RELIABILITY OF COW CASEIN QUANTITATION IN SHEEP MILK AND CHEESE BY ELISA METHOD

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ABSTRACT

In the present work we studied the use of ELISA method for the detection of the lab-prepared adulteration of sheep milk and cheese with cow milk. The analyses were focused on laboratory testing and evalutation of qualitative parameters commercially used ELISA tests (Casein ELISA set, SEDIUM R&D) based on detection of cow milk casein. Casein was determined in 16 samples of milk and 16 samples of cheese. Measurement of absorbance values were repeated twice under different combination of mixing (0; 0.5; 5; 50; 75 and 100 % of raw and heat treament cow milk in sheep milk). The results showed that this assay takes only about three hours and is suitable for detection of lots of sheep milk adulterated with 0.5 to 50 % cow milk (regression equations with R^2 determination coefficient: $R^2 = 0.965$). Our experiments shown that used ELISA test is not suitable to reliably detect the presence of cow milk casein in sheep cheese ($R^2 = 0.022$).

INTRODUCTION

The substitution of milk from one species by a milk of lower economic importance during the manufacture or manipulation of cheese is a fraudulent practice for consumers an is disloyal competition for other producers (Veloso et al., 2002). The identification of the species that originally produced the milk represents a considerable problem for food analysts (Bottero et al. 2002). ELISA is the most widely used form of immunoassay in milk analysis and has advantages of high sensitivity, low cost and fast application. It is easy to use, reliable, rapid and readily automated. Target antigens are caseins, lactoglobulins, immunoglobulins and other whey proteins (Hurley et al., 2004; Ruprichová et al., 2010). The largest share of pure proteins (on average 80 %) is attributed to proteins of casein fraction. Casein of cow milk represents a set of four phosphorprotein fractions designated as α_{S1}-, α_{S2}-, β- and κ-caseins (Madureira et al., 2007; Buňka et al., 2009). The caseins feature advantage in being more or less

stable under high temperature conditions. Therefore they can be successfully used as the main antigens in the heat treatment (pasteurization, UHT) of milk and milk products. Their major disadvantage is weak immunogenicity and higher sensitivity to protheolytic degradation (García-Risco et al., 2002).

MATERIALS AND METHODS

Raw sheep milk and raw cow milk gained from primary production, respectively heat treatment cow milk was mixed in defined amounts (0; 0.5; 5; 50; 75 and 100 % of cow milk in sheep milk). Cow milk was pasteurized for 15 seconds at 72 °C and 3 seconds at 85 °C. Process of cheese production included: cheesing of milk itself, processing of cheese curd, turning of cheese curd surface, its cutting, harping and miwing and finally formation of cloddish cheese. During the period of 12 days the temperature and pH in individual clods had been observed. The supernatant liquids were stored in freezer until specific preparation for ELISA analysis. The determination of casein is based on its immunochemical reaction with a specific antibody. The intensity of colouration thus developed is proportional to the concentration of casein in calibrators and samples. The measurement of the absorbance was made photometrically at 450 nm (STAT FAX 321/plus microwell reader - Awareness Technology, Palm City, FL).

RESULTS AND DISCUSSION

The objective of analyses was to find out whether the used ELISA test can really detect the defined concentration of cow milk in sheep milk and sheep chesse samples. The detection and quantification of cow milk was based on the presence of the specific caseins.
In line with the test instructions there was performed laboratory analysis of 16 samples of sheep milk and 16 samples of sheep cheese. At the beginning were intentionally contaminated by different cow milk additions. In accordance with the producer´s declared quantitation range contained in the manual to ELISA kit, it is possible to correctly quantify the contamination between 0 – 45 ppm (mg/kg) of cow casein presence in the examined samples. The starting point for obtaining of relevant data was to create 2 calibration curves (for milk: $y = -3.1375x - 0.398$; $R^2 = 0.9485$; for cheese: $y = -2.9743x - 0,3976$; $R^2 = 0.9753$). Majority of the samples were successfully quantified but only thanks to their multiple dilution prior to the analysis itself (0,5; 5 % with 10^{-1} and 10^{-2}; 50 % with 10^{-3}; 75; 100 % with 10^{-4} and 10^{-5}). In some samples, for the purpose of higher quality determination, we propose to increase the dilution by one decimal

order, because even in the dilution used by us, the addition of cow milk was not detected.

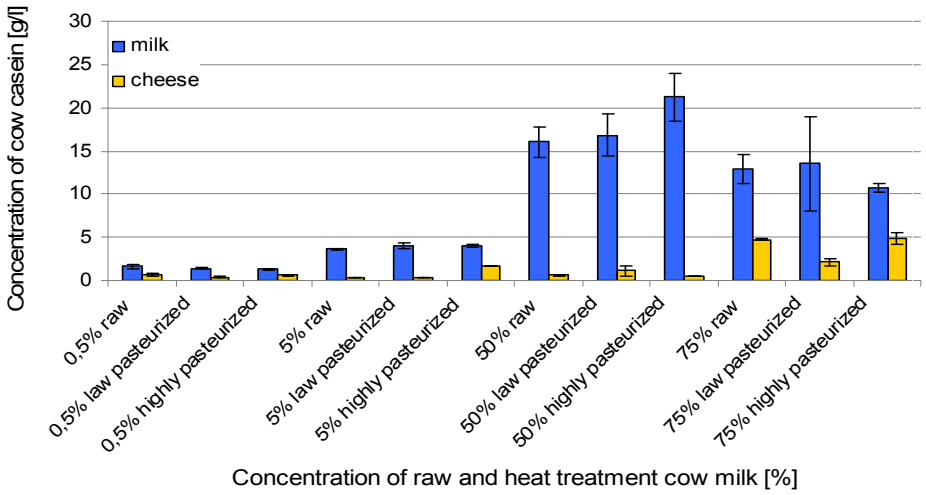

Figure 1.
Amount of detected cow casein in milk and cheese depending on mixing type of cow and sheep milk [g/l]

Results obtained with ELISA applied to the analyses of milk samples showed a correlation of < 0,001 between the detected amount of cow casein and cow milk admixture. The method proved to be specific, precise and accurate within the percentage range 0.5 – 50 %. Hereby it can be observed that on changes in contents of cow casein does not affect the heat treatment of milk within various falsified ratios. However, the test revealed to be more accurate for milk samples than for cheese samples. The presence of cow casein with the increasing proportion of either raw or pasteurized cow milk was not correlated (Figure 1).

As is shown in Figure 2, in sheep milk samples by interpolation of detected content of casein can be reliably determined corresponding content of the addition of cow milk (determination coefficient: $R^2 = 0.965$). In contrast, from samples of cheese the trend of detected amount of cow casein was not reflecting actual addition of cow milk ($R^2 = 0.022$).

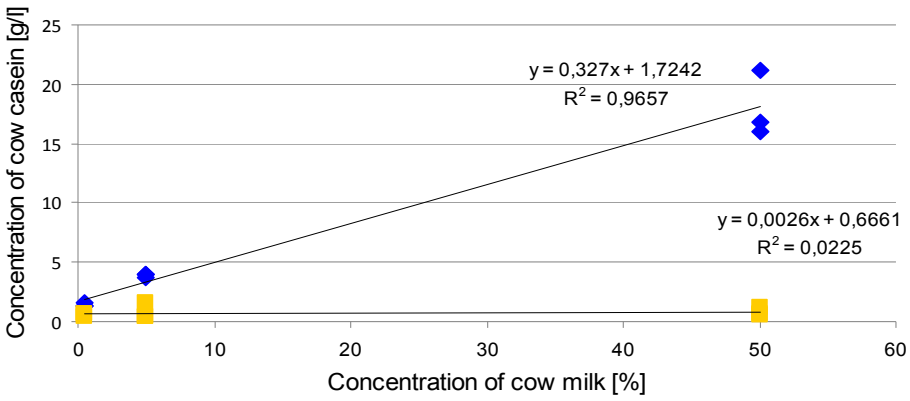

Figure 2
Trend of detected cow casein in milk and cheese [g/l]

CONCLUSION

Various mixtures of cow and sheep milk were analysed, leading to a reproducible calibration curve, which has been successfully employed in determining the percentage of cow milk fraudulently added to ewe milk in the production of ewe cheese. ELISA have been successfully applied to the detection of cow milk adulteration in sheep milk using a range of adulteration percentages (0.5 –50 %). Use of ELISA test is not adequate for routine surveillance of cheeses, especially for mixed cheeses, when the amount of milk from different species used for cheese making is unknown. Our experiments show that for better quality determination, especially of low concentrations, it is necessary to find an appropriate dilution for various concentrations of cow milk in the context with the way of its thermal treatment as well as further technological processing.

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