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Evaluation of a commercial ELISA method for the quantitative detection of goat and cow milk in ewe milk and cheese☆

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ABSTRACT

Portugal produces traditional protected designation of origin (PDO) cheeses, being the majority exclusively made with raw ewe's milk.

A PDO cheese must be genuine, thus these products have to be controlled in order to preserve its commercial quality. The possibility of adulteration of ewe cheesemilk with cow or goat milk can result in inferior characteristics than that expected by the consumer.

The purpose of the present work was to evaluate and validate an available commercial ELISA method, for quantitative determination of adulterations in ewe milk and cheese with cow or goat milk. For this, ewe milk was mixed with cow and goat milk using a range of adulteration percentages (1–50%) and model cheeses were manufactured according to traditional Portuguese ewe milk cheesemaking. Ewe milk cheese was also manufactured as control. The milk samples and respective cheeses were analysed to detect and quantify the amount of cow and goat milk added using specific commercial ELISA tests: Quantispeed Bov Test: QBT® and Quantispeed Goat Test: QGT®, respectively.

Results obtained with QBT® and QGT® showed a correlation of ~1 between the experimental and the detected value. The method proved to be specific, precise and accurate within the work domain and the detection and quantification limits were ~0.2% adulteration for both cow and goat milk. However, both tests revealed to be more accurate for milk samples than for cheese samples. The detected value in cheese samples was ~10% lower than the experimental value for QBT® and ~20% lower for QGT®, when more than 40% cow or goat milk were added. Therefore, the use of this test is not adequate for routine surveillance of cheeses in the market, especially for mixed cheeses, when the amount of milk from different species used for cheesemaking is unknown.

1. Introduction

Portugal has a strong tradition of regional cheesemaking. Traditional cheese varieties are produced from raw ewe and goat milk or from the mixture of both types of milk in various proportions. Some of them are still produced on a farmhouse scale and are traded directly within their production area or to cheese dealers. Despite the dominance of "industrial" pasteurised cow milk cheese in the market, the artisanal ewe and goat milk cheeses are considered as the most representative of Portugal culture and they are strongly demanded by the consumers. The production of some of these cheese varieties is now protected either by a protected designation of origin (PDO) or by the protected geographic indication (PGI). This makes compulsory the use of local pure ovine raw milk and *Cynara* L. as coagulant (usu-

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ally *Cynara cardunculus* L.) for the ewe milk cheeses and pure caprine raw milk for the goat cheese.

Particularly ewe milk cheeses have an important socio-economical contribution to the dairy sector at local and regional areas of each country and thus play an important part in the local agricultural economy. These cheeses are named after the region in which they are produced and the manufacturing techniques used by the farmers are very straightforward. Coagulation takes place at temperatures between 27 and 30 °C, the curd is cut, partially drained and then kneaded slowly by hand to aid drainage; dry salting is used. Ripening typically takes place at 8–13 °C and 85–90% RH.

Consumer demand for these products is increasing, which encourages some manufacturers to increase their production. The production of most of these cheeses is limited to the seasonal production of ewe's milk, although some attempts to produce them all year round have been carried out. Their popularity and recent increasing demand by consumers might lead to the addition of milk from other species in the cheesemilk, with the aim to extend production. This procedure is considered as fraud (Roseiro et al., 2003a).

Nevertheless, mixtures of bovine, caprine and ovine milk are also used for cheese production at a semi-industrial cheesemaking scale, not having the PDO quality indication. In these cases, it is necessary to indicate on the label the species of milk used in the manufacture of cheeses, in order to keep the consumer informed and able to choose the products they are buying. However, in such cases, the proportion in which these mixtures are present are not indicated on the label and most of the time, depending on the season of the year, particularly ovine milk is present at a very low percentage or sometimes it is even absent. There are often queries for detection of milk kind in ewe cheeses, both qualitative and quantitatively. Cheeses that are already in the market are analysed, and very rarely the analysis is done in a milk sample from bulk cheesemilk before cheesecurd manufacture.

Different protein-based methods have been reviewed for species identification in milk and dairy products, such as electrophoretic, chromatographic and immunological techniques (Chianese et al., 1990; EC Regulation, 2001; Molina et al., 1999). Also recently, immunoassays capable of distinguishing between milk species have received special attention (Moatsou and Anifantakis, 2003; Hurley et al., 2004). Among these, the enzyme-linked immunosorbent assay (ELISA) is the most widely used due to the fact that is a fast and easy procedure (Giovannacci et al., 2004).

The present work refers to the application of a commercial ELISA method for the quantitative determination of adulterations of ewe milk and cheese with cow or goat milk. Model cheeses were manufactured according to traditional Portuguese ewe milk cheesemaking using mixtures of cow, goat and ewe milk at known proportions (1–50%). A 100% ewe milk cheese was also manufactured as control. The milk samples and respective cheeses were analysed to detect and quantify the amount of cow and goat milk added using specific commercial ELISA tests: Quantispeed Bov Test: QBT® and Quantispeed Goat Test: QGT®, respectively.

Table 1
Composition of milk mixtures for the cheesemaking trials

Trial	Cheese	Milk origin (%)			Milk volume (mL)		
		Ewe	Cow	Goat	Ewe	Cow	Goat
	100	100	0	0	5000	0	0
1	98	98	1	1	5000	51.02	51.02
	90	90	5	5	5000	278	278
	60	60	20	20	4000	1333	1333
2	10	10	45	45	889	4000	4000
	0	0	50	50	0	5000	5000

2. Materials and methods

2.1. Reagents and milk samples

All reagents used throughout the study were P.A. grade (SIGMA, Aldrich) and the water used for all the analyses in the present work was Milli-Q water (Milli-Q® Ultrapure Water Purification Systems, Millipore, Portugal).

Pure raw ewe and goat milk were obtained from local certified milk producers in the outskirts of Lisbon (Azeitão for ewe milk and Azambuja for goat milk, respectively). Pasteurised whole cow milk was purchased from a local supermarket.

2.1.1. Microbiological analyses

All milk samples were subjected to routine microbial analyses for hygienic quality survey before cheesemaking (total viable count at 30 °C, presumptive coliforms with confirmation of *E. coli*, *Staphylococcus aureus* and *Listeria monocytogenes*), according to internal methods based on Portuguese standards (data not shown).

2.1.2. Biochemical analyses

Biochemical analyses were also performed in all milk samples in order to characterise them. Methods of analysis used were mainly according to Portuguese standards (NP) or internal methods based on Portuguese and/or IDF standards. Each determination was carried out in duplicate. pH was determined for all samples by direct measurement using a Metrohm 713 pH Meter (Metrohm Ltd., Herisau, Switzerland). Titrable acidity (NP-470, 1983) fat (NP-469 and NP-2105, 1983) total nitrogen (IDF-20B, 1993), dry matter (NP-475-1983), freezing point (IDF Standard 108B, 1991) and antibiotics (kit Delvotest® SP/SPMINI, *DSM Food Specialities*, *Dairy Ingredients*, Holland) were also determined for all samples.

2.2. Cheese manufacture

2.2.1. Coagulants

For the traditional cheesemaking procedure, dried cardoon flowers (*Cynara cardunculus*, L) obtained from a cheese dairy in Alentejo, were used to prepare the vegetable coagulant. Traditionally, the amount of dried flowers is calculated empirically by the cheesemaker, e.g. for 50 L milk, a handful of flowers was used (ca. 30 g). These were then soaked in ca. 0.5 L of cold water, squeezed, and the wet flowers were macerated in a mortar. The macerated flowers were added again to the same water, mixed well and then squeezed resulting in an aqueous extract of a purple-brownish colour. This aqueous extract was then filtered through a cloth and added to the milk (Roseiro et al., 2003b). Bovine animal rennet (Chr. Hansen, Denmark) was also used for the manufacture of model cheese with low or no ewe milk content.

2.2.2. Cheesemaking process

Milk mixtures for cheese trials were prepared according to the experimental design shown in Table 1. Six cheeses were manufactured in two different trials (within 15 days apart), following the traditional procedure for ewe's milk cheesemaking in Portugal, signed as follows: 100 (pure ewe milk), 98 (ewe adulterated with 1% cow milk and 1% goat milk), 90 (ewe adulterated with 5% cow milk and 5% goat milk), 60 (ewe adulterated with 20% cow milk and 20% goat milk), 10 (ewe adulterated with 45% cow milk and 45% goat milk) and 0 (50% cow milk and 50% goat milk).

The first step was to warm up the milk to 30–32 °C in a water bath, followed by the addition of 10 mL of the coagulant prepared just before use, as described above. For cheeses 10 and 0 in trial 2, the vegetable coagulant