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ANALYSIS OF THE MINERAL PROFILE OF MILK AND SWISS CHEESE FROM ŢARA DORNELOR IN RELATION TO SEASONAL AND TECHNOLOGICAL FACTORS

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ABSTRACT. Milk and cheese are important sources of macro and microelements, their amounts depend on the feeding method of the animal, the climatic and environmental conditions, the state of health and the lactation period, as well as the technology of milk processing. The purpose of this paper is to evaluate the mineral profile of raw milk and Swiss cheese produced in Ţara Dornelor in different seasons and obtained by different technologies. The results showed that during the summer season, milk is richer in K and Ca and poorer in Cu. Swiss cheese presented a lower content of Na, Ca and Mg in the summer period compared to the winter one, a similar trend being observed for Mn and Fe. The processing technology also determined significant variations ($p < 0.05$) in the content of micro and macro elements. The processing of Swiss cheese in a copper boiler determined an increase in the Cu content of up to 6 times compared to the product obtained in a stainless-steel boiler. These results are important both for processors interested in increasing the quality of their products, and for consumers aware of the importance of food with high nutritional value.

Keywords: *micro and macroelements, dairy products technology, season, Emmental cheese*

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INTRODUCTION

Dairy products are rich in micronutrients such as minerals that are implied in multiple vital processes for the body. Minerals from milk and dairy products are classified into macroelements (Ca, Mg, Na, K, P and Cl) and microelements (Fe, Cu, Zn and Se), being often found in various associations with other ions or organic molecules [1]. The distribution of minerals in milk takes place depending on the nature of the elements, for example K, Na and Cl being present in the aqueous phase, and Ca, P and Mg being bound to the casein micelles [1].

The content of micro- and macroelements in cheeses largely depends on the processing technology. Traditional Swiss cheese is made in copper boilers, but in countries such as Finland a practice is found that involves the manufacture of Emmental type cheeses in stainless steel boilers and the addition of copper sulfate solution in certain concentrations to ensure the copper level in the product finished similar to traditional cheese [2]. Copper in the technological process can inhibit the activity of lactic and propionic bacteria depending on the amount present, large quantities leading to ripening defects of the final product due to the modification of propionic fermentation [3]. Studies have shown that the presence of copper could reduce the risk of defects caused by C. tyrobutyricum and other clostridial species, thus improving the overall quality of Emmental cheeses [3]. The amount of micro and macro elements in cheeses depends on a series of factors such as the mineral content of the raw milk, which in turn is influenced by the state of health of the animal, breed, lactation period, feed, and growing environment, climatic conditions; the technological process of cheese manufacturing, which differs depending on its type (fresh, matured); in the case of matured cheeses, the ripening conditions (temperature, humidity) that influence the microbial activity, as well as the salting procedures on the surface of the products, have a major contribution [4].

The consumption of adequate amounts of macroelements is essential for the proper functioning of the body. Milk and dairy products can contribute to the supply of macroelements such as Ca, Mg, K, Na. Ca is a structural element of bones and teeth and is responsible for regulating the functioning of the nervous and muscular system, the transformation of prothrombin into thrombin, ensuring the permeability of the cell membrane involved in muscle contraction, being also a constituent of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase [5]. Na is the cation found in the largest quantity in the extracellular fluid and has an essential role in maintaining its volume, in blood pressure, in the transport of nutrients to and from the cells [6]. K is the predominant cation in the intracellular fluid and is involved in the acid-base balance, the regulation of osmotic pressure, the proper functioning of the nervous and muscular system and in glycogenesis [5]. Mg is a component of over 300 enzymes and has an important role in muscle contraction, the functioning of the nervous system, regulation of blood sugar and blood pressure, transmembrane transport of other ions, energy production and nucleic acid synthesis [7].

The microelements present in milk have an important role for human nutrition, as the consumption of dairy products can partially or totally cover the daily requirement. It is part of the structure of the enzyme glutathione peroxidase and some selenoproteins, also participating in a series of biological processes such as the reduction of oxidative stress, the activity of the immune system and the synthesis of thyroid hormones [8]. Fe is essential for ensuring the good functioning of the body as it is involved in the synthesis of hemoglobin by erythroblastic cells, Fe absorption being dependent on the interaction with other elements (for example Cd, Pb, Mn and Zn are Fe antagonists) [9]. The importance of Cu derives from its involvement in cellular energy, the synthesis of some neurotransmitters, in the biochemical process of collagen and elastin production, as well as in the absorption, storage and metabolism of Fe [9]. The role of Mn in the human body is to modulate the synthesis and metabolism of carbohydrates, fats and cholesterol, being also of major importance for the correct functioning of the brain and muscle tissue, but also for the production of DNA and RNA molecules [5]. Zn is part of many enzymes, thus being involved in DNA and RNA synthesis, in protein synthesis, macronutrient metabolism, in cell replication, in the proper functioning of the immune system and in the inhibition of the oxidative processes of unsaturated fatty acids [5,9].

The purpose of this paper is to highlight the effects of season and processing technology on the mineral content of cow's milk and Swiss cheese from Ţara Dornelor, a region with a history of producing this variety of cheese. To the best of our knowledge, there are no studies on the mineral profile of these products from Ţara Dornelor region, and the results are of major importance for the support of the producers and for the certification of the Dorna Swiss cheese.

RESULTS AND DISCUSSION

Mineral profile of milk

The content of some of the macro and microelements in the raw milk studied showed differences both between seasons and between producers (Table 1). Thus, the amount of K and Ca for unit A was significantly higher ($p < 0.05$) in the summer season (increase by 9.88% and 10.65% respectively), the content of Mg and Na not being significantly influenced. Conversely, for the milk from unit B, significant increases ($p < 0.05$) were observed in the amounts of Na (increase by 462.44%), Mg (increase by 121.08%), K (increase by 371.96%)

and Ca (increase by 68.24%) in the summer season compared to the winter season. On the other hand, significant differences ($p < 0.05$) were observed between the two processors regarding the amount of macroelements in both seasons. Thus, in the winter season, higher amounts of Na (4.90 times), Mg (1.64 times), K (5.40 times) and Ca (1.18 times) were obtained in the milk from unit A compared to unit B. In the summer season, higher amounts of Na (1.18 times), Mg (1.38 times) and Ca (1.29 times) were observed in milk from unit B and K content (1.28 times) lower compared to unit A. The content of micro and macro elements in milk depends on a series of factors such as animal species, season, animal nutrition, soil nature and farm location [10]. Macroelements such as Ca and Mg are found in milk in the form of calcium phosphate, calcium phosphocaseinate and free magnesium, associated with the colloidal suspension of casein micelles [11]. The amount of Na in milk is influenced by the animals feeding, the practice of supplementing the diet with sodium salts being known [11]. The presence of lactose, ascorbate, citrate, phosphopeptides and lactoferrin can have a significant effect on the absorption of macro and micro elements from milk in the human body [9]. Król et al. [12] reported lower values of the content of Ca and Mg in milk in summer compared to winter, similar to Gulati et al. [13] who obtained higher Ca, Mg and Na values in the autumn-winter period compared to the summer season. The differences could be related to the different feeding: in summer the animals were grazed outside, while in winter they were fed with hay.

Table 1. Minerals content (expressed as mg/kg d.b.) of raw milk in different seasons

Element	Unit A		Unit B	
	ITCL	VTCL	ICAL	VCAL
Macroelements				
Na	369.90 ± 4.10^{aA}	360.90 ± 4.00^{aY}	75.50 ± 1.20^{yB}	425.40 ± 6.50^{xX}
Mg	75.60 ± 3.80^{aA}	73.50 ± 1.20^{aY}	46.00 ± 1.50^{yB}	101.70 ± 1.20^{xX}
K	1869.80 ± 28.0^{bA}	2105.60 ± 26.60^{aX}	346.00 ± 2.10^{yB}	1633.00 ± 18.80^{xY}
Ca	2424.40 ± 89.70^{bA}	2682.50 ± 31.90^{aY}	2058.60 ± 125.60^{yB}	3463.00 ± 41.50^{xX}
Microelements				
Mn	0.02 ± 0.00^{aB}	0.03 ± 0.00^{aY}	0.11 ± 0.00^{xA}	0.09 ± 0.00^{yX}
Zn	1.23 ± 0.02^{bB}	2.99 ± 0.10^{aY}	3.27 ± 0.04^{xA}	3.24 ± 0.11^{xX}
Se	0.01 ± 0.00^{aA}	0.02 ± 0.00^{aX}	0.02 ± 0.01^{xA}	0.01 ± 0.01^{xX}
Cu	0.27 ± 0.01^{bA}	0.09 ± 0.01^{aY}	0.26 ± 0.01^{xA}	0.11 ± 0.01^{yX}
Fe	0.53 ± 0.01^{bB}	0.78 ± 0.03^{aY}	4.43 ± 0.04^{xA}	1.01 ± 0.04^{yX}

ITCL - milk sample from unit A from the winter period, VTCL - milk sample from unit A from the summer period, ICAL - milk sample from unit B from the winter period, VCAL - milk sample from the unit B from the summer period; a-b (for unit A) and x-y (for unit B) mean values followed by distinct lowercase letters in the same row indicate significant differences ($p < 0.05$) between seasons, A-B (for the winter season) and X-Y (for the summer season) mean values followed by distinct capital letters in the same row indicate significant differences ($p < 0.05$) between the processing units in the same season.

Regarding the microelement content of raw milk, significant differences ($p < 0.05$) were observed between seasons in the case of unit A, where Zn, Cu and Fe were found in greater quantities in summer compared to winter (increase with 143.09, 66.66, respectively 47.17%), while Mn and Se had no significant variations ($p > 0.05$). The same trend of increasing the concentration of Mn (by 22.22%), Cu (by 136.36%) and Fe (by 338.61%) during summer compared to winter was obtained in the case of milk from unit B, while the Se and Zn did not vary significantly ($p > 0.05$). Sola-Larrañaga and Navarro-Blasco [11] reported differences in Fe, Mn, Zn and Cu content of cow's milk in different seasons probably due to feed and metabolic adaptation to climate change. The amount of Fe in milk depends on the presence of lactoferrin and xanthine oxidase transferase enzymes, Fe, Zn and Cu from ruminant milk being mostly related to the casein fraction [14]. Our results regarding the content of Zn, Fe, and Mn are comparable to those obtained by Król et al. [15] for milk from different areas of Poland. The same authors reported higher values for Cu and Zn content in summer compared to winter [15], while Górska and Oprządek [16] reported an opposite trend. According to the opinion of some authors, Zn from animal feed decreases Cu absorption due to the interaction with its absorption system [15]. The values reported by the literature for Fe, Cu, Zn and Se differ due to the sample preparation protocol and the quantification methodology, generally the amounts being around 0.5 mg/L for Fe, 0.1 mg/L for Cu, 3-4 mg/L for Zn and 0.03 mg/L for Se [1]. The results of the present study are close to these values (Table 1).

Mineral profile of Swiss Cheese

The macroelement content of the Swiss cheeses from unit A was significantly different ($p < 0.05$) between the winter and summer periods (Table 2). If in the case of Na, Mg and Ca, in the summer periods the quantities were significantly lower ($p < 0.05$), as for K, it was found in larger quantities in the summer products compared to the winter ones. The Swiss cheese produced by unit B showed higher amounts of Na and Ca in the winter periods, while Mg and K were found in higher proportions in the summer samples compared to the winter ones. The differences between seasons for unit A in terms of Mn and Se content were not significant ($p > 0.05$), while the amounts of Zn and Fe recorded higher values in the winter periods compared to the summer ones. A higher content of microelements in the winter samples, except Zn, was also observed in the samples from unit B, the differences being significant ($p < 0.05$). The most significant change was observed regarding the Fe content which was much higher (up to 8 times higher) in the winter samples compared to the summer samples from both producers. Król et al. [12]

reported higher values for Ca and Mg in the curd obtained in the winter period compared to the summer season, which is consistent with the results of the present study. Minerals are usually present in the aqueous phase of milk or in an insoluble state, bound to casein micelles, and a higher concentration of macroelements in cheese could be due to the dry matter content of milk and/or reduced losses during processing [12].

Table 2. Minerals content (expressed as mg/kg d.b.) Swiss cheese in different seasons

	Unit A				Unit B			
	ITC1	**ITC2**	**VTC1**	**VTC2**	**ICA1**	**ICA2**	**VCA1**	**VCA2**
Macroelements								
Na	2181.40± 30.50^{cB}	4359.10± 61.00^{aE}	2172.10± 25.60^{cH}	2941.60± 30.40^{bM}	9095.10± 63.70^{xA}	7055.2± 35.50^{yD}	5786.00± 77.90^{zG}	3410.10± 44.60^{wL}
Mg	361.70± 14.50^{bA}	413.80± 34.30^{aD}	339.60± 5.10^{cH}	358.40± 5.60^{bL}	378.90± 24.20^{xA}	288.00± 6.30^{yE}	405.10± 5.80^{xG}	281.40± 5.00^{yM}
K	933.80± 14.00^{cB}	1379.30± 26.20^{aD}	815.60± 9.50^{dH}	12690± 20.10^{bL}	1311.20± 15.70^{yA}	1049.60± 4.20^{zE}	1693.30± 17.90^{xG}	1029.00± 15.40^{zM}
Ca	41047.20± 738.80^{bB}	58352.00± 700.20^{aD}	18157.50± 177.40^{dG}	22125.50± 200.10^{cL}	51948.90 ±987.00^{xA}	22908.30± 778.90^{yE}	18519.50 ±197.30^{zG}	15865.00± 227.80^{wM}
Microelements								
Mn	0.76± 0.01^{bA}	1.63± 0.02^{aD}	0.78± 0.03^{bG}	0.67± 0.02^{cL}	0.64± 0.01^{xB}	0.64± 0.01^{xE}	0.40± 0.01^{yH}	0.32± 0.01^{zM}
Zn	29.89± 0.51^{cB}	43.83± 0.61^{aD}	30.30± 1.03^{cH}	32.64± 1.11^{bL}	33.14± 0.36^{yA}	26.33± 0.37^{wE}	36.47± 1.24^{xG}	29.52± 1.00^{zM}
Se	0.02± 0.00^{aA}	0.02± 0.00^{aD}	0.01± 0.00^{aG}	0.002± 0.00^{aL}	0.04± 0.00^{xA}	0.004± 0.00^{yE}	0.02± 0.00^{xyG}	0.02± 0.00^{xyL}
Cu	19.54± 0.27^{aA}	10.80± 0.15^{bD}	19.81± 1.46^{aG}	12.50± 0.92^{bL}	0.67± 0.01^{yB}	1.69± 0.03^{xE}	0.57± 0.04^{zH}	0.50± 0.04^{zM}
Fe	12.50± 0.14^{bA}	52.59± 0.58^{aD}	12.67± 0.55^{bG}	10.24± 0.44^{aL}	20.89± 0.50^{yB}	44.87± 0.01^{xE}	4.70± 0.20^{wH}	5.75± 0.25^{zM}

ITC1 - Swiss cheese sample from unit A from winter period I, ITC2 – Swiss cheese sample from unit A from winter period II, VTC1 – Swiss cheese sample from unit A from summer period I, VTC2 - Swiss sample from unit A from the summer period II, ICA1 – Swiss cheese sample from unit B from the winter period I, ICA2 – Swiss cheese sample from unit B from the winter period II, VCA1 - Swiss cheese sample from unit B from the summer period I, VCA2 - Swiss cheese sample from unit B from the summer period II; a-d (for unit A) and x-w (for unit B) mean values followed by distinct lowercase letters in the same row indicate significant differences ($p < 0.05$) between seasons, A-B (for the winter period I) / D-E (for the winter period II) / G-H (for the summer period I) and L-M (for the summer period II) mean values followed by distinct capital letters in the same row indicate significant differences ($p < 0.05$) between the processing units in the same season.

The results presented by Kirdar et al. [4] for Çanak cheese (a type of Turkish semi-hard cheese) showed a decrease in the content of Ca, Mg, K and Zn in the summer season compared to the winter season, while for Cu, Fe and Mn the trend was opposite. These differences could be due to variations in the chemical composition of raw milk due to different metabolic rates and feed characteristics offered in the two seasons [4]. This finding can be confirmed in the present study since in summer the cows were grazed, while in winter they were feed with hay. On the one hand, Cu and Fe have an essential role in the nutritional and biological value of cheeses. On the other hand, they can create problems due to their catalytic effect on the oxidation processes of lipoproteins and proteins supporting the membrane of fat globules that generate unpleasant odor [4].

Regarding the comparison between the two producers, Swiss cheese from unit A presented a lower Na content in all the studied periods, compared to the samples from unit B. The amounts of Mg, Ca and K in Swiss cheese were significantly different between the two producers, the Ca content being higher in the samples from unit B due to the exogenous addition of calcium chloride. The Swiss cheese from unit A showed a higher content of Mn and Fe, significant variations ($p < 0.05$) being also observed in the case of Zn. There was no large variation in Se content between samples, while Cu was observed in up to 35 times higher amounts (Table 2) in the products from unit A due to the manufacturing technology involving the use of the copper boiler. The results obtained for Mg and Zn are comparable to those reported by Manuelian et al. [17] for Emmentaler type cheese, while the amounts of Ca, K, Na, Fe and Cu were higher compared to the mentioned work. Ca represents an important macroelement present in hard cheeses and indispensable in human nutrition. Walther et al. [18] state that this macroelement can be found in amounts of 6 to 11 g/kg in semi-hard and hard cheeses, a 50g portion of cheese providing a daily intake of about 1200 mg of calcium in the human nutrition. The results of the present study show a much higher Ca content of the Swiss cheeses which can ensure the necessary Ca by consuming a smaller portion. In general, the amount of Ca in cheeses is much higher compared to milk and it is found in the form of precipitates of calcium phosphate, calcium lactate and calcium carbonate [1]. It has been shown that phosphopeptides and free fatty acids in hard cheeses can form phosphopeptide-Ca or soap complexes [1]. The Na content correlates with brining and salting of the wheels in the ripening technology, which may explain its presence in Swiss cheese [19]. Mn content together with other rare elements has been shown to be an important predictor for traditional cheeses, and Mn itself can represent a geographic marker [20], a correlation that we can consider a geographic footprint considering that most of the Ţara Dornelor area is located on a rock rich in Mn associated with other elements. Another study showed that elements such as Cu, Mg, Mn, Ca, K and Na can be used as markers for authenticating the origin area of Emmental and Edam cheeses [21]. The transfer of mineral elements from the soil to the plants used as animal feed occurs only if they are found in inorganic form. If the minerals are found in organic form, the respective compounds must go through biochemical processes generated by the soil microflora that transform them into inorganic compounds to be absorbed by plants, especially through the roots [22].

Relationships between variables

The analysis of the relationships between the variables considered in the winter period (Table 3) highlighted significant direct correlations ($p < 0.05$) of the Na content with the content of Ca, Zn, Mg and Fe ($0.61 < r < 0.70$). Mg content was directly correlated with Ca, Zn, Mn, Cu and Fe ($0.57 < r < 0.98$, $p < 0.05$), while Ca content was directly correlated with Mn, Zn, Se, Cu and Fe ($0.51 < r < 0.96$, $p < 0.05$). Significant correlations ($p < 0.05$) were observed between some of the microelements studied, as follows: Mn directly correlated with Zn, Cu and Fe ($0.56 < r < 0.92$) and Zn directly correlated with Cu and Fe ($0.53 < r < 0.79$). Kirdar et al. [4] also reported significant correlations between Zn and Mn, Mg and Zn, Ca and Zn, Mn and Mg, Mn and Ca, Mg and Ca, Fe and Mn in the winter season.

Table 3. Pearson correlations coefficients for the winter season

Variable	Na	Mg	K	Ca	Mn	Zn	Se	Cu	Fe
Na	1.00								
Mg	0.70**	1.00							
K	0.16	0.17	1.00						
Ca	0.65**	0.96***	0.18	1.00					
Mn	0.42	0.85***	0.12	0.87***	1.00				
Zn	0.68**	0.98***	0.11	0.96***	0.92***	1.00			
Se	0.37	0.37	-0.06	0.51*	0.16	0.33	1.00		
Cu	-0.14	0.57*	-0.09	0.53*	0.56*	0.53*	0.03*	1.00	
Fe	0.61**	0.69**	0.10	0.63**	0.84***	0.79***	-0.12***	0.18	1.00

* significant at $p < 0.05$, ** * significant at $p < 0.01$, *** * significant at $p < 0.001$.

The Principal Components Analysis (Figure 1) for the winter period showed that the first component (PC1) explains 58.51% of the data variation, while PC2 explains 14.80% of it. Thus, K, Se and Cu variables and the ITC1 sample were associated with PC2, while Mn, Fe, Zn, Mg, and Ca and the rest of the analyzed samples were associated with PC1. A grouping of the milk samples is observed in the left quadrants, while the Swiss cheese samples are grouped in the right side, depending on the manufacturer (those from unit A in the upper quadrant, and those from unit B in the lower quadrant).

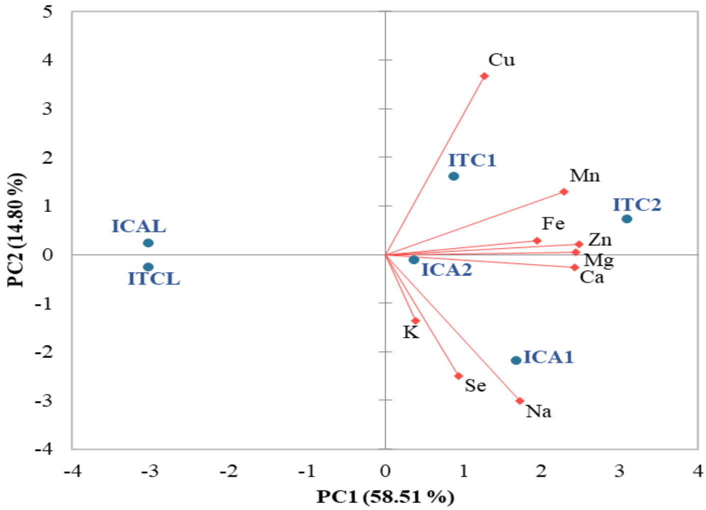

Figure 1. Principal Component Analysis bi-plot for the winter season

For the summer period, significant direct correlations ($p < 0.05$) were observed between the content of Na and Mg, Ca, and Zn ($0.77 < r < 0.87$), as shown in Table 4. The Mg content correlates directly with Ca, Mn, Zn, Cu ($0.75 < r < 0.99$, $p < 0.05$) and indirectly with K ($r = -0.58$, $p < 0.05$). Significant inverse correlations ($p < 0.05$) were also obtained for the content of K with Ca, Mn, Zn, Cu and Fe ($-0.83 < r < -0.62$).

Ca was directly correlated with Mn, Zn, Cu and Fe ($0.57 < r < 0.97$, $p < 0.05$), while Mn was directly correlated with Zn, Cu and Fe ($0.80 < r < 0.99$, $p < 0.05$) . Fe was significantly correlated with Cu, respectively Zn ($r = 0.92$, respectively $r = 0.74$, $p < 0.05$). Similar to our results, significant correlations were reported between Fe and Mn, Mg, Zn, respectively K, between Zn and Mn, Mg, Ca and K, between Mn and Mg, Ca, respectively K, between Mg and K, respectively K, as well as between Ca and K by Kirdar et al. [4] for cheese in the summer season.

The relationships between the studied variables during the summer period were highlighted through the Principal Components Analysis (Figure 2). The first component (PC1) explains 69.05% of the data variance, and the second one explains 22.13%. The PC1 component is associated with Fe, Mn, Ca, Mg, Zn and K content, while the PC2 component is associated with Na, Se and Cu. Regarding the analyzed samples, the milk samples (VTCL and VCAL), and the Swiss cheese samples VTC1 and VTC2 are associated with PC1, while the VCA1 and VCA2 samples are associated with PC2. A grouping of milk samples is observed in the left half of the graph, and the Swiss cheese samples from unit A are positioned in the negative right quadrant, while the cheese samples from unit B are located in the positive right quadrant. The content of K is placed in opposition to the content of Cu, Fe and Mn, according to the graph presented in Figure 2.

Table 4. Pearson correlations coefficients for the summer season

Variable	Na	Mg	K	Ca	Mn	Zn	Se	Cu	Fe
Na	1								
Mg	0.87***	1.00							
K	-0.25	-0.58*	1.00						
Ca	0.77****	0.97***	-0.67**	1.00					
Mn	0.44	0.82***	-0.78***	0.88***	1.00				
Zn	0.87***	0.99***	-0.62**	0.97***	0.80***	1.00			
Se	0.24	-0.05	0.22	-0.16	-0.33	0.02	1.00		
Cu	0.01	0.48*	-0.69**	0.57*	0.89***	0.44	-0.44	1.00	
Fe	0.34	0.75***	-0.83***	0.83***	0.99***	0.74***	-0.32	0.92***	1.00

* significant at p < 0.05, ** * significant at p < 0.01, *** * significant at p < 0.001.

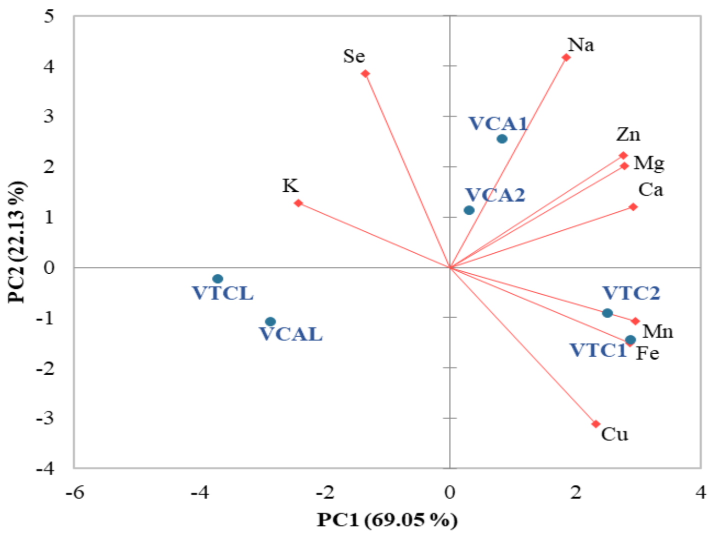

Figure 2. Principal Component Analysis bi-plot for the summer season

CONCLUSIONS

As the season and the milk processing technology to obtain Swiss cheese exhibited significant influences on the mineral profile of milk and Swiss cheese. The content of K and Ca in raw milk was higher in the summer period compared to the winter season in the case of both producers and with regard to the content of microelements, significant variations being observed for Zn and Fe. The season had a significant effect on the amounts of Na, Ca and Mg in Swiss cheese, which recorded lower values in summer compared to winter, with the content of Mn and Fe being also lower in the Swiss cheese samples from the summer season compared to the winter one. The influence of the processing technology was observed especially for the samples obtained in a copper boiler which presented more than 6 times higher content of Cu compared to the sample manufactured in a stainless-steel boiler. The obtained results highlight the rich nutritional value of the analyzed products and can be useful both to consumers for information and to producers to control the quality of the final product. As expected, our study presents some limitations regarding the variations regarding the quality of Swiss cheese samples due to the long ripening time and the spontaneous microflora that may not be constant, as well as to the limited study area since the Ţara Dornelor is the only area where Dorna Swiss cheese is produced. The results of our study are able to encourage the development of new research on the influence of season on the spontaneous microflora that outline the sensory and physico-chemical characteristics of Dorna dairy products.

EXPERIMENTAL SECTION

Sample collection and unit characteristics

To determine the mineral content of raw milk, samples were collected from two producers (UnitUnit A and UnitUnit B characterized in Table 5), in different seasons, winter and summer respectively (n=4). To determine the mineral content of Swiss cheese, the samples were collected from the same producers, in different seasons, namely two periods of winter 2022 and two periods of summer 2022 (n=8). The samples were investigated at a laboratory within the INCDTIM (National Research and Development Institute for Isotopic and Molecular Technologies) in Cluj-Napoca.

Table 5. Characteristics of the processing units

Characteristic	Unit A	Unit B
Production capacity	600 – 1000 L of which 420-840 L for Swiss cheese	2000 – 2400 L of which 500 L for Swiss cheese
Milk origin	The cooperative "Caşcaşaru Dornei"	Own farm, 40 micro farms and 4 commercial farms
Feeding type	Hay in winter and fresh grass in summer	Hay in winter and fresh grass in summer
Swiss cheese processing technology	Copper boiler and wood heating are used, lactic cultures are added	Double-walled stainless-steel boiler with water is used, no lactic cultures are used, calcium chloride is added

The stages of Swiss cheese production include: slow pasteurization of milk, addition of raw milk and homogenization, normalization, addition of lactic cultures (only in the case of unit A), coagulation, processing of the curd, scalding of the curd and draining of the whey, forming of the Swiss cheese wheel, pressing, brining, drying, maturation for 70-90 days and storage. Since the manufacturing period of the Swiss cheese exceeds 90 days, the data related to the atmospheric conditions were selected from the period prior to the processing of the analyzed milk (Table 6).

Table 6. Atmospheric conditions in 2022 (mean values) [23]

Period	Temperature* (°C)	Humidity* (%)	Rain* (L/m^2)	Air pressure* (mb)
Winter I	1.50	89.00	74.45	850.60
Winter II	-2.15	87.50	60.10	857.45
Summer I	12.55	86.00	61.15	850.35
Summer II	14.50	82.00	127.85	855.60

* mean value between the data reported at the Călimani meteorological station an those reported at the Poiana Stampei station.

Samples preparation

To determine the concentration of Na, Mg, K, Ca, Mn, Zn, Se, Cu and Fe, a closed microwave digestion unit (Speed ENTRY by Berghof) equipped with 10 Teflon containers was used to mineralize 0.1 g dry sample. Each sample was accurately weighed into a PTFE digestion vessel and 6 mL of

HNO_3 (60% v/v) and 2 mL of H_2O_2 (30% v/v) were added for sample digestion, according to the method of Lee et al. [24]. The microwave system was set as follows: step 1 (a ramp time of 3 min to reach a temperature of 145 °C and a hold time of 5 min), step 2 (a ramp time of 5 min to reach a temperature of 170 °C and a hold time of 10 min), step 3 (a ramp time of 2 min to reach a temperature of 190 °C and a hold time of 15 min) and step 4 (a ramp time of 1 min to reach a temperature of 75 °C and a hold time of 10 min). The obtained solution was allowed to cool to room temperature and was then diluted with ultrapure water to a final volume of 50 mL. Digestion of blank (6 mL of HNO_3 and 2 mL of H_2O_2) was done using the same preparation steps.

Determination of the concentration of macroelements by atomic absorption spectrometry (AAS)

The concentration of macroelements (Na, Mg, K and Ca) in milk and cheese samples was determined by atomic absorption spectrometry (AAS) with a Contra AA 800D spectrometer (Analytic Jena, Germany) equipped with a xenon lamp as a lamp with continuous supply for all elements. Mg, Na and K concentrations were measured by air-acetylene flame, while Ca was determined by nitrous oxide-acetylene flame. The samples were further properly diluted to analyze in the range of 0.1-0.5 mg/L for AAS analysis. The results were expressed in mg/kg of product on a dry basis.

Determination of trace elements concentration by inductively coupled plasma mass spectrometry (ICP-MS)

The concentrations of Mn, Zn, Se, Cu and Fe in cheese and milk samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) using an ELAN DRC mass spectrometer (PerkinElmer SCIEX, Billerica, MA, USA) equipped with a Meinhart nebulizer. Operating conditions for ICP-MS were optimized daily using a 10μg/L solution of Ba, Cd, Ce, Cu, In, Mg, Pb, Rh and U. A certified multi-element standard solution composed of Ag, Al, As, Ba, Fi, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn (10 μg/mL, PerkinElmer Pure Plus, Billerica, MA, USA) was used for the preparation of calibration solutions. The standard calibration solutions were prepared in the range of 0.1-100 μg/L (Zn, Fe and Cu) and 0.1-25 μg/L (Mn and Se), respectively. The results were expressed in mg/kg of product on dry basis.

Statistical analysis of the experimental data

Determinations were performed in triplicate for each sample. The evaluation of the differences between the mean values was carried out by applying the Fisher test and the t-test, the Analysis of Variance (ANOVA) with Tukey test, at a significance level of 95%. The relationships between the studied variables were evaluated by Principal Component Analysis (PCA). The statistical processing of the experimental data was performed using the XL STAT program, version 2022.

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