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## Analytical, Nutritional and Clinical Methods

## Evaluation of cheese authenticity and proteolysis by HPLC and urea-polyacrylamide gel electrophoresis

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## Abstract

Chromatographic and electrophoretic methods have been established as useful tools in characterising cheese ripening and in the detection of milk adulteration. The purpose of this work was to evaluate casein proteolysis of cheeses made from bovine, ovine or mixtures of bovine and ovine milks, as well as ovine cheese authenticity, for 30 days of ripening by HPLC and urea–polyacrylamide gel electrophoresis.

Complementary information was obtained by both techniques when applied to the study of casein proteolysis during 30 days of ripening of ovine milk cheeses, ovine milk cheeses with 10% and 20% of bovine milk and bovine milk cheeses, manufactured according to the traditional Terrincho technology. For ovine cheeses,  $\alpha$ -casein was the fraction that showed the higher degradation during cheese ripening. A similar behaviour was observed for ovine milk cheese with 10% of bovine milk. The profile for ovine milk cheese with 20% of bovine milk was more similar to that obtained for bovine cheese. Concerning bovine milk cheeses, electrophoresis was the most sensitive technique for the evaluation of proteolysis in these cheeses.

Ten and 20% of bovine milk could be detected in ovine milk cheeses by urea-polyacrylamide gel electrophoresis and HPLC, respectively, even after 30 days of ripening.

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## 1. Introduction

The origin of the milk used to manufacture cheese must be declared by the producer, especially in the case of protected denomination of origin (PDO) cheeses. However, adulteration of ovine milk with bovine milk is relatively common due to: (i) seasonal fluctuations of the availability of ovine milk; (ii) the higher price of ovine milk compared to bovine milk; and (iii) opportunity to use the overproduction of bovine milk without loss of profit (Herrero-Martínez, Simǿ-Alfonso, Ramis-Ramos, Gelfi, & Righetti, 2000). Therefore, the development of reliable detection methods that can be applied to cheeses after ripening is of great interest in countries that produce or import ovine milk cheeses.

In recent years, several methods were reported in the literature for the detection of milk and cheese adulteration based on the analysis of casein fractions or whey proteins, and the study of cheese ripening: (i) electrophoretic techniques using polyacrylamide gels with urea (urea–PAGE) (Carretero, Trujillo, Mor-Mur, Pla, & Guamis, 1994; Farkye, Kiely, Allshouse, & Kindstedt, 1991; Gobbetti et al., 2002; Mayer & Hörtner, 1992; Veloso, Teixeira, & Ferreira, 2002) or SDS (SDS– PAGE) (Basch, Douglas, Procino, Holsinger, & Farrell, 1985; Jin & Park, 1996) and isoelectric focusing (IEF) (Kim & Jimenez-Flores, 1994), (ii) high-performance liquid chromatography (HPLC) by ion-exchange (Andrews, Taylor, & Owen, 1985; Kaminarides & Anifaantakis, 1993), hydrophobic interactions (Bramanti

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