Possibilities of Different Animal Milk Detection in Milk and Dairy Products – a Review

Markéta BORKOVÁ and Jana SNÁŠELOVÁ

MILCOM, a. s, Dairy Research Institute, Prague, Czech Republic

Abstract

Borková M., Snášelová J. (2005): **Possibilities of different animal milk detection in milk and dairy products – a review**. Czech J. Food Sci., **23**: 41–50.

Adulteration of milk and dairy products with different types of milk, other than declared, presents a big problem for food monitoring. The evidence of milk adulteration is a difficult task considering similar compositions of various types of milk. The presented review is therefore focused on the study of the composition of milk from different animal species. The aim is to find a useful marker component for the adulterant detection. The analysis of milk proteins is a suitable solution of this problem. The techniques used for research in this area were also studied. As prospective techniques, immunological techniques and techniques based on DNA analysis are especially considered. The first ones are able to determine 0.5% of different milk adulterant, and the second ones even as little as 0.1%. Reverse-phase high-performance liquid chromatography is successfully applied in the quantitative analysis of individual milk adulterants in samples. The most frequent adulteration of ewe and goat milk is its replacement with less expensive and more plentiful bovine milk. Not so typical adulteration is the presence of goat milk in ewe milk or the detection of bovine milk as adulterant in buffalo mozzarella cheese.

Keywords: milk adulteration; adulterants; milk protein; dairy products; cow milk; ewe milk; goat milk

The adulteration of food products is a significant problem in the food production. This is how fraudulent producers try to cheat consumers and authorities. The adulteration affects all commodities in the food processing. Most frequently, such products are adulterated that are produced in big quantities and further, the expensive products whose adulteration brings a profit. The subject of this review is to summarise the data on substituting one milk type for another one. In the literature, especially reported is the substitution of ewe and goat milk by cow milk. Cow milk is frequently used for adulteration because of its prevailing production in the world and its lower price as compared to other types.

Marker components for the detection of adulteration

First of all, it is necessary to familiarise with the composition of individual milks from different species and to find the suitable marker components for the detection of adulteration with other species. The basic chemical components of cow, ewe, goat and buffalo milk are presented in Table 1.

The determination of fat, crude protein, lactose, ashes, and total dry matter in cow, ewe and goat buffalo milk and colostrum was dealt with by Hadjipanayiotou (1995). The highest contents of fat, crude protein, ashes, and total dry matter were found in ewe milk followed by goat milk, the

lowest contents of these components having been discovered in cow milk (Hadjipanayiotou 1995). We also obtained the basic composition of buffalo milk (Drbohlav & Vodičková 2001). Table 2 presents the milk yields of individual animal species. It is apparent that the yield of cow milk is significantly higher than those of other species. It also follows from Table 2 that the highest content of non-protein nitrogen (NPN) can be found in goat milk and the lowest one in cow milk.

It follows from the literature that the detection of adulteration by the substitution of one milk type for another one is made by protein analysis (Strange *et al.* 1992). This problem is a very complicated one as it is necessary to take into account that the composition of milk and milk proteins is very variable (Tietze & Majewski 1997), both between individual types of milk and within one type. It depends on the breed or on the lactation level.

The quantitative determination of milk proteins is complicated by the existence of genetic and non-genetic polymorphism, and by the technological treatment and processing of milk (Recio *et al.* 1997b). Thermal denaturation or proteolysis, that is common with the manufacturing of many milk products, incurs a risk of complex formation, the formation of insoluble new compounds, smaller peptides and amino acids whose analysis is fairly complicated. The information on the occurrence and quantity of individual proteins or derived compounds is, for the reasons mentioned above, very important for the estimation of processing, quality, and adulteration. Protein content in individual milks and their abundance in casein and whey fractions is shown in Table 3.

Many studies were published on bovine casein. Its composition in both raw and processed milk is well known. However, few studies deal with the composition of milk casein in other types of milk (e.g. goat, ewe or buffalo). Jensen (1995) stated that caseins make 82, 87, 80, and 77% of proteins in ewe, buffalo, bovine and goat milk, respectively.

Casein micelles consist of four caseins: α_{S1}-, α_{S2}-, β- and κ-caseins that occur in cow milk in the ratio of 39:10:36:13 (Davies & Law 1980). The distribution of individual casein fractions in raw cow, ewe, goat, and buffalo milk according to Bramanti *et al.* (2003) is shown in Table 4. The main casein component in cow milk is α_S-casein. This casein is also the main constituent of ewe and buffalo milk. β-Casein is the dominant casein component in goat milk.

In milk are also present the products of proteolysis of all four primary caseins. γ-Casein and some proteoso-peptone compounds are fragments of

Table 1. Basic composition (in g/kg) of ewe, goat (Hadjipanayiotou 1995*)* and buffalo milk (Drbohlav & Vodičková 2001)

Component	Bovine	Ewe	Goat	Buffalo
Number of measurements	861	432	721	–
Total dry matter	112.0 ± 0.35	162.2 ± 1.08	132.1 ± 0.50	182.3
Lipids	32.8 ± 0.28	49.2 ± 0.65	42.6 ± 0.43	72.6
Crude protein	31.3 ± 0.13	57.7 ± 0.47	40.9 ± 0.18	45.9
Ashes	7.4 ± 0.02	9.4 ± 0.04	8.3 ± 0.02	7.9

Table 2. Output of cow, ewe, goat and buffalo milks and their composition (Hadjipanayiotou 1995)

Component	Bovine	Ewe	Goat
Number of animals	44	30	30
Milk output (kg/day)	21.09 ± 7.86	2.45 ± 0.549	3.49 ± 0.504
Fat (g/kg)	38.0	55.2	45.2
Crude protein (N × 6.38) (g/kg)	33.0	58.0	41.1
NPN (g/kg)	2.18	2.70	2.91
NPN (expressed in % of crude protein)	7.43	4.66	7.13

Table 3. Protein composition of bovine, ewe, goat (Velíšek 1999) and buffalo milk (Drbohlav & Vodičková 2001)

Component (g/100 g)	Bovine	Ewe	Goat	Buffalo
Proteins	3.2	4.6	3.2	4.6
Caseins	2.6	3.9	2.6	4.5
Whey proteins	0.6	0.7	0.6	0.5

β-casein, originating from the action of plasmin, the endogenous alkaline milk protease. λ-Caseins are presumably fragments of α_{S1}-caseins, having also originated through plasmin cleavage. Glycomacropeptides and para-κ-caseins are fragments of κ-caseins emerging as a result of chymosin action (Strange *et al*. 1992).

Whey proteins contain proteins soluble at pH 4.6 and 20°C. To these proteins belong β-lactoglobulin (β-Lg), α-lactoalbumin (α-La), immunoglobulins (IgG, IgA, IgM) and serum albumin (BSA). The following minor proteins are also present: lactoferrin, lactoperoxidase, enzymes, protein compounds of milk fat globule membrane (MFGM), proteosopeptone compounds, and glycomacropeptides. Well known are the primary sequences α-La, β-Lg

and BSA. In bovine milk, β-Lg and α-La occur approximately at a ratio of 3:1 (de Jong *et al*. 1993). β-Lg is the main whey protein in all types of milk studied (Table 5). Its highest abundance was found in ewe milk, the lowest one in goat milk. In ewe milk, immunoglobulins are contained in significant amounts; after β-Lg they represent the second largest fraction. In bovine and goat milks, the second most represented whey fraction is α-La.

The discovery of two variants of β-lactoglobulin in cow milk by Aschaffenburg and Drewry in 1955 (Moioli *et al*. 1998) generated considerable interest in the research of milk proteins. The polymorphism of milk proteins is caused either by the substitution of amino acids or by their deletion. All caseins and main whey proteins show genetic polymorphism that can affect milk composition and some parameters of milk processing. For this reason, genetic variants of milk proteins are considered to be potential selection criteria in cattle husbandry (Visser *et al*. 1995).

The identification of the genetic variant of milk protein also enables the determination of adulteration with various animal milk types. The variants of the main milk proteins found in the literature are summarised in Table 6.

Table 4. Representation of casein fractions in raw bovine, ewe, goat and buffalo milk (Bramanti *et al*. 2003)

Milk type	Bovine	Ewe	Goat	Buffalo
Total protein content (g/l)	27.8 ± 2.2	59.4 ± 3.3	33.4 ± 1.6	49.2 ± 1.9
Caseins relating to total protein content (%)	83 ± 10	93 ± 10	99 ± 12	90 ± 5
α_{S1}-casein (%)	37 ± 7	33 ± 8	10 ± 6	31 ± 2
α_{S2}-casein (%)	7 ± 1	14 ± 2	–	13 ± 1
β-casein (%)	42 ± 8	30 ± 5	63 ± 11	28 ± 2
γ-casein (%)	6 ± 2	9 ± 1	18 ± 4	22 ± 1
κ-casein (%)	9 ± 4	14 ± 2	8 ± 2	7 ± 1

Table 5. Composition and content of whey proteins in skimmed cow, ewe and goat milk (Law 1995)

Component	Bovine	Ewe	Goat
Whey proteins (g/l)	6.46	10.76	6.14
β-lactoglobulin (%)	59.3	61.1	54.2
α-lactalbumin (%)	16.2	10.8	21.4
Immunoglobulins (%)	15.0	20.0	11.5
Serum albumin/lactoferrin (%)	9.5	8.1	12.8

Table 6. Genetic variants of main milk proteins

Component	Bovine (Recio *et al.* 1997b)	Ewe (Amigo *et al.* 2000)	Goat (Moioli *et al.* 1998)
α_{S1}-casein	A, B, C, D, E	A, B, C, D, E	A, B_1, B_2, B_3, C, D, E, F, G, O
α_{S2}-casein	A, B, C, D	A,B	A, B, C
β-casein	A^1, A^2, A^3, B, C, D, E, X (named F 5P) *(*Visser *et al.* 1995)	A, B, C	A, B, O
κ-casein	A, B, C, E	none existing	A, B
α-lactalbumin	A, B (Voelker 1997)	A, B	A, B
β-lactoglobulin	A, B, C, D, E, F, G	A, B, C	A, B

Analytical methods for the detection of milk adulteration by analysis of milk proteins and their genetic variants

For the detection of adulteration of one type of milk with another one, namely the following methods are used: electrophoresis, isoelectric focusing (IEF), capillary electrophoresis (CE), reverse phase high-performance liquid chromatography (RP HPLC) and ion exchange high-performance liquid chromatography (IE HPLC), hydrophobic interactive chromatography (HIC), immunochemical methods (ELISA), PCR techniques, and mass spectrometry.

In the subsequent part, we will only mention several examples of applications relevant to the problems studied.

Table 7 gives a summary of the techniques studied except PCR, and the protein marker analytes used. Owing to the orientation of our research, we will deal in detail especially with the HPLC technique.

Electro-migration methods

Electrophoresis. Electrophoresis plays a significant role in the research of milk proteins and genetic variants of main milk protein components. The classification of caseins was carried out by electrophoretic analysis; minor casein components γ_1, γ_2, γ_3 and para-κ caseins were detected (Strange *et al.* 1992).

Polyacrylamide gel electrophoresis (PAGE) was used for the analysis of milk proteins. This employs the separation of individual molecules both according to their electric charge and size. In PAGE, the proteins have a negative or a positive charge, depending on the buffer conditions. They migrate with a speed based on their charge and size. The advantage of this technique lies in the fact that the individual groups of milk proteins are well separated and that the genetic protein variants and the levels of their phosphorylation can be detected.

Tamine *et al.* (1999) proved the addition of bovine milk (25% and more) into the goat Kish product by PAGE technique. The analysis was based on the mobility of bovine α_{S1}-casein. This technique was also used for the identification of bovine milk in ewe yoghurt (Kaminarides & Koukiassa 2002). The marker component was bovine para-κ-casein and 1% addition of bovine milk was successfully detected.

For the detection of adulteration also SDS and urea electrophoresis can also be used. Urea and anion solvent sodium dodecyl sulphate (SDS) possess the ability of dissolving various types of proteins and of decomposing polymer proteins to polypeptide components. SDS binds to individual protein molecules and gives them a strong negative charge thus removing the differences in the total charge. The electrophoretic separation takes place only on the basis of their molecular weights. On the contrary, with the application of urea, proteins are separated according to their charge.

Tamine *et al.* (1999) used for the cow milk detection in goat Kish product besides the above mentioned PAGE also SDS electrophoresis. A good separation was achieved of κ-, β-, α-caseins of cow and goat milk. Nevertheless, the detection of adulterants was not successful due to the extensive proteolysis of the sample.

By urea-PAGE electrophoresis, the presence of 5% and more of bovine milk in ewe and goat milk was detected. The detection was based on the analysis of bovine α_S-casein (Veloso *et al.* 2002).

Table 7. Summary of protein marker components used for detection of various types of animal milks

Techniques	Marker components	References
Electro-migration		
Electrophoresis	bovine para-κ-casein	Kaminarides & Koukiassa (2002)
	bovine α_{S1}-casein	Tamime *et al*. (1999)
	bovine α-casein	Veloso *et al*. (2002)
Isoelectric focusing	bovine γ_2 and γ_3-caseins reference method EU	Anonym (2001)
	goat and ewe para-κ-casein	Mayer *et al*. (1997)
Capillary electrophoresis	goat α-lactalbumin and bovine β-lactoglobulin A	Cartoni *et al*. (1999)
	complete analysis of bovine caseins and whey proteins	Herrero-Martínez *et al*. (2000)
	complete analysis of bovine, ewe and goat caseins and whey proteins	de Jong *et al*. (1993)
	bovine α_{S1}-casein	Lee *et al*. (2001); Cattaneo *et al*. (1996)
Immunochemical methods		
Indirect ELISA	bovine κ-casein	Bitri *et al*. (1993)
	bovine immunoglobuline G	Hurley *et al*. (2004)
	goat α_{S2}-casein	Haza *et al*. (1996)
	bovine β-casein	Pizzano *et al*. (2000)
	bovine α_{S1}-casein	Rolland *et al*. (1995)
Sandwich ELISA	bovine β-casein	Anguita *et al*. (1997)
	goat whey proteins	Garcia *et al*. (1993)
	bovine β-lactoglobulin	Levieux & Venien (1994)
Chromatography		
RP HPLC	bovine, goat and ewe β-lactoglobulins	Ferreira & Caçote (2003)
	bovine α-lactalbumin and β-lactoglobulin B	Romero *et al*. (1996)
	bovine α-casein	Veloso *et al*. (2002)
HIC	bovine, goat and ewe α_{S1}-, α_{S2}-, β- and κ-caseins	Bramanti *et al*. (2003)
Mass spectrometry		
MALDI-MS	bovine α-lactalbumin, β-lactoglobulin A and B	Cozzolino *et al*. (2001)

Isoelectric focusing (IEF). The protein separation according to their isoelectric points is especially suitable for the analysis of caseins that form many genetic variants. For example, by using IEF instead of PAGE electrophoresis for the analysis of the genetic variants of bovine β-casein, the procedure is significantly simplified. If for the same determination PAGE electrophoresis is to be used, it has to apply both alkali and acid PAGE for the differentiation of A variants from B, C and D, and A1, A2, and A3 variants (Strange *et al*. 1992).

IEF is an EU reference method for the determination of the presence of cow milk and caseinate in cheeses made from ewe, goat or buffalo milk or their blends. It is based on the identification of γ-caseins after plasminolysis. The method is suitable for the sensitive and specific detection of raw and thermally processed cow milk and caseinate in fresh and ripened cheeses made from ewe, goat or buffalo milk or their blends. This detection is based on γ_2- and γ_3-caseins determination. Their isoelectric points lie between pH 6.5 and 7.5. By

using two milk reference standards (with 0 and 1% of cow milk), the samples positive for the presence of cow milk can be detected. In the case, that the amounts of bovine γ_2-casein and γ_3-casein are equal or greater that their amounts in 1% standard, the presence of cow milk is confirmed. The method allows the detection of 0.5% addition (Anonym 2001). The disadvantage is that it is impossible to determine the adulteration with goat milk in ewe milk and *vice versa*. This may be changed by selecting an appropriate marker analyte, e.g. para-κ-casein (Mayer *et al.* 1997).

Capillary electrophoresis (CE). Capillary electrophoresis is a modification of electrophoresis that is carried out as a carrier-free electrophoresis via free capillary. CE is a quickly developing technique that enables a rapid casein and whey protein separation with a high resolution and good quantification (De Jong *et al.* 1993). The use of CE resulted in the development of expedient and automated analyses with a very high resolution and with the demand for only very small amounts of samples and buffers (Recio *et al.* 1997a).

De Jong *et al.* (1993) carried out a complete analysis of caseins and whey proteins in bovine, ewe and goat milk by capillary electrophoresis. Based on this analysis, he succeeded in identifying adulterants (starting from 1%) of bovine, ewe and goat milk in all milk blends.

The employment of capillary zone electrophoresis in the detection of adulterants of cow milk in ewe and goat milk is described in the paper of Cattaneo *et al.* (1996). α_{s1}-Casein fraction was the marker component for the detection of cow milk and a successful detection was carried out of the addition of cow milk to ewe or goat milk starting from 8%. Lee *et al.* (2001) used the detection of α_{s1}-casein fraction with this technique. The authors succeeded in improving the detection and they were able to determine 1% of adulterants in raw and reconstituted milk.

Whey fraction was also studied for the detection of cow milk presence in goat milk and cheeses (Cartoni *et al.* 1999). As suitable marker analytes, caprine α-lactalbumine and bovine β-lactoglobuline A were determined. Minimal detectable amount of cow milk was 2% in milk mixtures and 4% in cheeses.

An unusual method using CE analysis of ethanol-water protein fractions with isoelectric iminodiacetic acid buffer was also described as a possibility to fast identify and quantify cow milk adulterants

in goat and ewe cheeses (Herrero-Martínez *et al.* 2000). The authors declare that the amount of cow milk in goat and ewe cheese can be estimated with the relative standard deviation of 6–7%, based on electroforegrams and statistical PLS (partial last squares) multivariable regression.

Immunochemical methods

Immunochemical methods are suitable for the detection of specific fractions of bovine, ewe or goat milk proteins. The principle of the immunochemical methods resides in the reaction of the antigen (transgenic protein) with the antibody. The immunoanalysis employs specific mono- or polyclonal antibodies that react with whey proteins, the individual casein fractions, and peptide fragments of caseins. Reactions were described of the antibodies with, e.g., bovine β-Lg (Levieux & Venien 1994), bovine IgG (Hurley *et al.* 2004), bovine κ-casein (Bitri *et al.* 1993), bovine β-casein (Anguita *et al.* 1997), bovine α_{S1}-casein (Rolland *et al.* 1995), and goat whey proteins (Garcia *et al.* 1993). By means of cross-reactions with individual milk proteins, it was proved that the antibodies are highly specific and manifest minimum cross-interactions. The sensitivity of these methods is less than 0.5%.

ELISA (Enzyme Linked Immunosorbent Assay). Immunochemical methods, as mentioned above, are very specific. In the literature many examples can be found of employing these techniques for the detection of milk adulteration. For this purpose, both indirect (competitive) and sandwich (uncompetitive) ELISA was used. ELISA method was used for the detection of the cow milk presence in goat, ewe, and buffalo milk (Hurley *et al.* 2004), further in goat and ewe cheese (Pizzano *et al.* 2000), and of the presence of goat milk in ewe milk (Haza *et al.* 1996).

For example, the addition of goat milk to ewe milk was detected both by sandwich ELISA (from 0.5%) (Garcia *et al.* 1993) and indirect ELISA, where 0.5–15% (v/v) of goat milk in ewe milk was detected (Haza *et al.* 1996). In the case of indirect ELISA, a monoclonal antibody (MAb B2B) against goat α_{s2}-casein was used. For the detection of basically similar types of milk, such as bovine and buffalo, it seems to be prospective to use monoclonal antibody against bovine immunoglobuline G. The reason is that immunoglobulines are not easily liable to proteolysis and are stronger immunogenes than

caseins and other whey proteins. This way of the detection of cow milk in goat, ewe and buffalo milk using indirect ELISA was used by Hurley *et al.* (2004) who were able to detect 0.1% of bovine IgG in all types of milk. This test is suitable both for raw milk and pasteurised or frozen. It is not suitable for UHT or powdered skimmed milk due to denaturation of bovine IgG.

Techniques based on DNA analysis

PCR (Polymerase Chain Reaction) is used for the amplification of DNA part situated between two regions of a known sequence. It was found in the literature that the PCR technique was used for the detection of cow milk in ewe (López-Calleja *et al.* 2004) and goat milk (Bania *et al.* 2001), and also in goat cheeses (Maudet & Taberlet 2001) and in buffalo mozzarella cheese (Bottero *et al.* 2002). In buffalo cheese, cow milk was successfully detected if the cheese exclusively of buffalo origin. In ewe and goat milk and cheeses, the detection of the presence of cow milk was possible starting from 0.1%. Cow milk in goat and ewe milk was determined with the detection limits mentioned above not only in raw milk samples, but also in pasteurised and sterilised samples (López-Calleja *et al.* 2004). The advantage of DNA techniques lies in their considerable sensitivity.

For the detection of one type milk adulteration with another one, combined techniques of immunochemical and DNA methods were also used. Such an example is the PCR-LCR (Ligase Chain Action)-EIA (Enzyme Immunoassay) technique (Klotz & Einspanier 2001) based on the detection of one specific nucleotide presence in bovine milk (*Bos taurus β-casein* gene, position 8411) and sheep, goat and buffalo milk. The first quantitative analysis of the blend enabled to discover the presence of 5% bovine DNA in sheep, goat and buffalo DNA.

Chromatography

High-performance liquid chromatography (HPLC). Chromatographic methods find an extensive application in the isolation of milk proteins. The advantage of the HPLC technique is its speed, simplicity, and possibility of automation. The analysis of milk proteins can be carried out by means of reverse phase, ion exchange (both anion and cation), molecularly exclusive, and hydrophobic liquid chromatography.

Reverse phase high-performance liquid chromatography (RP HPLC). Reverse phase high-performance liquid chromatography is a technique of chromatographic separation of analytes on the basis of their hydrophobic properties.

Ferreira and Caçote (2003) dealt in their paper with the detection and quantification of blends of bovine, goat and ewe milk and cheeses by RP HPLC determination of β-lactoglobulins. The determination was carried out by gradient elution under the following conditions: Chrompack P 300 RP column, mobile phase passage 0.5 ml/min, column temperature 45°C, gradient made of solvents A (0.1% trifluoroacetic acid in water) and B (0.1% trifluoroacetic acid in 80% acetonitrile, v/v), UV detection at 215 nm.

Binary mixtures were analysed of bovine and ewe or bovine and goat raw milk containing 1, 2, 5, 10, 20, 30, 50, 75 and 95% (v/v) of bovine milk, and also binary blends of ewe and goat milk containing 1, 2, 5, 10, 20, 30, 50, 75 and 95% (v/v) of ewe milk.

Of these model samples cheese was made by traditional methods. Milk blends and fresh and ripened cheeses made of them were analysed. Different chromatographic profiles were obtained for bovine, ewe, and goat whey proteins. Every milk species revealed different retention times for β-lactoglobulin peaks. β-Lg and α-La were separated in all milks. In ewe milk was also identified the presence of β-Lg A and B. In goat and bovine milk, only the presence of B genetic variant of β-Lg was identified.

The authors found that a linear relation exists between the area logarithm of β-lactoglobulin of the sample and the logarithm of relative percentage of bovine or ewe milk. In this way, the authors succeeded in identifying the addition of individual milk species with the detection limit of 2%. Quantification of milk species by this technique was carried out in the range of concentrations of 5–95%. The method was successfully used for the determination of authenticity and quantity of individual milk in commercial ewe and goat cheeses.

The detection of the addition of cow milk to goat and ewe milk by the analysis of whey proteins (α-La, β-Lg and BSA) using RP HPLC was also described (Romero *et al.* 1996). The determination was carried out on polymer reverse phase column (type PLRP-ES from the Polymer Laboratory, Church Stretton, UK) by gradient elution with the detection in UV range of 205 nm. The detection of adulterants resulted from

the retention times of whey proteins and genetic variants (t_r values in proteins are shown in Table 8). This technique enables the detection of adulterants in amounts lower than 1%. Peaks of bovine α-La and β-Lg (B) in 9.37 min and 18.10 min, respectively, manifest the presence of cow milk in ewe and goat milk. A disadvantage of this method lies in the fact that it does not make any difference between goat and ewe milk because their whey proteins have very similar retention times.

In the detection of different milk types by RP HPLC, also Veloso *et al.* (2002) were engaged. Despite their obtaining various chromatographic profiles of bovine, ewe and goat milk, they succeeded in detecting and quantifying the addition of merely 5 and more per cent of bovine milk in goat milk. This detection was based on the presence of α-casein peak. Chromatographic conditions were as follows: Chrompack P 300 RP column, gradient elution, detection 280 nm.

Ion exchange high-performance liquid chromatography (IE HPLC). Ion exchange chromatography is based on the separation by ion exchange between the mobile and stationary phase.

Anion exchange FPLC (fast protein liquid chromatography) was used for identifying the authenticity of the goat Kish product. The authors succeeded in the separation of caseins of bovine and goat milk, yet the sample separation was negatively influenced by its considerable proteolysis. It was not possible to identify the adulterants (Tamime *et al.* 1999). This technique is possible for the identification of bovine milk in goat milk by the detection of bovine α_{S1}-casein.

Hydrophobic interactive chromatography (HIC). The separation of substances takes place in the hydrophobic stationary phase by decreasing the ionic power of the mobile phase.

This technique was used for the separation and determination of α_{S1}-, α_{S2}-, β-and κ-caseins in bovine, ewe and goat milk, their blends and cheeses (Bramanti *et al.* 2003). The Eichrom Propyl HIC column was used. The UV detection was carried out at 280 nm, gradient elution was used. The method is based on a rapid and easy dissolution of real raw samples by 4.0M guanidin thiocyanate without the preliminary precipitation or casein separation, and is completed by HIC analysis. The method was applied on commercial casein blends and for qualitative and quantitative analysis of casein fractions in raw cow, ewe and goat milk (10 samples of every type), one sample of buffalo milk, and further for commercial cheeses (mozzarella, robiola, ricotta and stracchino). Binary milk mixtures were analysed (cow/goat and cow/ewe) and the relation was outlined between the areas of casein peaks (α_{S1}/κ , α_{S2}/β, β/κ and α_{S2}/α_{S1}) which was later discussed from the point of view of the possibility of applying this method for the detection of milk adulterants. By means of these model relations it was possible to estimate the addition of 10% and more of cow milk into ewe and goat milk.

Mass spectrometry

Recently, new technologies for the structural analysis of milk proteins have been developed based on mass spectrometric methodologies (Siciliano *et al.* 2000). In particular, matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) has shown to be a powerful analytical tool in providing a valid fingerprint of milk protein profiles. This technique is fast, simple, and does not require any complicated preparation of milk samples. Cozzolino *et al.* (2001) was dealing with the detection of cow milk in ewe and buffalo milk. The determination of the presence of cow milk in ewe milk was based on the detection of bovine β-lactoglobulin A and B. Cow milk in buffalo milk was detected by the presence of bovine α-lactalbumin; adulteration of 5% was possible to detect. The authors consider this detection limit to be sufficient and they claim that, in the case of adulteration, the adulteration with less than 5% of cow milk is uneconomical.

Table 8. Retention times t_r (min) of whey proteins given by Romero *et al.* (1996)

Component	Goat	Bovine	Ewe
α-lactalbumin	7.99	9.37	7.30
Serum albumin	13.91	12.30	13.50
β-lactoglobulin	17.77	17.99 (A), 18.10 (B), 19.90 (C)	14.50 (A), 17.40 (B)

Conclusion

For detecting the replacement of one type milk with another protein analysis is used. Especially prospective for the identification of adulteration with individual types of milk are the immunochemical techniques and techniques based on DNA analysis. They enable detecting as little as 0.5 and 0.1% of adulterants, respectively. For the authenticity of milk samples and products (namely cheeses), not only the identification of the milk type is important, but also the quantitative supply of the individual milk types in the samples. The reverse phase HPLC seems to be appropriate to solve this problem.

References

Amigo L., Recio I., Ramos M. (2000): Genetic polymorphism of ovine milk proteins: its influence on technological properties of milk – a review. International Dairy Journal, **10**: 135–149.

Anguita G., Martin R., Garcia T., Morales P., Haza A.J., Gonzales I., Sanz B., Hernandez P.E. (1997): Detection of bovine casein in ovine cheese using digoxigenated monoclonal antibodies and a sandwich ELISA. Milchwissenschaft, **52**: 511–513.

Anonym (2001): Commission regulation (EC) No 213. Official Journal of the European Communities.

Bania J., Ugorski M., Polanowski A., Adamczyk E. (2001): Application of polymerase chain reaction for detection of goats' milk adulteration by milk of cow. Journal of Dairy Research, **68**: 333–336.

Bitri L., Rolland M.P., Besancon P. (1993): Immunological detection of bovine caseinomacropeptide in ovine and caprine dairy products. Milchwissenschaft, **48**: 367–371.

Bottero M.T., Civera T., Anastasio A., Turi R.M., Rosati S. (2002): Identification of cow's milk in buffalo cheese by duplex polymerase chain reaction. Journal of Food Protection, **65**: 362–366.

Bramanti E., Sortino Ch., Onor M., Beni F., Raspi G. (2003): Separation and determination of denatured α_{s1}-, α_{s2}-, β- and κ-caseins by hydrophobic interaction chromatography in cows', ewes' and goats' milk, milk mixtures and cheeses. Journal of Chromatography A, **994**: 59–74.

Cartoni G., Coccioli F., Jansionowska R., Masco M. (1999): Determination of cows' milk in goats' milk and cheese by capillary electrophoresis of the whey protein fractions. Journal of Chromatography A, **846**: 135–141.

Cattaneo T.M.P., Nigro F., Greppi G.F. (1996): Analysis of cow, goat and ewe milk mixtures by capillary zone electrophoresis (CZE): preliminary approach. Milchwissenschaft, **51**: 616–619.

Cozzolino R., Passalacqua S., Salemi S., Malvagna P., Spina E., Garozzo D. (2001): Identification of adulteration in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Journal of Mass Spectrometry, **36**: 1031–1037.

Davies D.T., Law J.R. (1980): The content and composition of protein in creamery milks in south-west Scotland. Journal of Dairy Research, **47**: 83–90.

Drbohlav J., Vodičková M. (2001): Tabulky látkového složení mléka a mléčných výrobků. ÚZPI, Praha.

Ferreira I.M.P.L.V.O., Caçote H. (2003): Detection and quantification of bovine, ovine and caprine milk percentages in protected denomination of origin cheeses by reversed-phase high-performance liquid chromatography of beta-lactoglobulins. Journal of Chromatography A, **1015**: 111–118.

Garcia T., Martin R., Morales P, Gonzalez I., Sanz B., Hernandez P. (1993): Sandwich ELISA for detection of caprine milk in ovine milk. Milchwissenschaft, **48**: 563–566.

Hadjipanayiotou M. (1995): Composition of ewe, goat and cow milk and of colostrum of ewes and goats. Small Ruminant Research, **18**: 255–262.

Haza A.I., Morales P., Martín R., García T., Anguita G., González I., Sanz B., Hernández P.E. (1996): Development of monoclonal antibodies against caprine rS2-casein and their potential for detecting the substitution of ovine milk by caprine milk by an indirect ELISA. Journal of Agricultural and Food Chemistry, **44**: 1756–1761.

Herrero-Martínez J.M., Simó-Alfonso E.F., Ramis-Ramos G., Gelfi C., Righetti P.G. (2000): Determination of cow's milk and ripening time in nonbovine cheese by capillary electrophoresis of the ethanol-water protein fraction. Electrophoresis, **21**: 633–640.

Hurley I.P., Coleman R.C., Ireland H.E., Williams J.H.H. (2004): Measurement of bovine IgG by indirect competitive ELISA as a means of detecting milk adulteration. Journal of Dairy Science, **87**: 543–549.

de Jong N., Visser S., Olieman C. (1993): Determination of milk proteins by capillary electrophoresis. Journal of Chromatography A, **652**: 207–213.

Jensen R.G. (1995): Handbook of Milk Composition. Academic Press, San Diego.

Kaminarides S.E., Koukiassa P. (2002): Detection of bovine milk in ovine yoghurt by electrophoresis of para-κ-casein. Food Chemistry, **78**: 53–55.

Klotz A., Einspanier R. (2001): Development of a DNA-based screening method to detect cow milk in ewe, goat and buffalo milk and dairy products using PCR-LCR-EIA-technique. Milchwissenschaft, **56***: 67–70.

Law A.J.R. (1995): Heat denaturation of bovine, caprine and ovine whey proteins. Milchwissenschaft, **50***: 384–388.

Lee S.J., Chen M.Ch., Lin Ch.W. (2001): Detection of cows' milk in goats' by capillary zone electrophoresis. Australian Journal of Dairy Technology, **56**: 24–27.

Levieux D., Venien A. (1994): Rapid, sensitive two-site ELISA for detection of cows' milk in goats' or ewes' milk using monoclonal antibodies. Journal of Dairy Research, **61***: 91–99.

López-Calleja I., González I., Fajardo V., Rodríguez M.A., Hernández P.E., García T., Matín R. (2004): Rapid detection of cows' milk in sheeps' and goats' milk by a species-specific polymerase chain reaction technique. Journal of Dairy Science, **87**: 2839–2845.

Maudet C., Taberlet P. (2001): Detection of cows' milk in goats' cheeses inferred from mitochondrial DNA polymorphism. Journal of Dairy Research, **68***: 229–235.

Mayer H.K., Heidler D., Rockenbauer C. (1997): Determination of the percentages of cows', ewes' and goats' milk in cheese by isoelectric focusing and cation-exchange HPLC of γ- and para-κ-caseins. International Dairy Journal, **7**: 619–628.

Moioli B., Pilla F., Tripaldi C. (1998): Detection of milk protein genetic polymorphisms in order to improve dairy traits in sheep and goats: a review. Small Ruminant Research, **27**: 185–195.

Pizzano R., Nicolai M.A., Padovano P., Ferranti P., Barone F., Addeo F. (2000): Immunochemical evaluation of bovine β-casein and its 1-28 phosphopeptide in cheese during ripening. Journal of Agricultural and Food Chemistry, **48**: 4555–4560.

Recio I., Amigo L., López-Fandifio R. (1997a): Assessment of the quality of dairy products by capillary electrophoresis of milk proteins. Journal of Chromatography B, **697**: 231–242.

Recio I., Perez-Rodrlguez M.L., Ramos M., Amigo L. (1997b): Capillary electrophoretic analysis of genetic variants of milk proteins from different species. Journal of Chromatography A, **768***: 47–56.

Rolland M.P., Bitri L., Besancon P. (1995): Monospecificity of the antibodies to bovine α$_{s1}$-casein fragment 140-149: application to the detection of bovine milk in caprine dairy products. Journal Dairy Research, **62***: 83–88.

Romero C., Perez-Andújar O., Olmedo A., Jiménez S. (1996): Detection of cow's milk in ewe's or goat's milk by HPLC. Chromatographia, **42***: 181–184.

Siciliano R.A., Rega B., Amoresano A., Pucci P. (2000): Modern mass spectrometric methodologies in monitoring milk quality. Analytical Chemistry, **72***: 408–415.

Strange E.D., Malin E.L., Van Hekken D.L., Basch J.J. (1992): Chromatographic and electrophoretic methods used for analysis of milk proteins. Journal of Chromatography A, **624***: 81–102.

Tamime A.Y., Barclay M.N.I., Law A.J.R., Leaver J., Anifantakis E.M., O'Connor T.P.O. (1999): Kishk – a dried fermented milk/cereal mixture. 2. Assessment of a variety of protein analytical techniques for determining adulteration and proteolysis. Lait, **79***: 331–339.

Tietze M., Majewski T. (1997): Chemical composition and sanitary value of cow and sheep milk. Bulletin of International Dairy Federation, No. 9702*: 303–307.

Velíšek J. (1999): Mléko a mléčné výrobky. In: Chemie potravin, Ossis, Tábor: 43–48.

Veloso A.C.A., Teixeira N., Ferreira I.M.P.L.V.O. (2002): 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***: 209–218.

Visser S., Slangen Ch.J., Lagerwerf F.M., Van Dongen W.D., Haverkamp J. (1995): Identification of a new genetic variant of bovine β-casein using reversed-phase high-performance liquid chromatography and mass spectrometric analysis. Journal of Chromatography A, **711**: 141–150.

Voelker G.R., Bleck G.T., Wheeler M.B. (1997): Single-base polymorphisms within the 5' flanking region of the bovine a-lactalbumin gene. Journal of Dairy Science, **80***: 194–197.

Corresponding author:

Ing. Markéta Borková, MILCOM, a. s., Výzkumný ústav mlékárenský, Ke Dvoru 12a, 160 00 Praha 6-Vokovice, Česká republika
tel.: + 420 224 382 726; fax: + 420 235 358 107; e-mail: borkova@af.czu.cz