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

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Abstract


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

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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 spe-
cies. 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 in-
dividual 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: αS1+, αS2−,
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 αS-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 proteoly-
sis 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 & Vodič-
ková 2001)

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

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Bovine</th>
<th>Ewe</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>44</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Milk output (kg/day)</td>
<td>21.09 ± 7.86</td>
<td>2.45 ± 0.549</td>
<td>3.49 ± 0.504</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>38.0</td>
<td>55.2</td>
<td>45.2</td>
</tr>
<tr>
<td>Crude protein (N × 6.38) (g/kg)</td>
<td>33.0</td>
<td>58.0</td>
<td>41.1</td>
</tr>
<tr>
<td>NPN (g/kg)</td>
<td>2.18</td>
<td>2.70</td>
<td>2.91</td>
</tr>
<tr>
<td>NPN (expressed in % of crude protein)</td>
<td>7.43</td>
<td>4.66</td>
<td>7.13</td>
</tr>
</tbody>
</table>
β-casein, originating from the action of plasmin, the endogenous alkaline milk protease.  𝜆-caseins are presumably fragments of  𝛼S1-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>
<thead>
<tr>
<th>Component (g/100 g)</th>
<th>Bovine</th>
<th>Ewe</th>
<th>Goat</th>
<th>Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>3.2</td>
<td>4.6</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Caseins</td>
<td>2.6</td>
<td>3.9</td>
<td>2.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Whey proteins</td>
<td>0.6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Component (g/l)</th>
<th>Bovine</th>
<th>Ewe</th>
<th>Goat</th>
<th>Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein content (%)</td>
<td>27.8 ± 2.2</td>
<td>59.4 ± 3.3</td>
<td>33.4 ± 1.6</td>
<td>49.2 ± 1.9</td>
</tr>
<tr>
<td>Caseins relating to total protein content (%)</td>
<td>83 ± 10</td>
<td>93 ± 10</td>
<td>99 ± 12</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>αS1-casein (%)</td>
<td>37 ± 7</td>
<td>33 ± 8</td>
<td>10 ± 6</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>αS2-casein (%)</td>
<td>7 ± 1</td>
<td>14 ± 2</td>
<td>–</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>β-casein (%)</td>
<td>42 ± 8</td>
<td>30 ± 5</td>
<td>63 ± 11</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>γ-casein (%)</td>
<td>6 ± 2</td>
<td>9 ± 1</td>
<td>18 ± 4</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>κ-casein (%)</td>
<td>9 ± 4</td>
<td>14 ± 2</td>
<td>8 ± 2</td>
<td>7 ± 1</td>
</tr>
</tbody>
</table>

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

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

Table 5. Composition and content of whey proteins in skimmed cow, ewe and goat milk (Law 1995)
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 \( \gamma_1, \gamma_2, \gamma_3 \) and para-\( \kappa \)-caseins were detected (Strang 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. 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 \( \alpha_{S1} \)-casein. This technique was also used for the identification of bovine milk in ewe yoghurt (Kaminarides & Koukiassa 2002). The marker component was bovine para-\( \kappa \)-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 \( \kappa-, \beta-, \alpha \)-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 \( \alpha_S \)-casein (Veloso et al. 2002).
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 $\gamma_2$- and $\gamma_3$-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 $\gamma_2$-casein and $\gamma_3$-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-$\kappa$-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 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). $\alpha_1$-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 $\alpha_1$-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 $\alpha$-lactalbumine and bovine $\beta$-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-MARTÍNÉZ 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 electroforegments 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 $\beta$-Lg (LEVIEUX & VENIEN 1994), bovine IgG (HURLEY et al. 2004), bovine $\kappa$-casein (BITRI et al. 1993), bovine $\beta$-casein (ANGUITA et al. 1997), bovine $\alpha_{21}$-casein (ROLLAND et al. 1995), and goat whey proteins (GARCÍA 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%) (GARCÍA 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 $\alpha_{21}$-casein was used. For the detection of basically similar types of milk, such as bovine and buffaloo, 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 $\alpha$-La and $\beta$-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 $\alpha$-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 $\alpha S_1$-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 $\alpha S_1$-, $\alpha S_2$-, $\beta$- and $\kappa$-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 ($\alpha S_1/\kappa$, $\alpha S_2/\beta$, $\beta/\kappa$ and $\alpha S_2/\alpha S_1$) 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 $\beta$-lactoglobulin A and B. Cow milk in buffalo milk was detected by the presence of bovine $\alpha$-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>
<thead>
<tr>
<th>Component</th>
<th>Goat</th>
<th>Bovine</th>
<th>Ewe</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-lactalbumin</td>
<td>7.99</td>
<td>9.37</td>
<td>7.30</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>13.91</td>
<td>12.30</td>
<td>13.50</td>
</tr>
<tr>
<td>$\beta$-lactoglobulin</td>
<td>17.77</td>
<td>17.99 (A), 18.10 (B), 19.90 (C)</td>
<td>14.50 (A), 17.40 (B)</td>
</tr>
</tbody>
</table>
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

BRAMANTI E., SORTINO Ch., ONOR M., BENI F., RASPI G. (2003): Separation and determination of denatured \( \alpha_1 \), \( \alpha_2 \), \( \beta \)- and \( \kappa \)-caseins by hydrophobic interaction chromatography in cows’, ewes’ and goats’ milk, milk mixtures and cheeses. Journal of Chromatography A, 994: 59–74.


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