Authenticity of dairy products by Capillary Electrophoresis

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INTRODUCTION

Capillary electrophoresis (CE) represents a very powerful technique due to the multiple adjustable parameters that are unknown to other analytical techniques. In CZE mode it can easy separate charged molecules, such as proteins, in MEKC mode it can analyze neutral molecules, and in Electrochromatography mode it combines the separation driven by a stationary phase (chromatography) together with adjunctive resolution power provided by a high electrical voltage.

Milk and derivatives are a very important part in the diet of the world population. Products from goat, buffalo and sheep species have a greater economic value than the cow ones therefore authenticity frauds occur frequently: dairy products are among the seven more attractive foods for adulteration. Each milk from the above-cited animal species has its own definite pattern of whey proteins, essentially variants of β-Lactoglobulin (β-Lg) and α-Lactalbumin (α-LA) that can be usefully exploited as markers of authenticity by means of CE in CZE mode. This work presents case studies on the authenticity of cheeses and on some milk mixtures. Sample preparation simply required the adding of an acidic solution for milk samples and of distilled water for cheese samples. After centrifugation and filtration, samples were ready for CE analysis. Electrophoretic runs where carried out by means of an alkaline buffer and the UV detection which allowed the complete resolution of proteins of interest in few minutes. Instrument used was a Spectraphoresis 1000 from Thermo Quest Corporation (California, U.S.A.).

CASE STUDIES

Ewe-cow milk mixtures

Fig. 1. Ewe milk and cow milk. Conditions: 18kV, methyl deactivated column 68 cm (61 cm to the detector), 50 µm ID, borate buffer 80 mM, UV detection 200 nm, T 25°C.

Fig. 1a shows an electropherogram of whey proteins from a pure ewe milk collected in central Italy. With the conditions used the optimum resolution between ewe alfa-Lactalbumin (ewe α-LA) and ewe beta- Lactoglobulin (ewe β-Lg) can be observed (all the original high resolution figures of the present poster can be requested from the corresponding author). By using the same experimental conditions an electropherogram of pure cow milk is reported in Fig. 1b. The three whey proteins of cow milk are perfectly resolved.

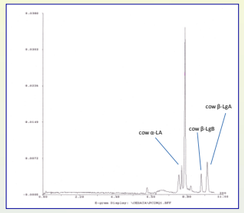

Fig. 2. Mixture of ewe milk and cow milk 50:50. Conditions: 28kV, methyl deactivated column 68 cm (61 cm to the detector), 50 µm ID, borate buffer 80 mM, UV detection 200 nm, T 25°C.

The three peaks in Fig. 1b refer to cow α-LA, cow β-Lg variant B (β-LgB) and cow β-Lg variant A (β-LgA). Run times are short, less than 15 min. By overlapping the two electropherograms 1a and 1b it may be expected that by injecting a mixture of ewe and cow milk ALL whey proteins coming from the two milk types could be separated (Fig. 1c). To test this hypothesis, a mixture of sheep and bovine milk 50:50 was prepared and subsequently injected. Instrumental conditions were essentially the same of those in Figs. 1a, 1b, 1c, but the potential was increased from 18kV to 28 kV to maintain a good peak efficiency of a complex protein mixture like this one. The result is shown in Fig. 2. As the Voltage increases the run time decreases but the whey proteins coming from the two milks are perfectly resolved in 10 min so demonstrating the great potential of Capillary Electrophoresis in CZE mode in the field of protein analysis. The characteristic "doublet" of cow β-LgB and cow β-LgA is clearly visible in Fig. 2. Such peaks can be used as markers for the presence of cow milk. Therefore they represent markers of authenticity.

A milk declared as "pure sheep's milk" should have a profile as in Fig. 1a without the presence of bovine whey proteins. By using cow β-LgA as a marker of cow milk (more intense than cow β-LgB so allowing a better limit of detection) a limit of detection of 2% of cow milk fraudulently added to ewe milk can be measured with the present method. Given the quick and simple milk preparation and by considering the fast analysis time, the method can be used to quickly check the authenticity of sheep's milk batches that could be subject to fraud with cow's milk adding. In fact cow's milk is much cheaper and more available than sheep's milk. This latter has limited availability depending on the season and is used to produce typical products with high economic and cultural value such as the "Pecorino cheese" which is PDO, that means it has Protected Designation of Origin status at European level.

Goat-cow milk mixtures

Goat-based dairy products have a thousand-year tradition and were widespread in all ancient civilizations. The tradition continues today, in some cases with the support of modern technologies, but in many other cases by means of traditional methods. From the nutritional point of view goat milk contains more short- and medium-chain fatty acids than cow milk.

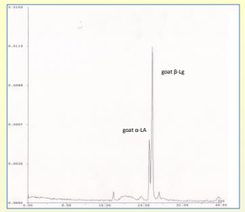

Fig. 3. Pure goat milk. Conditions: 4kV, methyl deactivated column 29 cm (22 cm to the detector), 50 µm ID, borate buffer 50 mM, UV detection 200 nm, T 25°C.

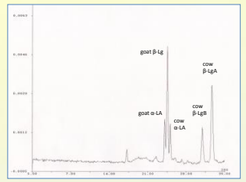

Fig. 4. Mixture of goat milk and cow milk 50:50. Conditions: 4kV, methyl deactivated column 29 cm (22 cm to the detector), 50 µm ID, borate buffer 50 mM, UV detection 200 nm, T 25°C.

These fatty acids are more rapidly metabolized and absorbed by the organism, therefore goat milk is more easy to digest with respect cow milk. Other characteristics are the following: the free fatty acids in goat milk are lower than in cow's milk, the price is higher, the flavor is stronger and the lactose content is a little lower. As regards goat cheeses, there is a vast tradition and an extensive market worldwide. The particular content of medium-chain fatty acids (caproic, caprylic, capric) confers typical organoleptic properties to goat cheeses which therefore have a good economic value and are sought after as typical products in many countries.

They can be marketed as fresh or aged cheeses. For all the reasons mentioned and also due to the more limited availability of goat's milk compared to cow's milk, it is important to check the correct composition of batches of milk declared as "pure goat".

Figs. 3 and 4 show the great performance of CE in ascertaining the authenticity of goat milk when fraudulently added with cow's milk. In Fig.3 the electropherogram of a pure goat milk sample collected in southern Italy is reported. The two main whey protein α-Lactalbumin and β-Lactoglobuiln are quite resolved. A mixture of goat-cow milk 50:50 was then prepared and injected. Result is shown in Fig.4. It may be noted that all whey proteins coming from the two types of milk are resolved. On the right it appears the typical profile of cow's milk, as in Fig. 1b, with cow α-LA, cow β-LgB and cow β-LgA. Applied Voltage was lower with respect Figs. 1 and 2 (ewe milk) therefore the runtime increased to about 33 min.

Under these conditions for a batch of goat's milk adulterated with cow's milk it is impossible to pass the authenticity check performed by Capillary Electrophoresis as the bovine whey proteins must be completely absent in the electropherogram.

Goat cheeses

Fig. 5 shows a goat cheese of French origin purchased on the market. It was commercialized in the form of fresh cheese. The whey proteins present in the milk, from which the cheese derived from, are well visible also in the electropherogram of the cheese. It can be noted that the relative intensities of goat α-LA and goat β-Lg are different from that observed in Fig.3 for the goat milk collected in southern Italy. This is probably due to the different breed of goats considered (genetic variability). From the electropherogram in Fig.5 it seems that the cheese was produced with goat milk only, as declared by the producer. But for a true confirmation an experimental verification is necessary, also by considering that a weak peak is present at about 22.4 min. For the confirmation a minimum amount of whey from cow milk was added to the solution of goat cheese already injected for the electropherogram of Fig.5. The spiking is to be deemed perfectly successful: the two weak peaks of cow β-LgB and cow β-LgA appear in an area previously devoid of peaks, on the sides of the weak pre-existing peak, thus confirming the total absence of cow's milk in the production of this declared "pure goat cheese".

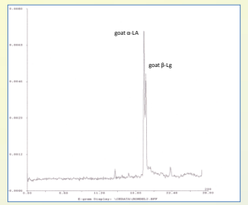

Fig. 5. Goat cheese. Conditions: 12kV, methyl deactivated column 29 cm (22 cm to the detector), 50 µm ID, borate buffer 80 mM, UV detection 200 nm, T 25°C.

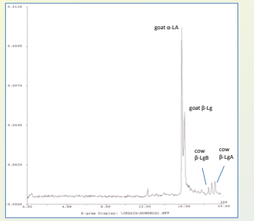

Fig. 6. Goat cheese spiked with a minimum amount of cow milk. Conditions: 12kV, methyl deactivated column 29 cm (22 cm to the detector), 50 µm ID, borate buffer 80 mM, UV detection 200 nm, T 25°C.

In other cases of goat cheeses found from local producers (but in the form of aged cheeses) we carried out the same control that was effective. However it was much more laborious due to the presence of some new-formed peptides which derived from the ripening process.

CONCLUSIONS

Case studies about ewe-cow and goat-cow milk mixtures together with the analysis of a goat cheese are presented. In all these cases Capillary Electrophoresis showed effective in determining the true composition of the sample by a simple operational procedure and a short time requested, so making the technique very suitable for checking the authenticity of dairy products. Some limitations are to be taken into account, mainly when aged cheese are examined since in such samples the peptides formed during the ripening process may interfere with the whey proteins used as markers of authenticity.

REFERENCES

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