parameters were calculated: hardness, adhesiveness, springiness and gumminess.

From the obtained results the mean, standard deviation and simple correlation coefficient (r) were worked out statistically in the software Statistica 10.0 (StatSoft, USA). Significance of differences between the averages ($p \leq 0.05$) was estimated with Tukey's test.

RESULTS AND DISCUSSION

The proper calcium intake from the diet is important in the prevention of diet-related metabolic disorders (diabetes, hypertension, obesity, atherosclerosis and

cancer). Due to relatively low consumption of milk in Poland, fortification of fermented milk beverages with calcium is becoming more common. The most popular products enriched with calcium are milk and dairy products with reduced fat content. According to Ziarno (2008) applied salts should have a good solubility, high chemical and thermal stability, and they cannot change the sensory characteristic of products. Moreover, the mineral compound must be well-absorbed. In the production of fermented milk beverages the fortification with calcium ions is possible directly after heat treatment, or as an additive to a final product. Bisglycinates available on the market are the source of calcium, zinc, copper and magnesium. They are composed of divalent metal ions, notably Ca²⁺, Zn²⁺, Cu²⁺ and Mg²⁺ which are connected to two molecules of glycine. The metal ion is bound to the carboxyl group, as well as α -amino group of glycine and it is forming the form of a heterocyclic ring. The 1 : 2 metal-ligand ratio reduces the reaction of the compound with contained in the diet magnesium absorption inhibitors and limits its entry in the free radical reactions. Calcium bisglycinate is characterized by high absorption and use by the human body. In order to increase the absorption of calcium there is used a nutritional cofactor – vitamin D, which maintains the appropriate level of calcium and helps to reduce its losses. In contrast to inorganic forms of calcium, bisglycinate does not affect the absorption of other minerals, including iron (Schuette et al., 1994).

On the first day of analysis the lowest general acidity was observed in kefir enriched with 5 mg of Ca (in 100 g of milk) (35.60°SH), while the highest general acidity was found in kefir fortified with 25 mg Ca/100 g of milk (40.20°SH; Table 1). During successive weeks of cold storage in all experimental groups a tendency to decrease general acidity was observed. Many authors (Cais-Sokolińska et al., 2008; Kok-Tas et al., 2013; Mituniewicz-Małek et al., 2009) reported an increase of total acidity of kefir made from cow and sheep milk during cold storage. On the 21st day of cold storage there were observed differences determined between the acidity of control kefir and acidity of fermented beverages enriched with the dose of 10 mg of calcium (for 100 g of milk) or more.

There were observed differences between pH of control samples and kefir enriched with 5 mg of

Table 1. Influence of dose of enrichment with calcium on general acidity ($^{\circ}\text{SH}$) of kefir during storage (means \pm SD, $n = 15$)

Dose of enrichment with calcium mg of calcium for 100 g of milk	Storage time, days			
	1	7	14	21
0	36.80 \pm 1.60 ^{Aa}	35.20 \pm 0.80 ^{ABa}	33.80 \pm 0.72 ^{Ba}	34.70 \pm 1.11 ^{ABa}
5	35.60 \pm 1.60 ^{Aa}	33.40 \pm 0.48 ^{Aa}	33.50 \pm 1.21 ^{Aab}	35.80 \pm 0.83 ^{Aab}
10	38.80 \pm 0.40 ^{Aa}	44.90 \pm 1.25 ^{Ba}	35.90 \pm 0.87 ^{Bb}	34.10 \pm 1.73 ^{Ba}
15	38.00 \pm 0.00 ^{Aa}	34.90 \pm 0.77 ^{Ba}	34.30 \pm 0.59 ^{Bab}	36.00 \pm 0.28 ^{Cab}
20	37.40 \pm 0.20 ^{Aa}	33.90 \pm 1.82 ^{Ba}	33.20 \pm 0.28 ^{Bab}	34.20 \pm 1.08 ^{Ba}
25	40.20 \pm 2.20 ^{Aa}	34.80 \pm 0.75 ^{Ba}	34.10 \pm 0.33 ^{Ba}	36.80 \pm 0.28 ^{Cb}
30	37.60 \pm 1.50 ^{Aa}	35.40 \pm 0.87 ^{ABa}	34.40 \pm 1.30 ^{Ba}	36.10 \pm 0.95 ^{ABab}
Simple correlation coefficient (r) between the dose of calcium enrichment and general acidity	0.065	0.189	0.121	0.122

A-C – different capital letters with mean values in rows indicate statistically significant differences at $p \leq 0.05$.

a-c – different small letters with mean values in columns indicate statistically significant differences at $p \leq 0.05$.

calcium (for 100 g of milk) and pH of kefir fortified with 20 mg, 25 mg and 30 mg of Ca (for 100 g milk) on the 1st day of analysis (Table 2). Higher pH values in kefir fortified in 15 mg of calcium and more (for 100 g of milk) were maintained throughout the

whole period of cold storage and presented differences were statistically significant ($p \leq 0.05$). The influence of the applied dose of enrichment also confirms high significant correlation coefficients between the dose of calcium and pH values on dates of each analysis

Table 2. Influence of dose of enrichment with calcium on active acidity of kefir during storage (means \pm SD, $n = 15$)

Dose of enrichment with calcium mg of calcium for 100 g of milk	Storage time, days			
	1	7	14	21
0	4.52 \pm 0.01 ^{Aac}	4.55 \pm 0.00 ^{Ba}	4.59 \pm 0.01 ^{Ca}	4.65 \pm 0.01 ^{Da}
5	4.51 \pm 0.02 ^{Ac}	4.56 \pm 0.01 ^{Bb}	4.62 \pm 0.01 ^{Cb}	4.66 \pm 0.01 ^{Da}
10	4.56 \pm 0.01 ^{Aac}	4.64 \pm 0.04 ^{Bc}	4.65 \pm 0.01 ^{Bc}	4.65 \pm 0.01 ^{Ba}
15	4.58 \pm 0.01 ^{Aab}	4.60 \pm 0.01 ^{Bd}	4.67 \pm 0.00 ^{Cd}	4.70 \pm 0.01 ^{Db}
20	4.63 \pm 0.05 ^{Ab}	4.77 \pm 0.01 ^{ABc}	4.70 \pm 0.01 ^{ABc}	4.75 \pm 0.03 ^{Bc}
25	4.63 \pm 0.01 ^{Ab}	4.63 \pm 0.01 ^{Af}	4.64 \pm 0.01 ^{Af}	4.69 \pm 0.01 ^{Bb}
30	4.62 \pm 0.02 ^{Ab}	4.64 \pm 0.01 ^{Af}	4.67 \pm 0.01 ^{Bf}	4.73 \pm 0.01 ^{Cc}
Simple correlation coefficient (r) between the dose of calcium enrichment and active acidity	0.837	0.695	0.734	0.759

A-D – different capital letters with mean values in rows indicate statistically significant differences at $p \leq 0.05$.

a-f – different small letters with mean values in columns indicate statistically significant differences at $p \leq 0.05$.

($r > 0.6$, $p \leq 0.05$). According to Pirkul et al. (1997) fortification of fermented beverages with calcium salts increases the buffer capacity, thereby preventing excessive acidification. Mituniewicz-Malek et al. (2009), Magra et al. (2012) reported a decrease of pH value in kefir during the cold storage. Irigoyen et al. (2005) reported that pH do not vary significantly during the storage of kefir and it might be related to the presence of yeasts in this fermented milk beverage. The lactic acid bacteria in the presence of yeast proliferate and produce lactic acid more slowly than in pure culture (Baranowska, 2009).

Table 3 shows the results of syneresis in kefir depending on the dose of enrichment and the time of storage. On the 1st and 7th days of cold storage there was observed a tendency for high doses of fortification with calcium (25 mg and 30 mg Ca/100 g of milk) to significantly reduce whey leakage. On the 1st and the 7th day of analysis there were calculated significant correlations between syneresis and applied quantities of calcium ($r > -0.5$, $p \leq 0.05$). Additional extension of storage time caused the effect of the dose of enrichment on the syneresis to become less significant, moreover, the correlation coefficients were low. Serum separation occurs as a result of aggregation of protein particles during storage and sedimentation under

gravity. Stabilizers, acidity, total solids, milk and culture type can also affect the whey leakage in fermented milk beverages (Montanuci et al., 2012). Montanuci et al. (2012) observed that an increase of syneresis was noted in kefir fermented with kefir starter culture compared to beverages fermented with kefir grains. The authors obtained less syneresis in whole milk kefir than in the beverages produced from skimmed milk. The same results were obtained by Baranowska (2009). Moreover, the concentration of Ca^{2+} ions has an influence on syneresis (Jovanovic et al., 2004). The authors reported that selected factors of coagulation (including the amount of added CaCl_2) have a big influence on the amount of whey leakage in gel by the application of various intensities of centrifugal force (induced syneresis) and the best rheological properties had gels produced with 400 mg/l CaCl_2 added (Jovanovic et al., 2004).

The major factor affecting the hardness of kefir in the experiment was the time of storage. Significant differences were found between the hardness of kefir on 1st day of storage and the hardness of kefir on the 14th and the 21st day of cold storage (Table 4). There were found correlations between the dose of enrichment with calcium and the hardness of kefir on the 7th day of storage ($r = -0.570$, $p \leq 0.05$), on the 14th

Table 3. Influence of dose of enrichment with calcium on syneresis of kefir during storage (means \pm SD, $n = 15$)

Dose of enrichment with calcium mg of calcium for 100 g of milk	Storage time, days			
	1	7	14	21
0	37.55 \pm 0.73 ^{Aa}	38.17 \pm 0.64 ^{Bab}	33.29 \pm 0.76 ^{Cac}	35.61 \pm 1.03 ^{Aa}
5	37.96 \pm 0.18 ^{Aa}	35.81 \pm 0.32 ^{BCab}	36.15 \pm 0.55 ^{Bb}	34.79 \pm 0.62 ^{Ca}
10	38.18 \pm 0.76 ^{Aa}	34.89 \pm 1.61 ^{Bab}	36.62 \pm 1.02 ^{ABb}	35.92 \pm 0.67 ^{ABa}
15	36.02 \pm 0.34 ^{Aa}	34.91 \pm 0.83 ^{Aab}	31.80 \pm 0.34 ^{Bc}	34.47 \pm 1.80 ^{Aa}
20	37.22 \pm 1.72 ^{Aa}	33.89 \pm 0.20 ^{Bab}	32.86 \pm 1.49 ^{Bac}	33.70 \pm 2.46 ^{Ba}
25	33.38 \pm 0.12 ^{Ab}	36.48 \pm 1.48 ^{Ba}	35.23 \pm 0.66 ^{ABb}	36.29 \pm 1.24 ^{Ba}
30	33.62 \pm 1.48 ^{Ab}	33.21 \pm 1.15 ^{Ab}	34.06 \pm 0.47 ^{Aa}	33.00 \pm 0.38 ^{Aa}
Simple correlation coefficient (r) between the dose of calcium enrichment and syneresis	-0.763	-0.507	-0.213	-0.383

A-C – different capital letters with mean values in rows indicate statistically significant differences at $p \leq 0.05$.

a-c – different small letters with mean values in columns indicate statistically significant differences at $p \leq 0.05$.

Table 4. Influence of dose of enrichment with calcium on hardness [N] of kefir during storage (means \pm SD, $n = 15$)

Dose of enrichment with calcium mg of calcium for 100 g of milk	Storage time, days			
	1	7	14	21
0	1.38 \pm 0.01 ^{Aabc}	1.61 \pm 0.02 ^{Ba}	1.76 \pm 0.03 ^{Ca}	1.73 \pm 0.04 ^{Ca}
5	1.44 \pm 1.00 ^{Aa}	1.74 \pm 0.04 ^{Bb}	1.93 \pm 0.11 ^{Cb}	1.83 \pm 0.04 ^{Ca}
10	1.36 \pm 0.66 ^{Aabc}	1.56 \pm 0.09 ^{Bac}	1.85 \pm 0.06 ^{Cab}	1.83 \pm 0.02 ^{Ca}
15	1.34 \pm 0.03 ^{Aabc}	1.57 \pm 0.06 ^{Bac}	1.65 \pm 0.10 ^{Bad}	1.81 \pm 0.03 ^{Cab}
20	1.32 \pm 0.05 ^{Aabc}	1.48 \pm 0.03 ^{Bc}	1.67 \pm 0.02 ^{Cad}	1.76 \pm 0.09 ^{Dab}
25	1.28 \pm 0.04 ^{Ab}	1.54 \pm 0.01 ^{Bac}	1.42 \pm 0.08 ^{Bc}	1.69 \pm 0.04 ^{Cb}
30	1.42 \pm 0.02 ^{Aac}	1.50 \pm 0.05 ^{Aac}	1.75 \pm 0.02 ^{Bad}	1.66 \pm 0.93 ^{Bb}
Simple correlation coefficient (<i>r</i>) between the dose of calcium enrichment and hardness	-0.260	-0.570	-0.529	-0.541

A-D – different capital letters with mean values in rows indicate statistically significant differences at $p \leq 0.05$.

a-d – different small letters with mean values in columns indicate statistically significant differences at $p \leq 0.05$.

day of storage ($r = -0.529$, $p \leq 0.05$) and on the 21st day of cold storage ($r = -0.541$, $p \leq 0.05$). Calculated negative correlation coefficients mean that with increasing doses of enrichment with calcium the hardness of kefir decreases.

In all experimental groups there was not showed the influence of storage time on the adhesiveness kefir (Table 5). There were found significant correlations between the dose of calcium enrichment and the adhesiveness of kefir on the 21st day of storage.

Table 5. Influence of dose of enrichment with calcium on adhesiveness [mJ] of kefir during storage (means \pm SD, $n = 15$)

Dose of enrichment with calcium mg of calcium for 100 g of milk	Storage time, days			
	1	7	14	21
0	2.67 \pm 0.66 ^{Aa}	3.60 \pm 0.43 ^{Aa}	3.07 \pm 0.76 ^{Aab}	4.10 \pm 0.92 ^{Aa}
5	2.73 \pm 0.31 ^{Aa}	3.20 \pm 0.22 ^{Aa}	3.97 \pm 1.08 ^{Aa}	2.90 \pm 0.36 ^{Aa}
10	2.47 \pm 0.05 ^{Aa}	3.37 \pm 1.38 ^{Aa}	3.43 \pm 0.29 ^{Ab}	2.77 \pm 0.47 ^{Aa}
15	1.63 \pm 0.30 ^{Aa}	2.97 \pm 0.12 ^{Aa}	2.10 \pm 0.14 ^{Ab}	3.07 \pm 0.59 ^{Aa}
20	3.03 \pm 0.98 ^{Aa}	2.07 \pm 1.57 ^{Aa}	3.17 \pm 0.49 ^{Abc}	2.60 \pm 0.28 ^{Aa}
25	1.57 \pm 1.11 ^{Aa}	2.35 \pm 0.15 ^{Aa}	1.27 \pm 0.90 ^{Ac}	2.20 \pm 0.10 ^{Aa}
30	3.13 \pm 1.32 ^{Aa}	3.07 \pm 1.16 ^{Aa}	2.37 \pm 0.33 ^{Abc}	2.27 \pm 1.62 ^{Aa}
Simple correlation coefficient (<i>r</i>) between the dose of calcium enrichment and adhesiveness	-0.019	-0.218	-0.499	-0.624

A – different capital letters with mean values in rows indicate statistically significant differences at $p \leq 0.05$.

a-c – different small letters with mean values in columns indicate statistically significant differences at $p \leq 0.05$.

Table 6. Influence of dose of enrichment with calcium on gumminess [N] of kefir during storage (means \pm SD, $n = 15$)

Dose of enrichment with calcium mg of calcium for 100 g of milk	Storage time, days			
	1	7	14	21
0	0.37 \pm 0.26 ^{Aa}	0.63 \pm 0.00 ^{Bab}	0.69 \pm 0.02 ^{Ba}	0.67 \pm 0.04 ^{Bab}
5	0.58 \pm 0.04 ^{Aa}	0.67 \pm 0.02 ^{ABa}	0.72 \pm 0.08 ^{Ba}	0.70 \pm 0.00 ^{Bb}
10	0.56 \pm 0.04 ^{Aa}	0.59 \pm 0.03 ^{Ab}	0.69 \pm 0.04 ^{Ba}	0.71 \pm 0.02 ^{Bc}
15	0.58 \pm 0.02 ^{Aa}	0.61 \pm 0.03 ^{ABb}	0.65 \pm 0.05 ^{Bab}	0.66 \pm 0.03 ^{Babc}
20	0.38 \pm 0.27 ^{Aa}	0.60 \pm 0.02 ^{ABb}	0.61 \pm 0.02 ^{Bb}	0.64 \pm 0.03 ^{Bab}
25	0.35 \pm 0.25 ^{Aa}	0.63 \pm 0.00 ^{Bab}	0.57 \pm 0.02 ^{ABb}	0.62 \pm 0.00 ^{Ba}
30	0.60 \pm 0.02 ^{Aa}	0.60 \pm 0.04 ^{Ab}	0.66 \pm 0.03 ^{Aab}	0.66 \pm 0.04 ^{Aabc}
Simple correlation coefficient (<i>r</i>) between the dose of calcium enrichment and gumminess	-0.280	-0.225	-0.479	-0.543

A-B – different capital letters with mean values in rows indicate statistically significant differences at $p \leq 0.05$.

a-c – different small letters with mean values in columns indicate statistically significant differences at $p \leq 0.05$.

Quantity of introduced calcium significantly differentiated the gumminess of kefir on the 14th and the 21st day of analysis (Table 6). The confirmation of occurring relationship is significant negative correlation coefficient indicating that with increasing dose of enrichment with calcium the gumminess of kefir decreases.

Mituniewicz-Małek et al. (2009) during the storage of fermented milk beverages, observed the greatest differences in the level of hardness in the first days of the experiment. Results obtained by these authors showed also that hardness and viscosity of kefir from sheep milk's were not significantly influenced by the composition of starter cultures used in the production. Sady et al. (2009) observed that supplementation of milk solid for kefir produced from skimmed milk caused the increase of the adhesiveness of products. The type of kefir (supplementation in skim milk powder (SMP) and/or whey protein concentrate (WPC) in comparison to the control kefir) did not have significant influence on the texture parameter obtained from TPA analysis.

According to Cais-Sokolińska et al. (2008) the rich microflora of kefir cultures contributes to the development and liberation of many compounds influencing the sensory characters of the final product. The substances

used as carriers of calcium should not affect the change of colour, taste and smell of the fermented beverage, shortening of expiry date, as well as cause changes in the product during transport and cold storage (Ziarno et al., 2009).

Figures 1–4 contain the results of sensory evaluation of selected experimental groups of kefir. Due to the presence of yeast in a starter culture there was evaluated the intensity of yeasty taste. With the increasing dose of calcium there increased the intensity of the yeasty ($r = 0.624$, $p \leq 0.05$) and a salty taste ($r = 0.745$, $p \leq 0.05$) of fermented milk beverages on the 1st day of analysis, while with increasing storage time there decreased the effect of the dose of enrichment on the perceptibility of yeasty and salty taste. It was observed that the quantity of applied calcium significantly reduces the intensity of sour taste of kefir assessed on the 7th and 14th days of cold storage ($r > 0.6$, $p \leq 0.05$). Moreover, there was no influence of the applied dose of calcium enrichment on the colour and consistency of kefir assessed in terms of subsequent analysis.

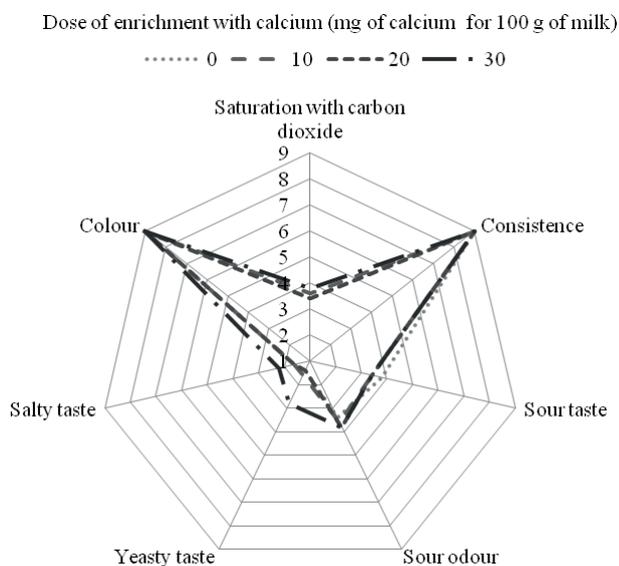


Fig. 1. Influence of dose of enrichment with calcium on sensory analysis of kefir on 1st day of storage (1–9 points)

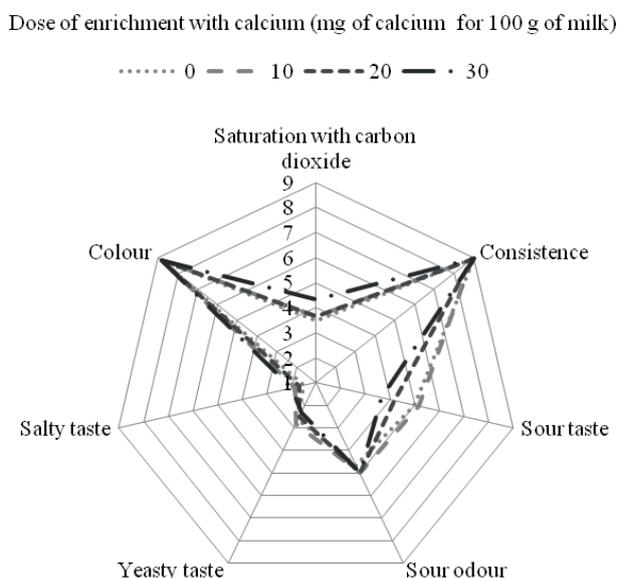


Fig. 2. Influence of dose of enrichment with calcium on sensory analysis of kefir on 7th day of storage (1–9 points)

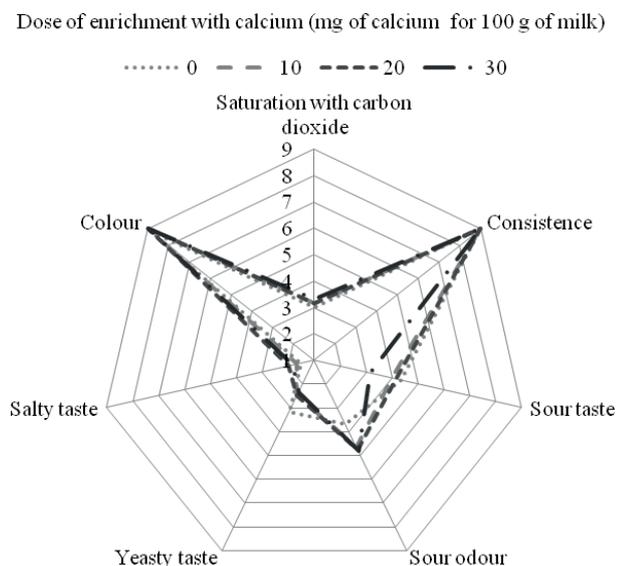


Fig. 3. Influence of dose of enrichment with calcium on sensory analysis of kefir on 14th day of storage (1–9 points)

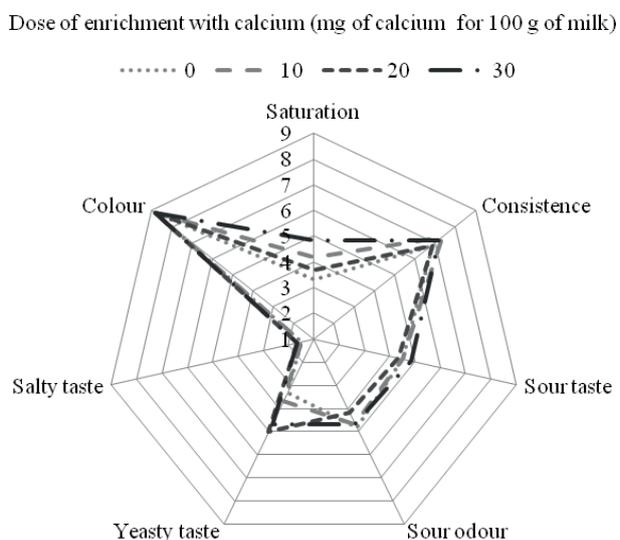


Fig. 4. Influence of dose of enrichment with calcium on sensory analysis of kefir on 21st day of storage (1–9 points)

CONCLUSIONS

Calcium bisglycinate may be used to enrich kefir with calcium even with 30 mg of calcium in 100 g of milk without the modification of the parameters of

production. With increasing doses of enrichment with calcium there decreased the hardness, adhesiveness and gumminess of kefir. The applied doses of calcium did not cause changes in the sensory characteristics

such as colour and consistency of the fermented beverages. Kefirs enriched with calcium bisglycinate were characterised by lower intensity of sour taste.

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