INVESTIGATION OF THE PROTEIN FRACTIONS IN GOAT MILK WITH RP-HPLC TO OPTIMALIZE THE MILK PROCESSING

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Abstract

T*he amount of protein in milk (which varies between different species of mammals) is critical to its commercial, technological and biological value. Caprine milk differs from cow milk in several physico-chemical characteristics, which explain major differences in the technological behaviour of the two milks. Goat milk also has different proportions of the four major caseins (α_{s1}, α_{s2}, β, κ) compared to cow counterparts, and there are great variations, especially between α_{s1}-casein and α_{s2}-casein contents between individuals and breeds of goats, because of the occurrence of genetic polymorphisms for all milk proteins, which influence greatly their cheese making properties.*

The aim of our study was to adopt BORDIN's (2001) RP-HPLC method for analysis the casein fractions of denatured goat milk samples. The separation of main caseins (α_s, β, κ) was successful by ion-pair reversed phase HPLC with the help of casein standards originated from cow milk. Our results are well correlated with the data of references.

Keywords: goat milk, casein fractions, separation, HPLC

INTRODUCTION

Goat milk is traditionally consumed directly or as handmade cheese (Medina and Nuñez, 2004). It is a source of proteins of excellent quality, thanks to the proportion of essential amino acids they provide (Park 2006). Goat milk is also highly digestible and the biological value of its proteins is superior to that of cow milk proteins (Haenlein, 2004, Almaas et al. 2008). Its nutritional properties and lower allergenicity in comparison to cow milk, especially in nonsensitised children (Haenlein, 2004; Park & Haenlein, 2006), has led to an increased interest in goat milk as a functional food, and it now forms a part of the current trend to healthy eating in developed countries (Michaelidou, 2008, Raynal-Ljutovac et al. 2008).

The amount of protein in milk (which varies between different species of mammals) is critical to its commercial, technological and biological value. Thus, the greater the quantity of proteins in the raw milk, the higher is its performance in the technological transformation required to prepare derivatives, such as fermented milks or cheeses (Raynal-Ljutovac et al. 2008, Pirisi et al. 2007). In recent years, increasing attention has been paid to the measurement of specific protein fractions in milk, obtained by digestion of the protein. These protein fractions, in addition

to their nutritional value, regulate physiological processes and can be considered as active ingredients with a beneficial effect on general health (Michaelidou, 2008).
Caprine milk differs from cow milk in several physico-chemical characteristics, which explain major differences in the technological behavior of the two milks (Park et al. 2007). The poorer cheese making ability of goat milk is largely attributable to the lower casein content, and to specific properties of casein micelles in caprine milk such as their composition, size and hydration. Goat milk also has different proportions of the four major caseins (α_{s1}, α_{s2}, β, κ) compared to cow counterparts, and there are great variations, especially between α_{s1}-casein and α_{s2}-casein contents between individuals and breeds of goats, because of the occurrence of genetic polymorphisms for all milk proteins, which influence greatly their cheese making properties (Park et al. 2007).
In the recent years different analytical methods (electrophoretic techniques, high-performance liquid chromatography and immunological methods) was applied to separate and quantify the casein fractions, though generally accepted or standardized method had not developed yet.
The aim of our study was to identify and quantify the casein proteins from denatured goat milk samples. The separation of main caseins (α_s, β, κ) was carried out by ion-pair reversed phase HPLC according to Bordin's (2001) with casein standards originated from cow milk. Originally Bordin's HPLC method was developed for cow milk, so we also analysed cow milk samples.

MATERIALS AND METHODS

The row goat milk samples (three samples) were purchased from different breeders from countryside of Szeged, as the cow milk samples from the Sole-Mizo Dairy PLC. Purified casein standards (κ-, α_S-, and β-CN) from bovine milk (Sigma) were lyophilized form, all chemicals were HPLC grade.

Determination of basic composition

The main chemical components (protein, fat, dry matter, lactose) of milk samples were determined by Infra Red Analyser (Bentley 150).

Analyses of protein fractions by HPLC

Sample preparation

The milk samples were defatted with cetrifugation (7000 rpm, 20 min, 35°C). The defatted samples were freezed (-20°C) till analyses.

The purified liophilysed casein standards were dissolved in ultrapure water, this stock solutions (10 mg/mL) were used for denaturation.
The milk samples or the purified casein stock solutions (50 and 100 μL) were dissolved in sample buffer consisting of 6 *M* guanidine–HCl, 20 m*M* dithiothreitol and 5 m*M* tri-sodium citrate (pH 7) is added. One hour of incubation is allowed at room temperature. A 1:3 dilution into the chromatographic eluent A was then carried out.

HPLC instrumentation and procedure

The HPLC analysis was carried out with the Varian LC Star system, incorporating a pump (9012), an autosampler (9100), a diode-array detector (9065) and a PC computer with Varian Star 5.3 software. Separations were made on a reversed phase analytical column ACE-5-C_4-300 (2.1 mm x 150 mm, 5 μm particle size). The temperature of the column was kept at 40°C. The separations were performed with two eluents according a program (Fig. 1). Eluent A consists of 10% (v/v) acetonitrile and 0.1% TFA in ultrapure water and eluent B: 10% water and 0.1% TFA in acetonitrile.
The flow rate of eluent was 0.25 mL*min^{-1}, the injected sample volume was 20 μL. The absorbance of eluate was recorded in the range 190-367 nm.

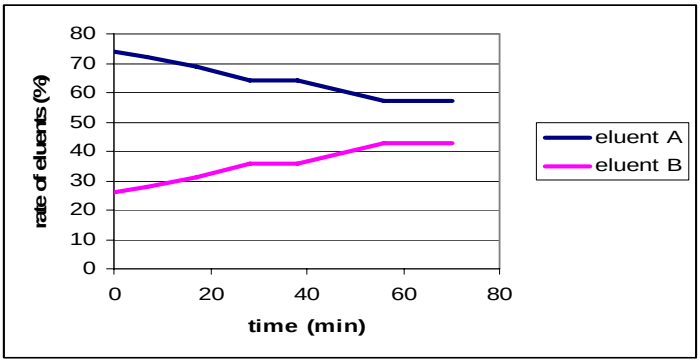

Fig. 1. Elution conditions

RESULTS AND DISCUSSION

Chemical composition of milk samples

The compositions of investigated goat and cow milk samples are in table 1. The composition of goat milk is similar to cow milk. Composition of goat milk varies with diet, breed, and animals within breed, parity, environmental conditions, feeding and management conditions, season, stage of lactation. The fat, total solids and protein contents of milk are

high in early lactation, fall rapidly and reach a minimum during the second to third months of lactation (PARK, 2006). Our results were well correlated with the data of Raynal-Ljutovac et al (2008) though the protein content of samples was relatively low.

Table 1.

Basic Composition of Goat and Cow Milks (g/100 g)

Constituents	**Cow**	**Goat**	***Goat*** *(RAYNAL-LJUTOVAC ET AL 2008)*
Fat	3.86	3.48	*3.4-5.6*
Protein	3.16	2.52	*2.6-4.0*
Lactose	4.62	4.68	*4.3-4.7*
Solids non Fat	8.64	7.77	
Total Solids	12.49	11.25	*11.6-14.8*

Evaluation of chromatograms

The milk protein chromatograms can be evaluated at λ=278 nm, it is typical for aromatic amino-acids and at λ=215 nm, which is characteristic for the peptide bounds. Comparing the chromatograms at two wavelength for further evaluation chromatograms at λ=215 nm were chosen because of better separation of different peaks.

Separation of casein standards

The different caseins standard (κ-, α_S-, β-CN) and their mixtures (κ- and α_S-CN; α_S- and β-CN) were analysed to determined the characteristic peaks and their retention time. The κ-casein had three peaks (at 11, 17 and 22 min retention time), The α_S casein had two fractions (at 24 min and at 32 min). The first appeared at 24 min was the α_{S2} and the second one at 32 min the α_{S1} according to BORDIN ET AL (2001). The β-casein had two fractions (at 41 and 54 min). In the mixture of two standards the sub-fractions were well separated.

Separation of milk samples

A typical chromatogram of cow and goat milk sample is presented in Fig. 2. and Fig. 3.

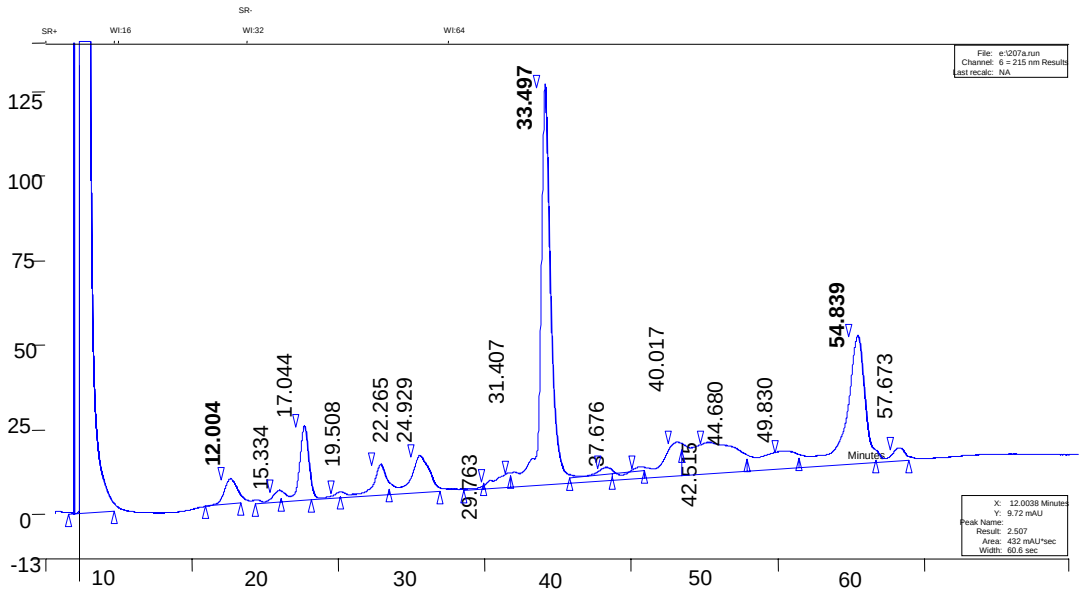

Fig. 2. Chromatogram of cow milk

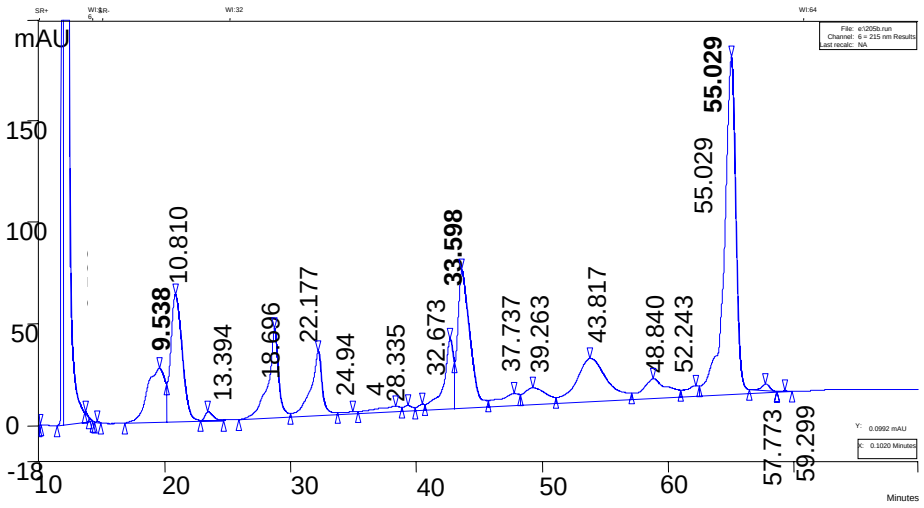

Fig. 3 Chromatogram of goat milk

To assess the amount of different casein fractions in milk we chose some peaks that are typical for each fraction. At cow milk κ-CN, rt: 12 min, αs_1-CN rt: 33 min, β-CN rt: 54 min. We had no standards for goat milk, but the sequence of casein fractions is the same according to MORA-GUTIERREZ ET AL (1991). The chosen peaks for casein fractions were the following: κ-CN, rt: 9.5 min αs_2-CN rt: 33.5 min, β-CN rt: 55 min. At goat milk the quantity of α_{S2} is higher than α_{S1}. As α_{S1} casein the major protein in cow milk, appears to be low or nonexistent in goat milk (Mora-Gutierrez et al. 1991). The quantities of caseins of different milk samples were calculated for these peaks' area comparing with the standards. The results are presented in table 3.

Table 3.

Evaluation of caseins fractions in milk samples

Casein fraction Cow milk samples	**κ-CN* (mg/cm^3)**	**α_S-CN (mg/cm^3)**	**β-CN (mg/cm^3)**
1	6.62	11.93	10.85
2	6.09	11.79	10.26
3	5.78	8.10	8.82
PARK (2006)	*2.7-6.1*	*9.9-15.6*	*6.1-14.1*
Goat milk samples			
1	5.38	3.18	9.82
2	6.05	3.64	9.19
3	5.40	2.27	8.69
PARK (2006)	*4.2-5.9*	*3.4-11.2*	*11.5-21.2*

*CN= casein

The quantity of each casein fractions well correlated with the data of PARK (2006). The α_S caseins in goat casein represent a much smaller proportion of total casein than that in bovine casein. The α_S-caseins are capable of being stabilized by κ-casein against precipitation. β-caseins are the major components of total goat milk casein (Park, 2006) In our samples the quantity of β-casein was just a little smaller, comparing Park's data, but the total protein content of our goat milk samples were also relatively low. These differences in the protein composition of milk can cause variances in the heat stability, renneting properties and allergenic properties of milk.

CONCLUSIONS

The applied analytical method was suitable to separate and quantified the three main casein fractions (κ-, α_S- and β-caseins) of denatured cow and goat milk samples. Our results well correlated with the data of references. The application of specific casein standards of different origin can help the future analyses to determine the uncertain peaks. In the method certification the use of skim milk powder as reference material can be a good help.

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