The use of mineral and trace elements profiles for cows' and goats' cheese species prediction

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Summary
Cluster, principal component, factor and canonical discriminant analysis were used for differentiation of cows' and goats' cheese species using the contents of minerals (Ca, Cu, K, Mg, Na) and risk elements (Ba, Cr, Hg, Mn, Mo, Ni, V). Slovakian cheeses' data output of cluster analysis concerning the membership of the samples to clusters resulted in 95.5% of correctly marked cheeses according to their species of origin. Recognition ability expressed as classification in canonical discriminant analysis conditions resulted in 99.3% of the total cheeses correctly classified, where Cu, Na, Ca, Hg and Mn showed the most discriminant impact on categorizing Slovakian cheeses by their affiliation to animal species. When discriminant analysis was applied to European cheeses, the classification resulted in 97.7% of cheeses correctly classified and in 97.5% of correctly classified samples after cross-validation in the prediction capability procedure. The most discriminating variables for European cheeses were Ba, Ca, Cr, Cu, Hg, K, Mg, and Na concentrations. Found results revealed that multielemental data selection and multivariate statistics are able to differentiate among animal species origin of cheeses produced in cheese-making manufactories on the territory of one or more countries.

Keywords
cheese; cow; goat; mineral elements; trace elements; multivariate statistics

In order to guarantee authenticity of cheeses, analytical traceability methods are required, which may be used by official authorities to control the correctness of the information given in the label. The ability to determine the species origin of milk products is important to protect consumer interests and prevent producers from fraud caused by the use of other than that declared milk in the cheese production.

Currently, extensive literature on research dealing with the detection of milk from different species in raw and processed milk products is available. Differences in the milk proteins are the primary route to discriminate milk and dairy products of different species. Separation of protein fractions by various forms of electrophoresis is one of the most applied techniques to authenticate cheeses of different species origin [1–9]. High-performance liquid chromatography is another route to protein separation and as such was effectively used to determine proteins from different species [10–13]. A number of enzyme-linked immunosorbent assays (ELISA) has been developed for milk species detection in dairy products [14–22]. Polymerase chain reaction (PCR) is one of the most used molecular biology tools, having been used by many authors for milk species identification [10, 22–29]. Recently, new strategies for the structural analysis of milk proteins based on mass spectrometric technologies, in particular matrix-assisted laser desorption – time of flight mass spectrometry (MALDI–TOF), have been developed [30]. The use of multivariate statistical methods has enabled a more global and objective approach to analyse the data generated by the measurement of basic or special physical-chemical descriptors in cheeses [31, 32].

In general, the existing literature about the use of trace elements composition for differentiation of cheeses according to species is rather scarce, but more works have been published on milk species discrimination [33–38]. The content ranges

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of some elements in milk and cheese are closely dependent on animal species and feeding, season, environmental conditions and manufacturing processes. Results of a study on selected trace elements (Al, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn) showed considerable differences between the ewes' and goats' milk as well as related products [33]. RINCON et al. (1994) used Cu, Fe, Zn, Mn, Ca, Mg, Na and K as markers for ewes' and goats' milk differentiation [34]. A statistical study of correlation, factorial and discriminant analyses applied to the metal composition (Se, Fe, Cu, Zn, Na, K, Ca, Mg) of milk (human, cows', goats', pasteurized and powdered infant formula) resulted in 98% of correctly classified samples [35]. The contents of nine mineral elements (P, Ca, Na, K, Mg, Zn, Fe, Cu and Mn) were treated using stepwise discriminant analysis and 76% of milk samples were classified correctly according to species origin [36]. The mineral composition of ewes', cows' and goats' milk and samples of different types of pure-milk cheeses was analysed and stepwise discriminant analysis of data demonstrated that variables K/Mg, Na/Ca, Zn, Cu/Zn and Cu/Na were the most useful in differentiating the samples, achieving correct classification in 98.2% of samples [37]. The most useful variables for cheese were ratios of Fe/K, Na/Ca, Zn/Cu, Na/Mg and Zn, which facilitated the correct classification in 97.1% of samples. Three goats' milk cheeses were successfully distinguished using variables K/Zn, Fe/Cu and P. Using classification functions obtained through discriminant analysis, 94.1% of cheese samples were correctly classified into traditional or industrial groups, and 89.7% of samples into groups of fresh, half-ripened, ripened and old ripened cheeses [38]. Some promising results were obtained from identification of specific sensory patterns for several cheese varieties with special attention to ewes', goats' and cows' cheeses. Linear discriminant analysis showed that ewes' milk cheese varieties with unique sensory characteristics were very different one from each other [39].

This paper summarizes results obtained from European cows' and goats' cheeses differentiation by using selected elemental data and multivariate statistics. Results of this article contribute to the development of a procedure based on the hypothesis that multielement profiling could distinguish animal species from which a foodstuff is derived [33, 40]. This study follows our previously published papers concerning the differentiation of cows' and ewes' cheeses according to their animal species origin [41] or the geographical authentication of some European cows' hard cheeses [42].

MATERIALS AND METHODS

A set of 162 goats' hard and semi-hard cheeses, with a declared fat content from 32% to 48% in dry matter, of Slovakian (40), Austrian (18), Czech (28), Italian (6), French (22), Dutch (26), German (6), Croatian (6), Portuguese (4) and Spanish (6) origin was obtained from retail during 2007–2008. Another set of 280 cows' hard cheeses (emmental and edam type, with a declared fat content from 32% to 48% in dry matter) of Slovakian (94), Austrian (30), Czech (16), Dutch (22), French (12), German (30), Hungarian (22), Italian (12), Polish (28) and English (14) origin was collected during 2006–2007. All samples were analysed for the contents of minerals (Ca, Cu, K, Mg, Na) and risk elements (Ba, Cr, Hg, Mn, Mo, Ni, V). The contents of mineral and trace elements were calculated on a wet weight basis and expressed in $mg \cdot kg^{-1}$.

Samples of cheeses (0.5 g) were digested with 4 ml of 65% HNO_3 (Suprapure; Merck, Darmstadt, Germany) and 0.5 ml H_2O_2 (p.a.; Merck) by a microwave digestion system MLS 1200 MEGA (Milestone, Sorisole, Italy). The microwave digestion programme was applied as follows: 250 W (1 min), 0 W (1 min), 250 W (5 min), 400 W (5 min) and 650 W (5 min). The digested samples were adjusted to the volume of 10 ml with ultrapure water and used for further analysis. A Perkin Elmer 4100 atomic absorption spectrometer (Perkin Elmer; Norwalk, Connecticut, USA) equipped with a deuterium lamp background-correction system, HGA 700 graphite tube atomizer with pyrolytically coated graphite tubes and flame was used for elements determination based on atomic absorption spectrometry. Mineral elements Ca, Cu, K, Mg, Na were determined by atomic spectrometry using an air/acetylene flame. Trace elements Ba, Cr, Mn, Mo, Ni and V were measured on graphite tube atomizer using argon as the inert gas. For the determination of mercury, atomic absorption spectrometer AMA 254 (Altech, Prague, Czech Republic) was used. This instrument is a single-purpose mercury analyser, in which mercury vapour is generated after thermal oxidation treatment of the sample in a stream of oxygen. All results were expressed as the average of triplicate measurements. Instrumental conditions and all analytical and validation parameters of this method have been in detail previously described [41, 42].

Statistical analysis

Multivariate statistical techniques using the cluster analysis (CA), principal component analysis (PCA), factor analysis (FA) and discriminant

(canonical, *k*-th neighbour and stepwise) analysis (DA) were done by statistical programme Unistat v. 5.6 (Unistat, London, United Kingom). The recognizability of a discriminant model was determined as the percentage of correctly classified samples in the training data set. In addition, the predictability was tested as percentage of the samples correctly classified in the *k*-fold cross-validation approach (jack-knife method) by assigning samples randomly to a training set and a test set, the latter containing about 20% of samples [43]. The same process of data splitting was repeated 5 times to ensure that all the cheese samples were at least once in the position of test sample.

Before analysis, data were checked for the presence of outliers. It was examined and confirmed that the data represent approximately normal within-class distribution which is one of the requirements for efficient discrimination. Some of the distribution data were found to be of Laplacian distribution, which is similar to normal distribution.

RESULTS AND DISCUSSION

All samples were analysed for the contents of twelve elemental markers, i.e. Ba, Ca, Cu, Cr, Hg, K, Mg, Mn, Mo, Na, Ni and V. Markers' selection was based on our previous experience with authentication of European cows' and ewes' hard cheeses [42]. Most of them were chosen as elements with the largest variance in the topsoil distribution according to the data accessible in the Geochemical Atlas of Europe [44]. The results on the distribution of mineral and trace elements in cows' and goats' cheeses originating from 10 European countries are shown in the Box-whisker and dot plot (Fig. 1). Some strong differences in element content variability between cows' and goats' cheeses are evident mainly in case of minerals Ca and Na, or trace elements Ba and Cu.

Slovakian cheeses

Recognition ability of multivariate statistics for distinguishing cheeses according to animal species origin was first examined with Slovakian cows'and goats' cheeses.

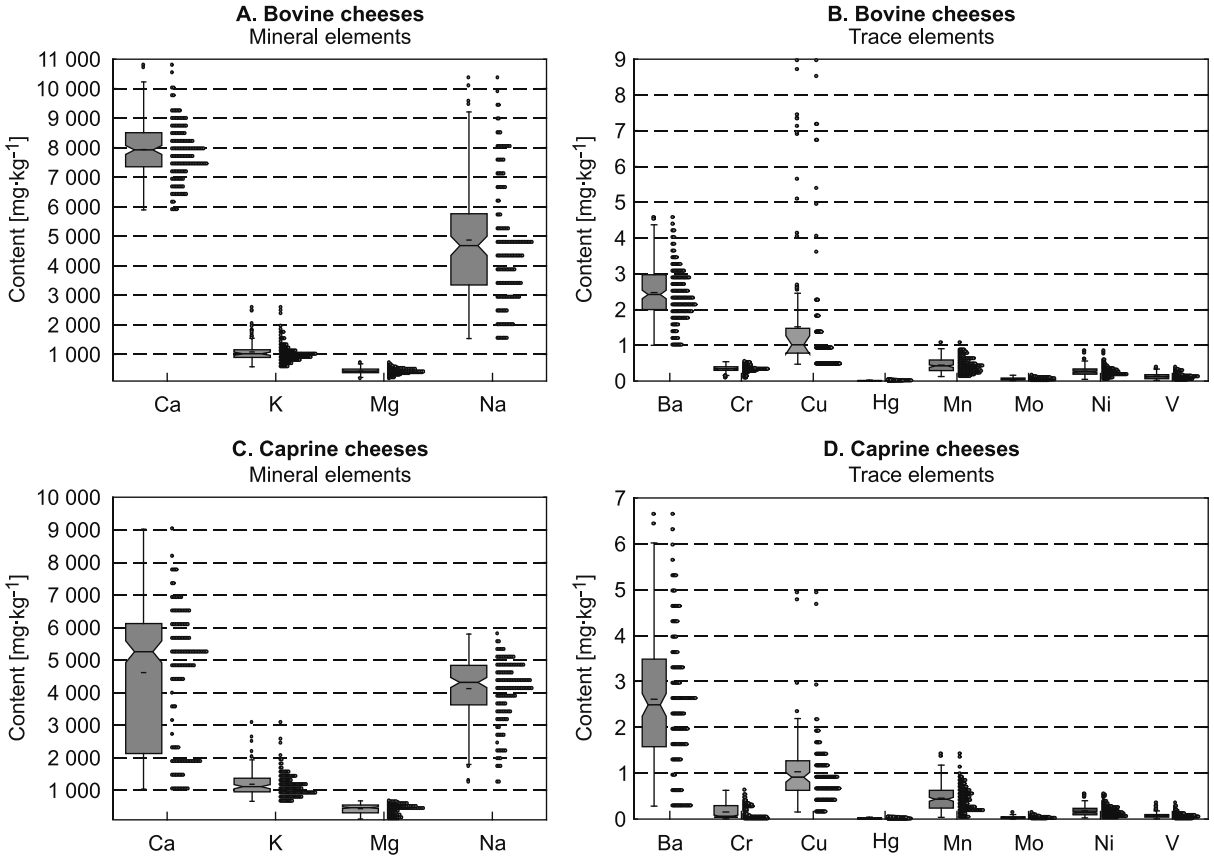

Fig. 1. Distribution of mineral and trace elements contents in European cows' and goats' cheeses.

280 cows' cheeses (Emmental and Edam types) and 162 goats' cheeses originated from 10 EU countries.

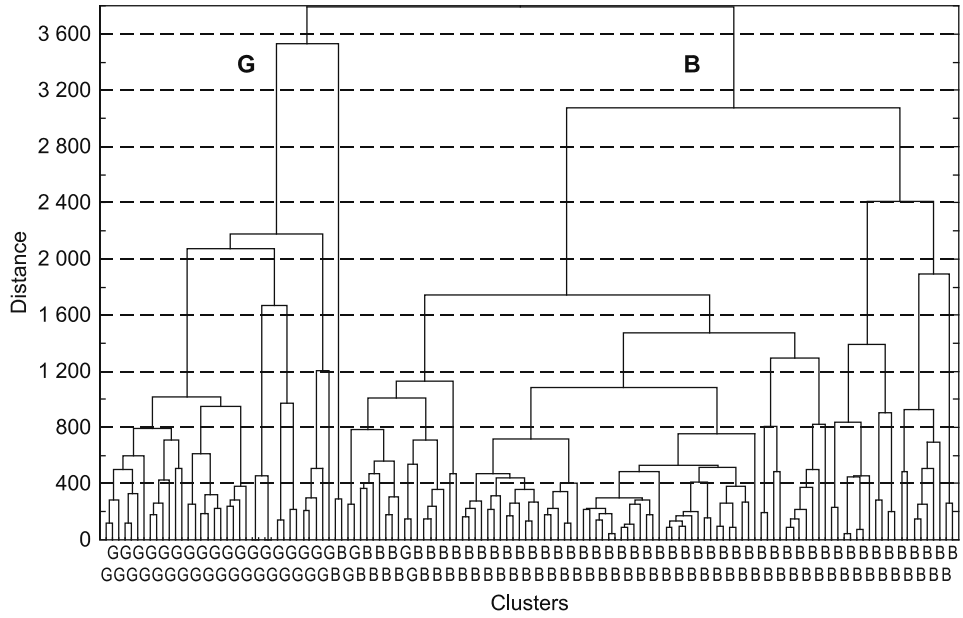

Fig. 2. Cluster analysis of contents of mineral and trace elements in Slovakian cows' (B) and goats' (G) cheeses.

94 cows' cheeses (Emmental and Edam types) and 40 goats' cheeses.

Cluster analysis is an exploratory technique that groups objects based on similarities in the observational data for the set of variables selected for the analysis [31]. In order to assess this tendency, a hierarchical cluster analysis of samples with a method of average within groups and Euclidean distance measure was performed. This procedure finds natural groupings of the data set presented in Fig. 2 in the form of a dendrogram. There, at a distance of about 3600, the cheese samples are divided into two main large clusters that correspond to the cheese species origin. The first cluster is mainly represented by goats' (G), and the second cluster consists mainly of cows' (B) cheeses. Data output of cluster analysis concerning the membership of the samples in clusters resulted in 95.5% of correctly marked cheeses according to their species origin.

Essentially, PCA provides the means to reduce the often large number of interdependent (correlated) variables (elemental data) present in the original data set to a few independent (uncorrelated) variables, or principal components, that are linear combinations of the original variables and explain most of the variation in the original data set [31]. PCA was applied to the Slovakian cows'and goats' cheese elemental data set (134 cheese samples, 40 goats'and 94 cows'). Despite the low covering (55.7%) of the total variance by the three principal components, good separation between cheese samples according to their species origin was achieved (Fig. 3). From the loadings of the variables on the principal components, Na and Mg were the dominant elements in the first principal component, Cr and V in the second one, while Ca and K dominated the third principal component. The method of factoring offered similar result (data not presented).

Discriminant analysis involves deriving linear combinations of the independent variables that discriminate between the *a priori* defined groups [31]. Correlation matrix (Tab. 1) showed that markers selection for cheese differentiation was done properly, because in the case of Slovakian cheeses only a medium correlation between Mg and Hg (0.535), Na and Hg (–0.634) and Na and Mg (–0.503) was found, and similarly, some sporadic weak correlations ($r < 0.5$: Mg–Ca, Mo–Ca and Mg–Ba) were found in the whole European data set.

When the canonical discriminant analysis was applied to the 12 elements, the classification in the recognition conditions resulted in 99.3% of the total cheeses correctly classified (only one sample was misclassified), Cu, Na, Ca, Hg and Mn being the most discriminant elements for categorizing samples into species.

All the used techniques of pattern recognition analysis applied on the Slovakian cows' and goats' cheese elemental data set demonstrated that multivariate analyses are capable of extracting information that is useful for differentiating of

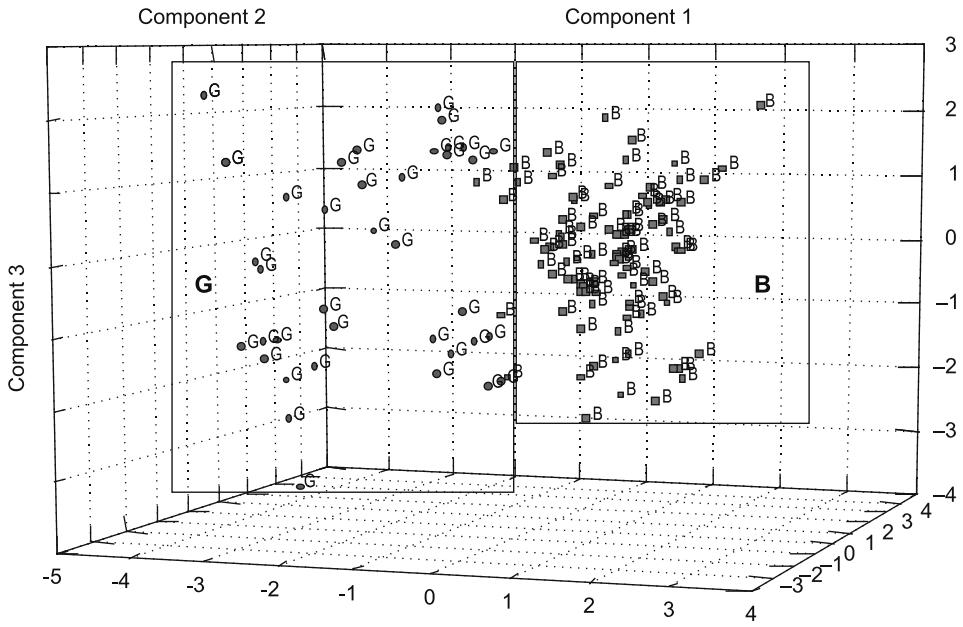

Fig. 3. Principal component analysis of mineral and trace elements contents in Slovakian cows' (□ B) and goats' (● G) cheeses.

94 cows' cheeses (Emmental and Edam types) and 40 goats' cheeses.

goats'cheeses from cows' ones or for their species classification. As shown below, such a good separation of cheese species was successful when multivariate approach was applied on the whole European elemental data set as well.

European cheeses

Recognition and prediction ability of multivariate statistics for distinguishing the cows' and goats' cheese species origin was examined on the European elemental data set consisting of 162 goats' and 280 cows' cheeses from 10 European Union countries. Fig. 4 demonstrates a very efficient separation of cows' and goats' cheeses achieved by principal component analysis. The most effective elements for cheese species differentiation were found to be Na, Ca, Mg and K.

Canonical discriminant analysis considering the 12 elements as cheese species origin markers was performed in the conditions of testing the recognition and prediction ability of the model. When DA was applied to the data, 97.7% of cheeses

Tab. 1. Correlation matrix between elements in the Slovakian cows' and goats' cheeses.

	Ba	Ca	Cr	Cu	Hg	K	Mg	Mn	Mo	Na	Ni	V
Ba	1.0000											
Ca	0.0326	1.0000										
Cr	–0.3990	0.0708	1.0000									
Cu	0.1649	–0.0121	–0.3864	1.0000								
Hg	–0.3030	–0.1621	–0.2483	0.0875	1.0000							
K	–0.0416	–0.2562	0.0459	–0.1704	0.2362	1.0000						
Mg	–0.4477	–0.0085	0.1875	–0.1253	0.5350	0.2210	1.0000					
Mn	0.2442	–0.0102	–0.0960	0.2290	–0.3550	–0.0696	–0.3934	1.0000				
Mo	0.2094	–0.2044	–0.2779	0.1988	–0.1538	–0.0303	–0.2268	0.4848	1.0000			
Na	0.2199	0.4837	–0.0025	0.2022	–0.6337	–0.2396	–0.5027	0.3502	0.1404	1.0000		
Ni	–01383	0.0690	0.3239	–0.0679	–0.2502	–0.1734	–0.1435	0.0327	–0.1098	0.1370	1.0000	
V	–0.3036	0.2213	0.4356	0.0557	–0.2265	–0.1648	–0.0018	0.1214	–0.1108	0.2234	0.2562	1.0000

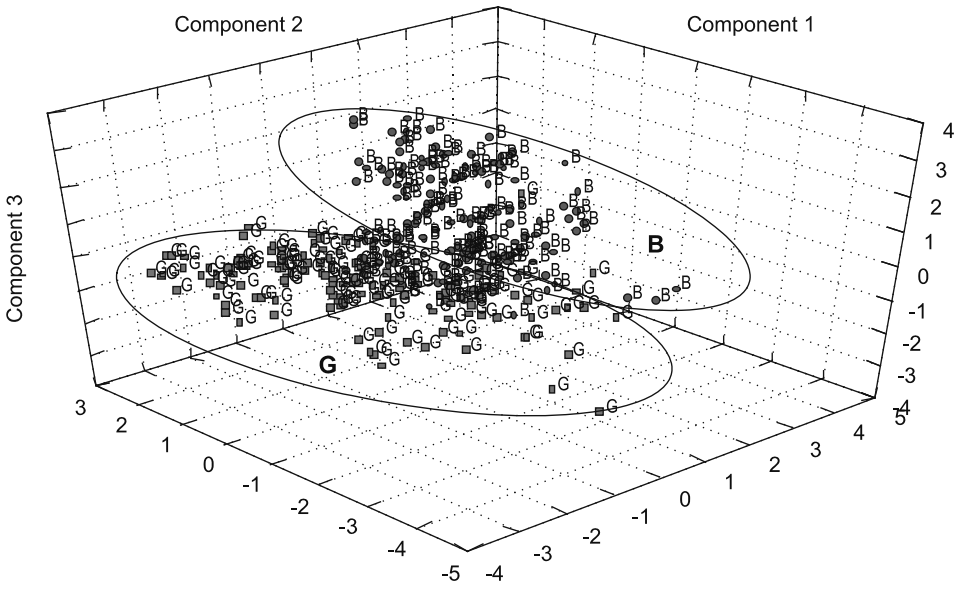

Fig. 4. Principal component analysis of mineral and trace elements contents in European cows' (● B) and goats' (□ G) cheeses.

280 cows' cheeses (Emmental and Edam types) and 162 goats' cheeses.

were correctly classified (recognition capability) and 97.5% samples were correctly classified after cross-validation in the prediction capability procedure. Elements Ca, Mg, Cr and Ba were found to be the most significant discriminant markers distinguishing goats' from cows' cheeses.

Recently, Benincasa et al. (2008) found that multielement data processing by discriminant analysis allows differentiation between milk from the two animal species, produced under identical environmental and husbandry conditions on one farm [40]. These results were by the authors explained as a consequence of different retention time and mineral absorption of feed in the digestive system of animals. Some authors recommended to study the discrimination of dairy products made from milks of different animal species coming from miscellaneous farms. Our results revealed that multielemental data processed by multivariate statistics are capable to differentiate cows' and goats' cheeses produced by different cheese-makers supplied with milks from many farms not only in the territory of one country (Slovakia) but also of several other European countries. It is evident that different environmental conditions of farms and pastures, as well as different cheese-making technologies, reinforce the differences between the elemental profiles in cheeses of different animal species. Further, results show that differences in mineral and trace element contents of cheeses originating from animal species with different physiology are greater than cheese mineral and trace elements variance across the regions and countries.

CONCLUSION

The results of this study demonstrate that multivariate analyses applied to an elemental data set is a useful tool for differentiation of cows' and goats' cheeses. The most discriminating variables for the differentiation and classification of cheeses were Ba, Ca, Cr, Cu, Hg, K, Mg, and Na concentrations. The results suggest that differences in elemental profiles between cheeses according to their species origin caused by natural physiological differences in animals producing milk may be intensified by diversification of feed sources and different environmental farm conditions on regional and multi-regional levels.

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REFERENCES

1. Commission Regulation (EC) No 1081/96 of 14 June 1996 establishing a reference method for the detection of cows' milk and caseinate in cheeses made from ewes' milk, goats' milk or buffalos' milk or mixtures of ewes', goats' and buffalos' milk and repealing Regulation (EEC) No 690/92. Official Journal of the European Union, L *142*, 1996, pp. 15–25.
2. Moio, L. – Chianese, L. – Rivemale, M. – Addeo, F.: Fast detection of bovine milk in Roquefort cheese with PhastSystem® by gel isoelectric focusing and immunoblotting. Lait, *72*, 1992, pp. 87–93.
3. Addeo, F. L. – Moio, L. – Chianese, L. – Nota, G.: Evaluation of bovine and water buffalo milk in mixtures of liquid milk and Mozzarella cheese by gel isoelectric focusing. Italian Journal of Food Science, *1*, 1989, pp. 71–80.
4. Moio, L. – Sasso, M. L. – Chianese, L. – Addeo, F.: Rapid detection of bovine milk in ovine, caprine and water buffalo milk or cheese by gel isoelectric focusing on Phastsystem®. Italian Journal of Food Science, *3*, 1990, pp. 185–190.
5. Mayer, H. K.: Quality control of grated Parmesan products using an electrophoretic ripening index. Milchwissenschaft, *52*, 1997, pp. 443–448.
6. Miralles, B. – Krause, I. – Ramos, M. – Amigo, L.: Comparison of capillary electrophoresis and isoelectric focusing for analysis of casein/caseinate addition in processed cheeses. International Dairy Journal, *16*, 2006, pp. 1448–1453.
7. Recio, I. – García-Risco, M. R. – Amigo, L. – Molina, E. – Ramos, M. – Martín-Álvarez, P. J.: Detection of milk mixtures in Halloumi cheese. Journal of Dairy Science, *87*, 2004, pp. 1595–1600.
8. Recio, I. – Pérez-Rodríguez, M. L. – Ramos, M. – Amigo, L.: Capillary electrophoretic analysis of genetic variants of milk proteins from different species. Journal of Chromatography A, *768*, 1997, pp. 47–56.
9. Veloso, A. C. A. – Teixeira, N. – Peres, A. M. – Mendonça, A. – Ferreira, I. M. P. L. V. O.: Evaluation of cheese authenticity and proteolysis by HPLC and urea–polyacrylamide gel electrophoresis. Food Chemistry, *87*, 2004, pp. 289–295.
10. Mayer, H. K.: Milk species identification in cheese varieties using electrophoretic, chromatographic and PCR techniques. International Dairy Journal, *15*, 2005, pp. 595–604.
11. Ferreira, I. M. – Mendes, E. – Marques, J. – Ferreira, M. A.: Development, validation and application of an HPLC/UV method for quantification of casein in infant formulae and followup milks. Journal of Liquid Chromatography and Related Technologies, *23*, 2000, pp. 2057–2065.
12. Ferreira, I. M. – Mendes, E. – Ferreira, M. A.: HPLC/UV analysis of proteins in dairy products using a hydrophobic interaction chromatographic column. Analytical Science, *17*, 2001, pp. 499–501.
13. Veloso, A. C. A. – Teixeira, N. – Ferreira, I. M.: Separation and quantification of the major casein fractions by reverse-phase high-performance liquid chromatography and urea–polyacrylamide gel electrophoresis. Detection of milk adulterations. Journal of Chromatography A, *967*, 2002, pp. 209–218.
14. Rolland, M. P. – Bitri, L. – Besancon, P.: Polyclonal antibodies with predetermined specificity against bovine α[s1]-casein: application to the detection of bovine milk in ovine milk and cheese. Journal of Dairy Research, *60*, 1993, pp. 413–420.
15. Rodríguez, E. – Martin, R.. – García, R. – Azcona, T. – Sans, B. – Hernández, P. E.: Indirect ELISA for detection of goats' milk in ewes' milk and cheese. International Journal of Food Science and Technology, *26*, 1991, pp. 457–465.
16. Anguita, G. – Matin, R. – Garcia, T.: Indirect ELISA for detection of cows' milk in ewes' and goat milks using a monoclonal antibody against bovine beta-casein. Journal of Dairy Research, *62*, 1995, pp. 655–659.
17. Ramos, M. – Juarez, M.: Analysis of raw and commercial milk and dairy products. International Dairy Federation Bulletin, *181*, 1984, pp. 3–9.
18. Ioannis, S. – Arvanitoyannis, A. – Nikolaos, E. – Tzouros, A.: Implementation of quality control methods in conjunction with chemometrics toward authentication of dairy products. Critical Reviews in Food Science and Nutrition Issue, *45*, 2005, pp. 231–249.
19. Hurley, I. P. – Ireland, H. E. – Coleman, R. C. – Williams, J. H. H.: Application of immunological methods for the detection of species adulteration in dairy products. International Journal of Food Science and Technology, *39*, 2004, pp. 873–878.
20. Costa, N. – Ravasco, F. – Miranda, R. – Duthoit, M. – Roseiro, L. B.: Evaluation of a commercial ELISA method for the quantitative detection of goat and cow milk in ewe milk and cheese. Small Ruminant Research, *79*, 2008, pp. 73–79.
21. Hurley, I. P. – Coleman, R. C. – Ireland, H. E. – Williams, J. H. H.: Use of sandwich IgG ELISA for the detection and quantification of adulteration of milk and soft cheese. International Dairy Journal, *16*, 2006, pp. 805–812.
22. López-Calleja, I. M. – González, I. – Fajardo, V. – Hernández, P. E. – García, T. – Martín, R.: Application of an indirect ELISA and a PCR technique for detection of cows' milk in sheep's and goats' milk cheeses. International Dairy Journal, *17*, 2007, pp. 87–93.
23. Rea, S. – Chikuni. K. – Branciari, R. – Sangamayya, R. S. – Ranucci, D. – Avellini, P.: Use of duplex polymerase chain reaction technique to identify bovine and water buffalo milk used in making mozzarella cheese. Journal of Dairy Research, *68*, 2001, pp. 689–698.
24. Plath, A. – Krause, I. – Einspanier, R.: Species identification in dairy products by three different DNA-based techniques. Zeitschrift für Lebensmitteluntersuchung und -Forschung, *205*, 1997, pp. 437–441.
25. Maudet, C. – Taberlet, P.: Detection of cows' milk in goats' cheeses inferred from mitochondrial DNA polymorphism. Journal of Dairy Research, *68*, 2001, pp. 229–235.

26. Feligini, M. – Alim, N. – Bonizzi, I. – Enne, G. – Aleandri, R.: Detection of cow milk in water buffalo cheese by SYBR green real-time PCR: Sensitivity test on governing liquid samples. Pakistan Journal of Nutrition, *6*, 2007, pp. 94–98.
27. Branciari, R. – Nijman, I. J. – Plas, M. E. – Di, A. E. – Lenstra, J. A.: Species origin of milk in Italian mozzarella and Greek feta cheese. Journal of Food Protection, *63*, 2000, pp. 408–411.
28. Abdel-Rahman, S. M. – Ahmed, M. M. M.: Rapid and sensitive identification of buffalo's, cattle's and sheep's milk using species-specific PCR and PCR–RFLP techniques. Food Control, *18*, 2007, pp. 1246–1249.
29. Bottero, M. T. – Civera, T. – Nucera, D. – Rosati, S. – Sacchi, P. – Turi, R. M.: A multiplex polymerase chain reaction for the identification of cow's, goat's and sheep's milk in dairy products. International Dairy Journal, *13*, 2003, pp. 277–282.
30. Smit, G.: Dairy processing. Improving quality. Cambridge : CRC Press LLC, Woodhead Publishing, 2003. 536 pp. ISBN 1 85573 676 4.
31. Coker, C. J. – Crawford, R. A. – Johnston, K. A. – Singh, H. – Creamer, L. K.: Towards the classification of cheese variety and maturity on the basis of statistical analysis of proteolysis data—a review. International Dairy Journal, *15,* 2005, pp. 631–643.
32. Castell-Palou, A. – Rosselló, C. – Femenia, A. – Simal, S.: Application of multivariate statistical analysis to chemical, physical and sensory characteristics of Majorcan cheese. International Journal of Food Engineering, *6*, 2009. doi: 10.2202/1556-3758.1417.
33. Coni, E. – Bocca, A. – Copplelli, P. – Caroli, S. – Cavallucci, C. – Marinucci, M. T.: Minor and trace element content in sheep and goat milk and dairy products. Food Chemistry, *57*, 1996, pp. 253–260.
34. Rincon, F. – Moreno, R. – Zurera, G. – Amaro, M.: Mineral composition as a characteristic for the identification of animal origin of milk. Journal of Dairy Research, *61*, 1994, pp. 151–154.
35. Rodríguez, E. M. – Alaejos, M. S. – Romero, C. D.: Chemometric studies of several minerals in milks. Journal of Agricultural and Food Chemistry, *47*, 1999, pp. 1520–1524.
36. Fresno, J. M. – Prieto, B. – Urdiales, R. – Sarmiento, R. M. – Carballo, J.: Mineral content of some Spanish cheese varieties. Differentiation by source of milk and by variety from their content of main and trace elements. Journal of the Science of Food and Agriculture, *69*, 1995, pp. 339–345.
37. Hernández, C. M. – Amigo, L. – Martín-Alvarez, P. J. – Juárez, M.: Differentiation of milks and cheeses according to species based on the mineral content. Zeitschrift für Lebensmitteluntersuchung und –Forschung, *194*, 1992, pp. 541–544.
38. Frau, M. – Simal, S. – Femenia, A. – Rosselló, C.: Differentiation and grouping of chemical characteristics of Mahon cheese. Zeitschrift für Lebensmitteluntersuchung und –Forschung, *207,* 1998, pp. 164–169.
39. Bárcenas, P. – Pérez-Elortondo, F. J. – Albisu, M.: Sensory comparison of several cheese varieties manufactured from different milk sources. Journal of Sensory Studies, *20,* 2005, pp. 62–65.
40. Benincasa, C. – Lewis, J. – Sindona, G. – Tagarelli, A.: The use of multi element profiling to diferentiate between cow and buffalo milk. Food Chemistry, *110*, 2008, pp. 257–262.
41. Suhaj, M. – Koreňovská, M.: Identification of cheese species origin by pattern recognition processing of elemental data. Journal of Food and Nutrition Research, *46,* 2007, pp. 174–180.
42. Suhaj, M. – Koreňovská, M.: Study of some European cheeses geographical traceability by pattern recognition analysis of multielemental data. European Food Research and Technology, *227*, 2008, pp. 1419–1427.
43. Berrueta, L. A. – Alonso-Salces, R. M. – Heberger, K.: Supervised pattern recognition in food analysis. Journal of Chromatography A, *1158*, 2007, pp. 196–214.
44. Salminen, R.: Geochemical Atlas of Europe [online]. Espoo : GTK Foregs, 2005 [cit. 10 April 2010]. <http://www.gsf.fi/publ/foregsatlas/> ISBN 951-690-913-2 (electronical version).

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