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Detection of bovine milk in ovine yoghurt by electrophoresis of para-κ-casein

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Abstract

The possibility of detection and determination of bovine milk in adulterated yoghurt by cationic polyacrylamide gel electrophoresis (PAGE) of yoghurt caseins, treated with rennet, was examined. Yoghurts made from bovine and ovine milk and from known mixtures of the two were examined. The evaluation of bovine milk in yoghurt was based on the optical density of the bovine para-κ-casein band, which was distinctly separated and bore a linear relationship to the percentage of bovine milk in the mixtures. By using PAGE of bovine para-κ-casein, levels of bovine milk as low as 1% were easily detected in ovine yoghurt.

Keywords: Adulterated yoghurt; Para-κ-casein; PAGE; Bovine and ovine milk detection

1. Introduction

Ovine milk is a very important commodity for the economy of some Mediterranean countries because it is used for the production of high quality traditional products, such as ovine cheeses and yoghurts. Ovine milk is always significantly more expensive than bovine milk, a fact which leads some milk producers to mix the two kinds of milk in order to increase their income. In Greece, yoghurts are made on a commercial scale from ovine and bovine milk. Yoghurt from ovine milk is more popular since it possesses unique organoleptic characteristics (a pleasant aroma and flavour, and a firm, creamy texture). In order to assure the authenticity of yoghurts and guarantee that they are unadulterated and accurately labelled, analytical techniques to detect mixtures of milk from different species are needed. A number of methods (chemical, immunological, physicochemical and their combination) proposed for the detection of bovine, ovine and caprine milk, in mixtures of milk and in cheese, have been reviewed by Ramos and Juarez (1986). The most accurate and sensitive of these methods are based on immunological and electrophoretic techniques. However, there are few publications concerning the detection of ovine, caprine and bovine milk in yoghurt (Anifantakis & Massouras, 1989) and no specific regulations within EU or Greek legislation for a reference method to detect and evaluate yoghurt adulteration. Food analysts are therefore challenged by the need to find an accurate analytical method for the detection and quantitative determination of bovine milk in ovine yoghurts. Immunological techniques were excluded from the analysis of yoghurt because heating of milk to 90–95 °C for 10 min, during yoghurt manufacture, is believed to cause denaturation of the milk immunoglobulins and perhaps affect the sensitivity of these methods (Calvo, Amigo, Olano, Martin, & Ramos, 1989). By contrast, the existing anionic PAGE analysis of yoghurt caseins, at alkaline pH, permits the determination of 2.5% bovine milk or more in caprine yoghurt (Anifantakis & Massouras, 1989). Isoelectric focusing (IEF) of γ_1 and γ_2 caseins (EU reference method, 1996) and para-κ-caseins (Mayer, Heidler, & Rockenbauer, 1997) for the detection of possible adulteration of milk and cheeses may be applicable to yoghurt but, to date, neither their sensitivity nor detection limit have been determined for yoghurt.

Electrophoresis of *para*-κ-caseins, rather than γ-caseins, has been preferred for the following reasons: (1)

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There were fewer zones in cationic electrophoresis than those in anionic electrophoresis of caseins treated with rennet, (2) the bovine and ovine *para*-κ-casein have different electrophoretic mobilities and isoelecric points; (3) it has been applied for the detection of bovine milk in ovine and caprine cheese (Addeo, Moio, Chianese, Stingo, & Di Luccia, 1990; Assenat, 1967; Kandarakis, Kaminarides, & Moschopoulou, 1995; Mayer, Heidler, & Rockenbauer, 1997) and (4) PAGE additionally offers lower cost and less technical skills than IEF.

The aim of this study was to evaluate whether cationic PAGE analysis of yoghurt caseins treated with rennet, under appropriate conditions, could be used for the detection of bovine milk in ovine yoghurt, using the bovine *para*-κ-casein as a protein marker.

2. Materials and methods

2.1. Milk

Bovine and ovine milks were collected from the herd of the Agricultural University of Athens.

2.2. Yoghurt manufacture

Traditional yoghurt was manufactured weekly in the pilot plant of the Dairy Laboratory of the Agricultural University of Athens, according to the procedure of Veinoglou (1980). Each week, seven milk types were used for yoghurt production, consisting of pure ovine and bovine milk, as well as mixtures containing 1, 2.5, 5, 10 and 20% bovine milk. This procedure was repeated for 5 weeks.

2.3. Casein isolation

Isolation of yoghurt caseins was achieved following the procedure described in the regulations of the European Commission (EU, 1996).

2.4. Rennet treatment of caseins

After a series of preliminary experiments to improve the detection limits, the following sample preparation was formulated. For each sample, 35 mg of isolated caseins were dissolved in 0.5 ml phosphate buffer (0.02 M Na_2HPO_4, 0.046 M KH_2PO_4, 6 M urea, 0.3% 2-mercaptoethanol) at pH 6.5 and mixed with a Vortex. Five microlitres of a 2.5% rennet solution (w/v) was added and the mixture incubated at 35 °C for 30 min with continuous shaking in a thermostatically-controlled water bath. Tubes were capped and rennet was inactived by heating to 80 °C for 5 min and the samples were submitted to electrophoresis or stored at −18 °C.

2.5. Cationic polyacrylamide gel electrophoresis (PAGE)

During the analysis, 60 μl from each sample were applied to each gel well for electrophoresis. PAGE was carried out using a vertical slab unit (Hoefer Scientific Instruments, SE 600, San Francisco, California) with slabs (160×140×1.5 mm) of acrylamide gel (12% T, 3% C, 0.06 M Tris–HCl, pH 6.7, 4.5 M urea, 0.01% NaN_3, 0.05% ammonium persulfate and 0.10% N,N,N,N tetramethylene diamine). Electrode vessels were filled with 0.008 M Tris and 0.03M 5,5-diethylbarbituric acid buffer, pH 7.4. After 30 min pre-electrophoresis at 150V, before sample application, the electrophoretic separation of yoghurt caseins treated with rennet was carried out at 6 °C using a constant current of 35 mA per gel slab for 6 h. The gels were then fixed in 12.5% and 5% trichloacetic acid (w/v) for 1h and 15 min, respectively, and stained for 1 h with 0.1% Coomassie Brillant Blue R-250 (w/v) in a solution of 50% water, 40% methanol and 10% acetic acid. The gels were destained in a solution of 10% acetic acid.

2.6. Densitometry

Gels were scanned by a scanner (Scan jet 4C/ TL, Hewlett Packard, USA) and image analysis of the electrophoresis patterns and densitometry curves of the bands were processed with the aid of PC software (Gel Compar 4 version 4, 1992–1994 by Applied Maths, Kortrijk, Belgium).

3. Results and discussion

For the detection of bovine milk, samples of yoghurt caseins treated with rennet were subjected to cationic PAGE and the results obtained are shown in Fig.1. This figure shows that: (a) on strip G, derived from genuine

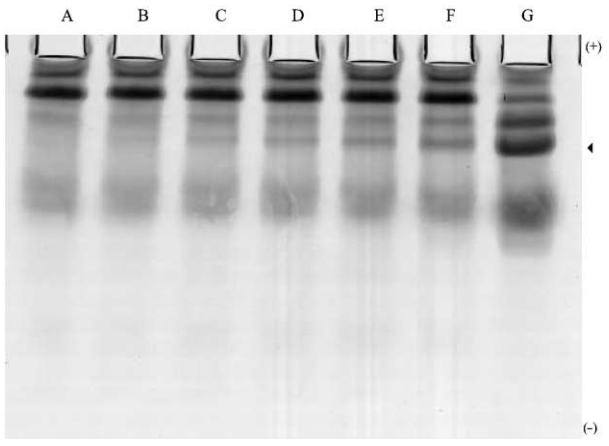

Fig.1. Polyacrylamide gel electrophoretic patterns of *para*-κ-caseins of seven model yoghurts made from genuine ovine (A), bovine (G) milk and mixtures containing 1% (B), 2.5% (C), 5%(D), 10% (E) and 20% (F) bovine milk.◀Indicates the band of bovine *para*-κ-casein.

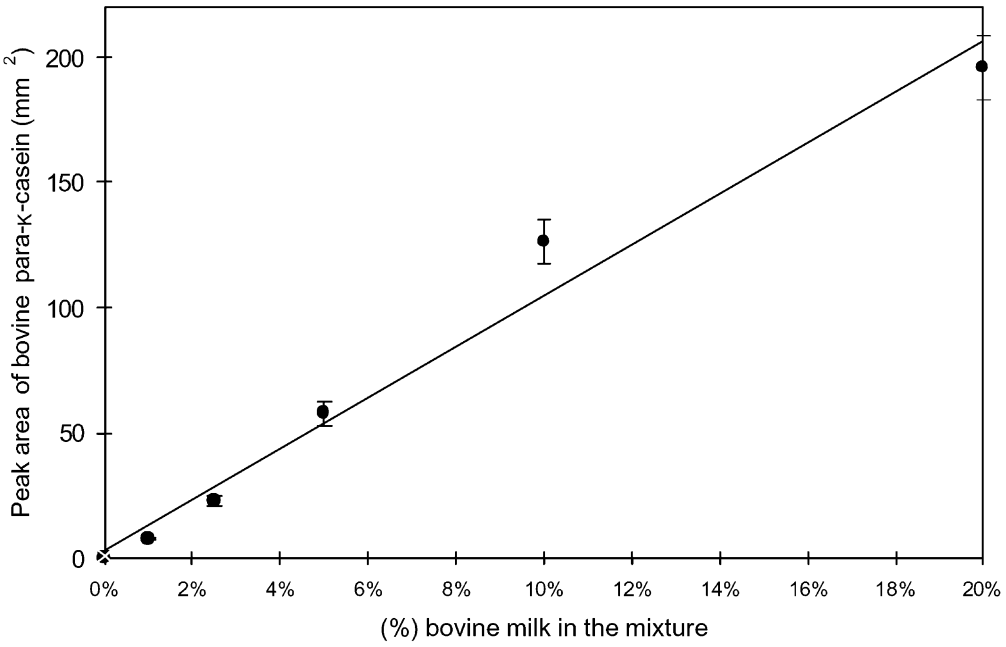

Fig. 2. Relationship between the bovine peak area (mm^2) of *para*-κ-casein (Y) and the relative percentage of bovine milk in model yoghurts made from different milk mixtures containing 1–20% bovine milk(X) (—●—) : $Y = 3.29 + 10.16X$, $r^2 = 0.99$, $n = 5$. Error bars represent standard error of mean.

bovine yoghurt, a distinctly separated zone of bovine *para*-κ-casein was observed, which was not present in the electrophoretic pattern of the genuine ovine yoghurt (strip A). (b) this bovine *para*-κ-casein zone may be used as a protein marker for the detection of bovine milk in ovine yoghurt as its intensity increased with increasing percentages of bovine milk in the mixture (strips B–F).

The content of bovine milk in the mixtures was estimated after densitometry of electrophoretic separations and based on the measurement of the peak area of bovine *para*-κ-casein in the densitograms. Regression analysis of the results revealed linear relationships between the area of the bovine *para*-κ-casein in the adulterated ovine yoghurt samples and the percentage of bovine milk in mixtures from 1–20% bovine milk (Fig. 2). The level of bovine milk in the mixtures was obtained by calculation of the percentage of peak area which corresponded to the bovine *para*-κ-casein in comparison with the peak area of *para*-κ-casein in the mixture containing 20% bovine milk. The values obtained were in good agreement with the true values.

By using this procedure under the same conditions, levels of bovine milk higher than 1% in adulterated yoghurt samples, of unknown composition, may easily be calculated using the respective regression curves. For the evaluation of the amount of bovine milk in adulterated samples it is advisable that both the adulterated samples and the sample from 20% bovine milk in ovine yoghurt be analysed simultaneously on the same gel. Then the bovine *para*-κ-casein band from 20% bovine milk in ovine yoghurt may be used as a reference standard, thus increasing the accuracy of calculation.

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