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INFLUENCE OF COAGULATION CONDITIONS ON THE MINERAL COMPOSITION OF ACID-COAGULATING CHEESES

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ABSTRACT

Cheese is a product of high nutritional quality, which is created by coagulation of milk. Acid-coagulating cheeses are produced by the action of high temperatures with the addition of organic acids. The aim of this study was to examine the influence of coagulation temperature and coagulant type on the mineral composition of cheeses, obtained by heat-acid coagulation of milk. Protein coagulation was performed at a temperature of 85 °C (samples 1, 3, 5) and 95 °C (samples 2, 4, 6), and three organic acids were used as coagulants, namely: citric acid (samples 1 and 2), tartaric acid (samples 3 and 4) and acetic acid (samples 5 and 6). The content of total ash in the cheese was determined by mineralization of the samples at 550 ºC. The instrument ICP-OES Optima 8000, manufactured by Perkin Elmer, was used to determine the content of macroelements and microelements in the produced cheeses. Cheese samples produced with acetic acid had statistically significantly higher ($p<0.05$) amounts of total ash compared to other samples. It was found that the coagulation temperature and the type of coagulant statistically significantly ($p<0.05$) affect the mineral composition of the produced cheeses. The following element concentrations were determined (in mg/100 g): calcium 84.10-246.62; sodium 79.07-114.09; potassium 29.96-40.28; magnesium 5.88-11.22; phosphorus 56.86-209.23; zinc 2.75-3.41. Other identified elements were present in much smaller quantities in the tested samples.

Keywords: milk, cheese, minerals.

INTRODUCTION

Mammalian milk is the first food for mammals, and as such, supplies all the energy and nutrients needed to ensure proper growth and development in the postnatal period. The consumption of milk in mammals generally stops after the weaning period, except in humans, who consume milk even during adulthood. Generally, dairy foods are commonly considered balanced and nutritive foods, being frequently included as important components of a healthy diet (Pereira, 2014). Although there are several milk products, the general term milk should only refer to cow's milk produced by healthy animals and excluding the lactic secretion between 30 days before and 8 days after birth, or until it is almost completely free of colostrum (Pravilnik o proizvodima od mlijeka i starter kulturama [POPMST], 2011). The chemical composition of milk can be influenced by several factors such as animal species and genetics, environmental conditions, lactation stage, and animal nutritional status (Caroli, Chessa, & Erhardt, 2009; Kalac, & Samkova, 2010).

Dairy products in general, and especially milk as their raw material, have a particular micronutrient composition. Minerals are present in all foodstuffs and they are a group of important nutrients. On average, mineral elements account for 4% of total body mass and part of every tissue, liquid, cell, and organ in the human body. Minerals, both independently or in proper balance with other minerals, have structural, biochemical, and nutritional functions that are very important for overall mental and physical human health (Vahčić, Hruškar, Marković, Banović, & Colić-Barić, 2010).

Milk has been naturally recognized as a rich source of calcium. Moreover, in its mineral fraction, several other elements can be distinguished such as phosphorus, magnesium, zinc, and selenium (Gaucheron, 2011; Pereira, 2014). Twenty minerals are nutritionally essential for humans. These are sometimes classified into two groups, i.e., the macrominerals (sodium, potassium, chloride, calcium, magnesium, and phosphorus) and trace elements (iron, copper, zinc, manganese, selenium, iodine, chromium, cobalt, molybdenum, fluoride, arsenic, nickel, silicon, and boron). A certain concentration of the 20 minerals is present in milk. Therefore, these could be considered as 'milk minerals' (Cashman, 2006). The mineral content of milk is not constant but is influenced by many factors such as the stage of lactation, nutritional status of the animal, and environmental and genetic factors (Cashman, 2006; Vahčić et al., 2010).

Large quantities of milk are processed to produce various kinds of cheese, which represent the most important and largest group of dairy products. Cheese is obtained by coagulation of milk along with the separation of whey. Cheese is the crown of dairy production, which is the oldest and safest form of concentrating and preserving the nutritional value of milk. Cheese is not only a valuable dairy product but has recently become a favourite food to enjoy, especially in countries with a high standard of living, where its consumption is also growing (Popović-Vranješ, 2015). There are many different types of cheese on the market today. Acid and acid–heat coagulated cheeses are cheese varieties produced by the coagulation of milk, cream, whey or blends thereof via direct chemical acidification, culture acidification or a combination of chemical acidification and high heat treatment (Chinprahast, Subhimaros, & Pattom, 2015; Farkye, 2017). Various organic acids can be used as coagulants in the production of cheese, like citric or lactic acid, calcium lactate, lemon juice, sour whey etc. (Khan, Pal, Wan, & Salahuddin, 2014; Kumar, Gupta, Kumar, & Kumar, 2015; Cao et al., 2017; Bankar, Raziuddin, & Zanjad, 2018). Depending on the coagulants used, various kinds of cheese with different physical, chemical and sensory properties are obtained (Zhang, Li, Feng, & Dong, 2013; Oštarić, Antunac, Prpić, & Mikulec, 2015; Shanaziya, Mangalika, & Nayananjalie, 2018). Acid-coagulated cheeses are formed by lowering the pH value to reach the isoelectric point of casein by lactic acid fermentation or by adding different types of organic acids to milk. A specific group of acid-coagulated cheeses consists of those obtained by heat-acid coagulation i.e., the activity of organic acids at high temperatures (Jovanović, Stanišić, & Maćej, 2000; Popović-Vranješ, 2015; Chinprahast et al., 2015).

This study aimed to examine the influence of coagulation temperature and type of coagulant on the mineral composition of cheeses obtained by heat-acid coagulation of milk.

MATERIAL AND METHODS

Pasteurized cow's milk containing 3.2% fat (produced by Pađeni Dairy, Bileća from the Entity of Republic of Srpska, Bosnia and Herzegovina) bought in a local supermarket was used to produce cheese. The milk was stored in the refrigerator at a temperature of +4 °C before it was processed into cheese. Cheese production was carried out in the milk processing plant (mini cheese factory) at the Agricultural School, Banja Luka. Six models of cheese samples were produced for this experiment (Table 1). Fresh pasteurized milk was heated to the appropriate temperature (85 °C and 95 °C), and various organic acids (citric, tartaric and acetic acid) were used to coagulate the milk. After the addition of acid, the mixture was mixed well and rested for 10 min to obtain curd. The resulting curd was filtered through gauze into a strainer. The hot drained curd was weighed and 1.5% of salt was added in it. After salting and mixing, the curd was placed for pressing in a mould. The pressing took one hour, and the load was 2 kg per one kilo of cheese. The finished cheese was cooled in a refrigerator to +4 °C and stored at the same temperature until the analysis.

The total content of ash in tested cheeses was determined gravimetrically after heating of sample in a muffle furnace (Micronal) at 550 °C. The ash is an inorganic residue after incineration of the sample. The ash content in the tested samples is expressed in g/100 g, ie in percent (%) (Carić, Milanović, & Vucelja, 2000).

Determination of minerals was performed by burning with a mixture of nitric and perchloric acid. The measured quantity of the test sample with 10 ml of concentrated nitric acid was poured into Kjeldahl tubes. The mixture was heated over an asbestos mesh on a low burner until the evolution of nitrogen gases ceased. The contents of the tube were kept at a gentle boil with frequent stirring. It was then evaporated almost to dryness and added was 10 ml of dilute nitric acid and 10 ml of concentrated perchloric acid. Afterwards, it was heated very carefully until the substance burned out, which could be determined by creating a colourless or weakly coloured solution. After evaporation, it was dissolved in dilute hydrochloric acid and heated until the salts were completely dissolved and filtered. The filtrate was diluted with distilled water to the mark and samples were taken from it for the necessary determinations (Trajković, Baras, Mirić, & Šiler, 1983).

Table 1. Acid-coagulated cheese production requirements.

Sample	Type of coagulant	Amount of coagulant (%)	Coagulation temperature (°C)
1	Citric acid	0.3	85
2	Citric acid	0.3	95
3	Tartaric acid	0.3	85
4	Tartaric acid	0.3	95
5	Acetic acid (9%)	1.5	85
6	Acetic acid (9%)	1.5	95

The instrument ICP-OES Optima 8000, manufactured by Perkin Elmer, was used to determine the content of macroelements and microelements in the produced cheeses. Before the measurement, argon and hydrogen gases were put into operation at the valves located outside the building. After that, the same gases were released through the internal valves, the compressor and the Chiller were turned on, and then the ICP-OES Optima 8000 instrument itself. It was necessary to turn on the instrument for about 15 minutes before measuring. After that time, the software in which the plasma was ignited was started. Its stabilization took a certain time (10 to 15 minutes).

Certified Reference Materials (CRM) from the same manufacturer at a concentration of 100 µg/mL were used as the standard for instrument calibration. Five standard solutions of different concentrations were prepared for calibration. The following concentrations were used as trace elements: 0.1; 0.5; 1.0; 2.0 and 5.0 µg/mL. The macronutrients used for the concentrations were 1.0; 5.0; 10; 15 and 20 µg/mL. The standards were prepared in 10 mL volumetric flasks. The pipetting was performed using different micropipettes depending on the desired concentration of the standard, and the volumetric flasks were then supplemented to the mark with a solution of 5% HNO3. After the preparation of the calibration curve, the samples and the blank were analysed. The blank of the standard was 5% HNO3.

Statistical processing of the obtained results was performed using the Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 computer program for Windows (Armonk, NY, United States). The results obtained in this paper are presented as mean values of the individual results of three randomly selected product samples ± standard deviation (SD). The significance of differences between arithmetic means was determined by analysing the variance with one independent variable (One way ANOVA) and multiple interval tests (*Tukey HSD – test*) and expressed with 95% probability ($p<0,05$).

RESULTS AND DISCUSSION

Ash was the residue obtained after incineration of the samples. It consisted of mineral residues after incineration of the sample. The ash content in the tested cheese samples was ranged from 1.25% in sample 3, which was produced at 85 °C with the addition of tartaric acids, to 2.04%, in sample 6, which was produced with acetic acid at 95 °C. Statistical analysis of the results showed that cheese samples produced with acetic acid had statistically significantly higher amounts of ash ($p<0.05$) compared to other samples. On the other hand, the ash content in samples produced with citric and tartaric acids was not statistically significantly different ($p>0.05$) (Table 2).

Table 2. Ash content in the tested cheese samples.

Sample	*Ash content* (%)
1	1.27[a] ±0.05
2	1.41[a] ±0.03
3	1.25[a] ±0.05
4	1.46[a] ±0.08
5	1.87[b]±0.07
6	2.04[b] ±0.05

[a-b] mean values with different letters in the same column differ statically significantly with 95% probability ($p < 0.05$)

The quality of dairy products is also determined by the quantitative content of minerals. The mineral content of cheese samples depends on various factors such as feeding, genetics, lactation period, geographical area of milk production, environmental conditions, lack of a standard technique in cheese production and possible contamination from the equipment during the cheese production (Kose, & Ocak, 2019). Milk and dairy products, especially cheese, are a good source of macrominerals and trace elements playing an important role in maintaining good health (Park, 2009).

Technological processes of dairy products production affect the content of Ca, Na, K and Mg in these products. These elements participate in coagulation processes and whey separation and affect the thermal stability and the ability of milk to coagulate, as well as the texture of the cheese. Calcium chloride is added to milk to compensate for the loss of Ca during pasteurization and to improve the cheese-making process. Sodium chloride may also be added during processing to compensate for lost sodium (Bilandžić, Sedak, Đokić, Božić, & Vebić, 2014).

Table 3 shows the mean values of macrominerals content in the examined cheeses. Based on the obtained results, it was determined that the coagulation temperature and the type of coagulant statistically significantly ($p<0.05$) affected the mineral composition of the produced cheeses.

Of the 20 essential minerals, calcium is certainly the 'milk mineral' that most people associate with bone health. It is present in milk in relatively high levels so that 200 mL of milk (a typical serving) would provide about 22% of the current US RDA (Cashman, 2006). The highest calcium content in the tested cheeses was recorded in samples produced with acetic acid (237.14 mg/100g in sample 5 and 246.62 mg/100 g in sample 6), while cheeses produced with citric and tartaric acid had a statistically significant ($p<0.05$) lower content of calcium (Table 3).

An important ingredient in cheese is sodium chloride. It reduces the amount of water in the cheese, affects the formation of the crust, promotes protein swelling, helps shape the plasticity of the curd, acts selectively on the microflora, affects the sensory properties of cheese, and improves cheese durability (Tratnik, 1998). Although sodium participates in many essential metabolic functions in the human body and plays an important role in maintaining water balance in cells, excessive sodium intake is associated with certain health problems in humans, such as high blood pressure and other chronic diseases (Felicio et al., 2013). Nowadays, there is plentiful evidence that food consumption is related to health. All nutritional trends are moving towards a reduction of fat, sugar, and salt in food. Increased sodium intake can be crucial for the incidence of hypertension, a phenomenon observed in modern societies, especially in the elderly. Sodium intake far exceeds nutritional recommendations, especially in modern and industrialized countries. The main source of sodium in food products is derived from sodium chloride i.e., table salt (Lilić, Borović, & Vranić, 2014). The sodium content of the tested cheeses ranged from 79.07 mg/100 g (sample 6) to 114.09 mg/100 g (sample 2) (Table 3).

The metabolism of water in the human body is affected and myoneural activities are stimulated by Na as well as K. According to nutritional recommendations, the sufficient Na and K proportion in the diet must be 0.6 for adults and 0.5 for children (Cruz et al., 2011).

In the examined cheese samples, the statistically lowest ($p<0.05$) potassium content was found in sample 6 (29.96 mg/100g), while sample 1 had the highest potassium content (40.28 mg/100g).

Although not so abundant, magnesium can also be found in milk as well as in other dairy products (Cashman, 2006; Pereira, 2014). The highest magnesium content was recorded in cheeses produced with acetic acid (9.73 mg/100 g and 11.22 mg/100 g in samples 5 and 6 respectively), while the magnesium content in cheeses produced with citric and tartaric acid was statistically significantly lower ($p<0.05$).

Milk and dairy products are also recognized as a good source of phosphorus, which is present in organic and inorganic forms. Organic phosphate is bound to organic molecules like proteins, phospholipids, organic acids, and nucleotides, which are present mainly in the micellar phase; whereas the inorganic form corresponds to the ionized phosphate, which depends on the pH value and is in the aqueous phase. Like calcium, both forms are in equilibrium and their distribution may depend on conditions like pH. The average concentration of phosphorus in milk is about 950 mg/L (Pereira, 2014). In this paper, the lowest phosphorus content was found in sample 2 (56.86 mg/100 g), and the highest in sample 6 (209.23 mg/100g).

Table 3. The content of macrominerals in the examined cheese samples.

Elements	Concentration (mg/100g)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Ca	$84.10^{a}\pm0.14$	$120.82^{b}7.99$	$85.27^{a}\pm9.37$	$105.42^{a,b}\pm4.70$	$237.14^{c}\pm3.83$	$246.62^{c}\pm8.31$
Na	$106.94^{b,c}\pm7.19$	$114.09^{c}\pm2.10$	$108.39^{b,c}\pm11.93$	$94.82^{a,b,c}\pm5.50$	$84.64^{a,b}\pm5.11$	$79.07^{a}\pm4.83$
K	$40.28^{c}\pm0.57$	$39.19^{b,c}\pm0.37$	$38.24^{b,c}\pm3.87$	$32.56^{a,b}\pm1.34$	$36.94^{b,c}\pm0.97$	$29.96^{a}\pm0.60$
Mg	$6.43^{a}\pm1.36$	$6.27^{a}\pm1.03$	$5.88^{a}\pm0.34$	$6.39^{a}\pm0.04$	$9.73^{b}\pm0.23$	$11.22^{b}\pm0.67$
P	$87.34^{a}\pm1.16$	$56.86^{a}\pm80.41$	$107.13^{a,b}\pm6.21$	$129.28^{a,b}\pm0.92$	$191.88^{b}\pm6.58$	$209.23^{b}\pm2.54$

$^{a-c}$ mean values with different letters in the same row differ statically significantly with 95% probability ($p<0.05$)

Milk is also a good source of microelements like zinc and selenium (Pereira, 2014). Zinc plays an important role in nucleic acid synthesis, transcription, and translation as a cofactor for some of the enzymes involved and may therefore participate in a broad range of metabolic activities in bones. Zinc has also been shown to be required by enzymes that have specific functions in bone metabolism (Cashman, 2006). One litre of milk supplies 3 to 4 mg of zinc, and selenium is present in an average concentration of 30 mg/L (Pereira, 2014). Iron is an essential trace element that is incorporated as a catalyst in various metabolic reactions. It is a component of haemoglobin, myoglobin, cytochrome, and other proteins and plays an important role in the transport, storage and use of oxygen. Milk and dairy products are insufficient sources for human nutrition in terms of Fe (Zamberlin, Antunac, Havranek, & Samaržija, 2012). Fe deficiency causes anaemia, a decrease in immunity and alteration in mental development (Gaucheron, 2000). Based on the results obtained in this paper, it was determined that the content of microelements in the tested cheeses ranged as follows (mg/100g): zinc 2.75-3.41; chromium 0.00-0.08; iron 0.001-0.38; manganese 0.00-0.024, molybdenum 0.00-0.081 and selenium 0.00-0.10 (Table 4).

The mineral composition of milk is not constant and depends on the stage of lactation, animal nutrition, environmental conditions, and genetic factors. Mineral elements are found in the form of inorganic ions and salts in milk and dairy products, or as part of organic molecules such as proteins, fats, carbohydrates, and nucleic acids. The chemical form in which the mineral elements are present is very important because it affects the absorption in the stomach and thus their biological utilization (Zamberlin et al., 2012). In every country, traditional or industrial production in cheese factories has its specific processes. Therefore, each cheese is characterized by a certain mineral composition that affects the nutritional values and sensory properties of the product. Bilandžic et al. (2014) determined the concentrations of macrominerals and microelements in different samples of cheese using the inductively coupled plasma emission method (ICP-OES). The following concentrations of elements (in mg/kg) were determined: calcium 802.2-7449, potassium 294.5-2014, sodium 414.6-11502, magnesium 85.1-288.2, zinc 21.8-63.1, iron 2.01-5.10, copper 2.06-2.84, selenium 0.075-0.56. Holland et al. (1995) determined the content of sodium 300-1440 mg/100 g, potassium 77-160 mg/100 g, calcium 73-1200 mg/100 g, phosphorus 100-810 mg/100 g, magnesium 9-45 mg/100g in different types of cheese.

Table 4. The content of microminerals in the examined cheese samples.

Elements	Concentration (mg/100g)					
	1	2	3	4	5	6
Zn	2.75±0.28	2.91±0.10	2.78±0.29	3.41±0.07	3.04±0.02	3.11±0.15
Cr	N. D	N. D	0.08±0.11	N. D	N. D	0.05±0.08
Fe	0.07±0.04	0.23±0.14	0.27±1.52	0.001±0.001	0.16±0.15	0.38±0.46
Mn	N. D	0.002±0.002	0.024±0.03	N. D	0.003±0.003	0.008±0.01
Mo	0.004±0.004	N.D	0.02±0.002	0.002±0.003	0.008±0.01	0.081±0.11
Se	$0.077^{b,c}$±0.02	0.101^{c}±0.00	$0.069^{a,b,c}$±0.03	$0.019^{a,b}$±0.02	N. D	N. D

$^{a-c}$ mean values with different letters in the same row differ statically significantly with 95% probability (p <0.05); N. D - not detected

CONCLUSIONS

Based on the obtained results, it can be concluded that coagulation conditions have a statistically significant effect (p<0.05) on the content of total ash, which consists of mineral residues after incineration of the tested cheeses. Compared to other cheese samples, the samples produced with acetic acid had statistically significantly higher (p<0.05) amounts of total ash. It was found that the coagulation temperature and the type of coagulant statistically significantly (p<0.05) affect the content of macroelements and microelements in the produced cheeses. The following element concentrations were determined (in mg/100 g): calcium 84.10-246.62; sodium 79.07-114.09; potassium 29.96-40.28; magnesium 5.88-11.22; phosphorus 56.86-209.23; zinc 2.75-3.41. Other elements identified in the tested samples were present in much smaller quantities.

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