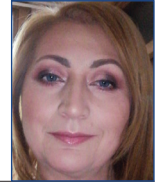


Mineral elements in sheep cheese



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Abstract

The indigenous Travnik (Vlasic) cheese is produced in central Bosnia in the area of Mt. Vlasic. This cheese belongs to the group of white soft cheeses, ripened in brine under anaerobic conditions, and made from raw, thermally untreated sheep's milk. The production technology is very simple and is adapted to mountainous conditions. Cheese is an important source of essential nutrients, in particular proteins, fat, vitamins and minerals. The aim of this study was to determine the concentration of macroelements (Ca, Na, K, Mg, P) and microelements (Zn, Fe, Cu) in 15

samples of Travnik sheep cheese using the atomic absorption spectrometry (AAS) and ultraviolet-visible spectrometry (UV-VIS) methods. A tendency of increasing Mg and K, Fe and Ca, Fe and Mg, and Fe and P was determined in the examined samples, but without statistical significance. The correlation coefficient values showed statistical validity at the level of high significance of differences ($P < 0.01$).

Key words: *sheep; cheese; mineral elements; AAS; UV-VIS*

Introduction

Travnik (Vlasic) cheese is an indigenous cheese of Bosnia and Herzegovina, traditionally produced in the area of Mt. Vlasic (elevation 1300 m). It belongs to the group of white soft cheeses matured in brine, also called white brined cheeses, made from raw, thermally unprocessed sheep's milk (Stojković et al., 2009; Hrković et al., 2011). In comparison with industrially

produced cheeses, which are produced under controlled conditions and strict procedures, indigenous cheeses have a variety of flavours, aromas and consistencies. Like most dairy products, cheese is a rich source of minerals, proteins, vitamins, fat and carbohydrates (Suliman et al., 2012). The mineral content of milk depends on numerous factors, including genetic characteristics,

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Table 1. Recommended nutrient intakes of the main minerals*

Analyte (mg/day)	Infants and Children (0 – 9 years)	Adolescents (9 – 18 years)	Male (19+years)	Female (19+ years)
Ca	300-700	1300	1000-1300	1000-1300
Mg	30-100	230	224-260	190-220
P	100-500	1250	700	700
Na	*	<2000*	<2000*	<2000*
K	*	3510	3510	3510
Fe	2.8-5.6	7.2-8.6	11.4	9.4-27.7
Cu	0.2-1	1.1-1.5	1.7	1.2
Zn	7.4-7.7	12.2-15.7	7	4.9

*According to the Sodium Intake for Adults and Children Guideline (WHO, 2012), sodium intake should be reduced to a maximum of 2000 mg per day for children for age of 2 to 16, and adults from the age of 16+. The recommendation for children does not address the recommended period of exclusive breastfeeding (0-6 months) or the period of complementary feeding with continued breastfeeding (6-24 months). *According to the Potassium Intake for Adults and Children Guideline (WHO, 2012), the recommendation for children does not address the recommended period of exclusive breastfeeding (0-6 months) or the period of complementary feeding with continued breastfeeding (6-24 months).

lactationstage, environmental conditions, type of pasture, soil contamination, etc. (Prpić et al., 2003; Gonzales-Martin et al., 2009). The mineral content of milk depends on the type of milk. Cow, sheep, goat and other types of milk contain certain amounts of sodium, potassium, magnesium, phosphorus and chlorine (Ćurin and Cetinić, 2007). The specificity of indigenous cheeses is also influenced by the traditional habits of the local population (Prpić et al., 2003). Accordingly, milk composition varies considerably. Cheese production requires milk with certain technological properties. Mineral elements are associated with compounds such as proteins, lipids and carbohydrates, and their effects on the body can vary significantly (Merdivan et al., 2004).

The chemical composition of milk is the basis that determines technological properties, and is directly dependant on mammary health. An increased somatic cell count in milk results in a change in milk composition, seen in the decrease in

content of casein, lactose and calcium, and an increase in content of chlorine, sodium and serum protein. The mineral content of cheese depends on the origin of the milk (cow, ewe, goat), and its ripening process. There is sufficient evidence that minerals, both independently or in proper balance with other minerals, have structural, biochemical and nutritional functions that are very important for human health, both mental and physical (Vahčić et al., 2010). Twenty mineral elements are essential to the human diet: sodium, potassium, chloride, calcium, manganese, selenium, iodine, chromium, cobalt, molybdenum, fluorine, arsenic, nickel, silicon and boron. These essential minerals are occasionally classified into two groups: major elements (macrominerals) and trace elements (or microminerals). The concentration of major elements (sodium, potassium, chloride, calcium, magnesium and phosphorus) in the human body exceeds 0.01% of total body mass, whereas trace elements (the remaining 14 elements) are

present at much lower concentrations, and their dietary intake may be lower than 100 mg/day (Zamberlin et al., 2012). Minerals are inorganic nutrients, usually required in small amounts from less than 1 to 2500 mg per day, depending on the mineral (Table 1). The recommended daily needs in nutrients for different age and gender groups are given in Table 1.

In general, cheese is rich in the fat and casein constituents of milk, which are retained in the curd during production, and it contains relatively small amounts of water soluble constituents (whey proteins, lactose, and water-soluble vitamins). The aim of this study was to determine the concentrations of macroelements (Ca, Na, K, Mg, P) and microelements (Zn, Fe, Cu) in 15 samples of indigenous Travnik sheep cheese.

Materials and methods

To determine the mineral content and determine the physiological values of indigenous Travnik cheese ($n=15$), approximately 300 g of cheese was sampled for testing. Cheese bearing the declaration "Travnik cheese" was purchased from individual producers and from the market. Samples were placed in pure polyethylene bags, labelled and kept at -18 °C until analysis. Analyses were intended to determine the values from the aspect of total mineral content for the Travnik area and individually from the aspect of nutrition, climatic conditions and altitude, and to determine the concentration of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), iron (Fe), copper (Cu) and zinc (Zn) in samples of Travnik (Vlasic) cheese. Analyses were performed using atomic absorption spectrometry (AAS) and ultraviolet-visible spectrometry (UV-VIS).

Cheese samples (5 g) were prepared by dry mineralization. The samples were first dried (ST-05 Dryer, Instrumentaria

Zagreb, Croatia) at 105 °C and placed in a muffle annealing furnace (LP-08 Muffle Furnace, Instrumentaria Zagreb, Croatia) at 550 °C, and incinerated for 3-5 h, up to light ash. Following mineralization, samples were digested with 5 mL HCL and quantitatively transferred to a 50 mL graduation vessel and made up to the mark with ultra-pure water. Prepared solutions were used for the analysis of all the minerals, except Fe. Samples for Fe analysis were digested with 10 mL HCL and quantitatively transferred to a 50 mL graduation vessel and made up to the mark with ultra-pure water. The same procedure was used for the blank probe and yield control samples. Process control was performed with the milk powder reference material, NMIJ-7512A, which was treated the same as the samples.

The calibration curve method was used to determine the concentration of the tested minerals. For each element, a stock standard was prepared, which was then used to prepare five standard solutions of known concentrations of minerals. Instrument calibration was performed with certified standard solutions of test elements: Ca, K, Na, Mg, Fe, Cu, Zn and P for Atomic Absorption Spectrometry (AAS) at a concentration of 1000 mg/L (Carlo Erba, UK). Working standards were prepared by diluting certified standards with the recommendations of Perkin Elmer Analytical Methods for Atomic Absorption Spectrometry (1994 Perkin Elmer Corporation).

Determination of Ca, K, Na, Mg, Fe, Cu, and Zn concentrations was performed using atomic absorption spectrometry, a flame technique (PinAAcle 900T Atomic Absorption Spectrophotometer, Perkin Elmer). P content was determined using UV-VIS spectrophotometry (UV-VIS Spectrophotometer Lambda 25, Perkin Elmer) at a wavelength of 650 nm.

Samples were diluted with 1% strontium chloride to determine Ca

and Mg concentrations, and with 1% caesium chloride to measure K concentration, as recommended by Perkin Elmer Analytical Methods for Atomic Absorption Spectrometry (1994 Perkin Elmer Corporation). The concentration range of the calibration curve was from 1-5 ppm for Ca and 0.1-0.5 ppm for Mg. Regarding the analysis of Na, Cu and Zn content, measurements were performed directly in the prepared solutions. The concentration range of the calibration curve was 0.1-0.5 ppm for the determination of Na, 0.2-2.0 ppm for Cu and 0.1-0.5 ppm for Zn.

The concentration of Fe in samples was measured directly in a solution prepared with 10 mL HCL. The concentration range of the calibration curve for the determination of Fe was in the range 1-5 ppm and all standards were treated the same as samples. The content of P was measured in solutions prepared with a specific aliquot of the sample, depending on the expected concentration and with the addition of the following reagents for the developed dyes: sodium sulfite, hydroquinone and sodium heptamolybdate. After adding the reagent, the measuring vessel was made up to the mark with ultra-pure water and left in the dark for 1 h until the colour developed, and P was measured in the prepared solutions at a wavelength of

650 nm in the concentration range of 2-10 ppm.

Based on these solutions, a calibration curve was obtained and was used to calculate the concentration of the test element in the system. The concentration of the standard was prepared so that there was a reproducible (linear) relationship between concentration and absorption. For each calibration curve, a blank probe containing all reagents except the sample was prepared. We measured the concentrations for Ca, Mg, Na, K, Fe, Cu, and Zn with AAS, and the concentration of P with UV-VIS. The results from the obtained calibration diagrams for each element were used to determine the concentration of these elements in the samples using mathematical calculations.

Statistical analysis

The results were statistically analysed using Microsoft Excel 2010, module Data Analysis. Element concentrations are expressed as the mean \pm standard error of mean ($X \pm S.E.M.$). Differences were considered statistically significant at the level of significance and correlation coefficient $P < 0.01^{**}$.

Results and discussion

Table 2 presents the minimum, maximum, mean and standard error

Table 2. The content of the main minerals in Travnik cheese

Analyte	N	Minimum	Maximum	Mean	S. E. M.
Ca (g/kg)	15	3293	7055	5088	0.319
Mg (g/kg)	15	0.115	0.555	0.223	0.028
P (g/kg)	15	1971	3911	2842	0.149
Na (g/kg)	15	3.14	46.87	26.46	3.333
K (g/kg)	15	0.245	0.732	0.487	0.033
Fe (mg/kg)	15	0.42	3.92	2.13	0.25
Cu (mg/kg)	15	0.27	0.79	0.46	0.04
Zn (mg/kg)	15	8.13	18	12.37	0.75

Table 3. Correlation factors (r) between the mineral components of Travnik cheese

Ratio	Correlation with P-value
Ca:P	0.719**
Ca:Mg	0.636**
Zn:Ca	0.671**
Fe:Zn	0.513**
Fe:Cu	0.650**
Cu:Mg	0.626**

**Statistical significance difference ($P < 0.01$)

for calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), iron (Fe), copper (Cu) and zinc (Zn) determined in the chemical analysis.

The highest value of macroelements was established for Na (26.46 g/kg) and the lowest for Mg (0.223 g/kg). Of the microelements, the highest value was found for Zn (12.37 mg/kg) and the lowest for Cu (0.46 mg/kg). Mineral content in cheese depends on a number of factors, including those related to milk (type of milk, animal breed and lactation period, feeding and geographical area of milk production, cheese making technologies, length of storage). Slaveska et al. (1987) monitored the dynamics of changing certain ingredients (minerals, Ca and P) during ripening and storage of indigenous Macedonian white cheese over a long period of time. They found lower values for Ca (1500-1550 mg/kg) and P (930-800 mg/kg) than reported here. Also, the Ca to P ratio was from 1.67 to 1.87, compared to the value of 0.719 obtained in this study. Cheeses made with rennet (ripe cheeses) contain a higher calcium and phosphorus content than fresh cheeses produced with the addition of microbial cultures, because the acid dissolves the Ca and P from casein and which then enters into the whey (Božanić, 2015). Martić, (2018) established that the concentration of almost all minerals in

fresh cheeses generally increases during storage. He reported a Ca concentration of 932,885 mg/g which is lower than in the present study (5,088 g/kg). In another study (Holland et al., 1995), the average concentration of calcium in fresh cottage cheese was 73 mg/g, while Tratnik and Božanić, (2012) reported a calcium concentration in fresh low fat cheese of 90 mg/kg. The recommended daily intake of calcium for adults is 1000-1300 mg, and dairy products are a major dietary source of this mineral (Zamberlin et al., 2012). Potassium is found in fresh cheeses mainly in top form (93%) with a smaller share in colloidal form (7%) (Abollino et al., 1998). Lante et al. (2006) found lower average values of potassium concentration in Italian fresh Crescenzi cheese (1069±52 mg/kg) and Squacquerone (1163±62 mg/kg) than reported here. Šnirc et al. (2019) reported a slightly lower average calcium concentration of 6850 mg/kg than obtained here, while zinc (23.2 mg/kg), copper (10.0 mg/kg) and iron (14.1 mg/kg) were several times higher than our obtained values.

The reason for this variation can be explained by the type of cheese, and by the time of ripening and storage of the cheese itself. Lukač Havranek et al. (2000) cited that about 80% of Ca and 38% of P remain in solid cheeses, as opposed to 20% and 37% in soft cheeses. The distribution of minerals in the tested samples of Travnik cheese was uneven, with a higher concentration of Na and Ca and a much higher Zn content compared to other minerals. In a study of the micro and macro elements in traditional cheese in the Banat area, Cozma et al. (2018) found that consuming 100 g of cheese covered 50% of the total recommended needs for Ca (10.51 - 13.79%), Mg (19.23 - 26.49%) and for Zn (4.52 - 10.21%). Magnesium is important in many physiological processes, such as protein and nucleic acid metabolism, neuromuscular transmission and muscle

contraction, bone growth and regulation of blood pressure. In Western countries, 16-21% of the total dietary magnesium is consumed from milk and dairy products (Cashman, 2002a; Zamberlin et al., 2012). The magnesium concentration was the lowest of all macroelements tested.

Unlike the major elements, microelements are present in the human body in concentrations less than 0.01% of the total body mass. Of the 20 essential minerals, 14 are trace elements: iron, copper, zinc, manganese, selenium, iodine, chromium, cobalt, molybdenum, fluorine, arsenic, nickel, silicon and boron. Scientific research on test animals have proven that some of these elements (arsenic, nickel, silicon and boron) are essential.

Therefore, it can be assumed that they are also essential for humans (Cashman, 2002b). Many micromineral elements are toxic; however, their concentrations in milk are too low to pose a threat to human health. Like the content of other minerals in milk, the concentration of trace elements is not constant. It depends on the lactation stage, nutritional status of the animal, and environmental and genetic factors (Cashman, 2002b). Zinc is very important for growth, sexual development, wound healing and normal functioning of the immune system and other physiological processes. Dairy products such as milk and cheese are very important in human nutrition, but are an insufficient source of zinc. It is estimated that in Western countries, the contribution of dairy products to total zinc intake ranges from 19 to 31% (Cashman, 2002b).

Table 2 shows the statistically significant correlations between individual microelements and macroelements. Calcium in the form of tricalcium phosphate is the basic substance of bone tissue, and is also part of the tooth. Insufficient dietary intake of calcium leads to bone disorders (rickets,

osteomalacia, osteoporosis), decreased blood coagulation and increased irritability of the nervous and muscular systems. The most significant source of calcium is milk and dairy products, which have the most favourable ratio of calcium to phosphorus. The recommended daily intake for men aged 19-65 years and for women aged 19-50 years is 1000 mg, while women over 51 years of age need 1300 mg. During pregnancy and lactation, the daily requirement is 1200 mg.

The content of calcium and certain minerals in the cheese depends on the type of cheese, or on the dry matter content of the cheese. The higher the dry matter content of the cheese, the harder it is and the higher is its mineral content (Kirin, 2011). The bioavailability of calcium from cheeses can be deduced from the fact that, for example, 100-150 g of Emmental cheese meets the recommended daily requirement for calcium. The nutritional value of cheeses also depends on the fat and the type of cheese.

Conclusions

Its high mineral content makes this cheese a highly valuable dietary product. The variations in mineral composition are due to the nutritional value and organoleptic properties of the product. The concentration of macronutrients and microelements in cheese is specific to each region and country, whether production is based on traditional or industrial methods.

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Minerali u ovčjem siru

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Autohtoni travnički (vlašički) se sir proizvodi u planinskom području centralne Bosne na planini Vlašić. Ovaj sir pripada skupini bijelih mekih sireva koji zriju u salamuri u anaerobnim uvjetima, proizvedeni od sirovog, termički neobrađenog ovčjeg mlijeka. Tehnologija proizvodnje je vrlo jednostavna i odgovara uvjetima proizvodnje u planinskim područjima. Sir je bogat izvor esencijalnih hranjivih sastojaka, posebice proteina, masti, vitamina i minerala. Cilj ovog istraživanja je određivanje koncentracija makro elemenata (Ca, Na, K, Mg, P) i mikro elemenata (Zn,

Fe, Cu) u 15 uzoraka autohtonog ovčjeg sira tehnikom atomske apsorpcijske spektrometrije (engl. *Atomic absorption spectrometry*, AAS) i spektrometrije ultraljubičastim zrakama (engl. *Ultraviolet-visible spectrometry*, UV-VIS). Tendencija povećanja Mg i K, Fe i Ca, Fe i Mg te Fe i P utvrđena je u uzorku ispitivanog sira, ali bez statističke značajnosti. Vrijednosti koeficijenta korelacije pokazale su statističku značajnost na razini velike značajnosti razlika ($P < 0,01$).

Ključne riječi: ovca, sir, makro elementi, mikro elementi, AAS, UV-VIS spektrometrija