



Nitrogen fractions of Andalusian goat milk compared to similar types of commercial milk

Manuel Olalla *, M^aDolores Ruiz-López, Miguel Navarro, Reyes Artacho, Carmen Cabrera, Rafael Giménez, Cristina Rodriguez, Raquel Mingorance

University of Granada, Nutrición y Bromatología, Facultad de Farmacia, Campus Universitario de Cartuja, 18071 Granada, Granada, Spain

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ABSTRACT

In this paper, part of a wider study on goat milk in Andalusia, the total nitrogen and the main protein fractions in commercial brands of Andalusian semi-skimmed cow milk and goat milk were measured and compared to the results for raw goat milk from the farms of a major Andalusian goat milk cooperative.

Mean total protein content was significantly higher in the untreated raw goat milk (3.67 g/100 g) than in the commercial goat milk (3.27 g/100 g) or cow milk (3.14 g/100 g). Mean non-casein content was 0.138 g/100 g in raw goat milk and 0.118 g/100 g in commercial cow milk, and mean non-protein nitrogen was significantly higher in raw goat milk (0.040 g/100 g) than in commercial cow milk (0.027 g/100 g). Nitrogen from casein peptides and from globulin was also determined.

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1. Introduction

Goat milk is a typical Mediterranean product traditionally consumed directly or as handmade cheese (Chandan, Attaie, & Sahani, 1992). It is a source of proteins of excellent quality, thanks to the proportion of essential amino acids they provide (Luquet, 1991). Goat milk is also highly digestible and the biological value of its proteins is superior to that of cow milk proteins (López-Aliaga et al., 2003; Ramos Morales et al., 2005). Its nutritional properties and lower allergenicity in comparison to cow milk, especially in non-sensitised 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 (Bevilacqua et al., 2001; Lara Villoslada, 2005).

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 (Fontecha Alonso, Baro Rodríguez, & Boza Puerta, 2004). 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 (Baro, Jiménez, Martínez-Pérez, & Boza, 2001).

Two nitrogenous groups can be distinguished in milk, nitrogen protein and non-protein nitrogen (NPN), which represent around 95% and 5%, respectively, of total nitrogenous compounds in milk. In turn, 80% of milk proteins are caseins (complex of phosphorized proteins), which are characterised by their precipitation when the milk is acidified to a pH of 4.6 (Baro Rodríguez, López-Huertas León, & Boza Puerta, 2005). The proteins that remain in a 4.6 pH solution are called milk serum proteins or soluble proteins, largely composed of albumens and globulins, which precipitate in the presence of 12% trichloroacetic acid. Milk serum proteins are of enormous interest to the Food Industry and are used in the manufacture of numerous different food products, including newborn and follow-on infant formulas. Proteose-peptones, glycoprotein substances with an intermediate molecular weight between proteins and peptides, are N-terminal fragments that derive from α -casein hydrolysis (Walstra, Geurts, Normen, Jellema, & Van Boekel, 1999).

In this study, the main nitrogenous fractions were compared amongst raw goat milk, commercial (processed) goat milk, and commercial cow milk in order to gather specific and reliable data on their composition. These data will be invaluable to complete food composition tables and evaluate possible protein losses in technological and sterilization processes.

2. Materials and methods

2.1. Samples

Ten brands of commercial milk (5 of goat milk and 5 of cow milk) were purchased from local shops. This milk was

* Corresponding author. Tel.: +34 958243864; fax: +34 958249577.

E-mail address: olalla@ugr.es (M. Olalla).

semi-skimmed (1.5–1.8% fat content) and heat sterilized (UHT), which is the only commercially available type of goat milk and is a widely consumed type of cow milk. Raw goat milk was also obtained over a period of eight months from a major Andalusian goat milk cooperative, using standard sampling techniques. All samples were analysed in triplicate.

2.2. Treatment of samples

Before their analysis, samples were heated to $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and carefully mixed. The raw goat milk samples (not homogenized), were slowly heated to 40°C , gently mixed and then cooled to $20^{\circ} \pm 2^{\circ}\text{C}$.

2.3. Determination of total nitrogen compounds in milk

Kjeldahl method (Barbano, Lynch, & Fleming, 1991; CEE 608/92, 1992).

Basic method: Milk was digested in H_2SO_4 , using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ – K_2SO_4 as catalyst.

Apparatus: Kjeldahl digester flasks (Bloc digest 12 Selecta). Distillation flasks (B-316 Büchi flasks).

Digestion method: The sample was progressively heated (initially very gradually) to 400°C and maintained at this temperature for at least 90 min until the solution achieves a transparent greenish blue colour (total digestion time should not be <150 min).

Blind assay: A blind assay using the same methodology was conducted using five millilitres of water instead of milk.

Nitrogen loss: Percentage recovery of >99% was obtained using 0.12 g of ammonium sulphate and 0.85 g of sucrose per flask.

Digestion efficiency: Percentage recovery of >98% was obtained using 0.15 g ammonium sulphate or 0.20 g tryptophan.

Precision: One sample was run ten times to test the repeatability (Table 1).

2.4. Procedure for evaluation of different protein fractions, Kjeldahl method

1. Total nitrogen. The nitrogen content of the milk, expressed as a percentage of mass, was determined by the Kjeldahl method as described above.
2. Non-casein nitrogen (NCN) (Lynch, Barbano David, Fleming, & Richard, 1998). NCN was determined by precipitation and filtration (acetic/acetate) of the casein at a pH of 4.6, applying the Kjeldahl method to the filtrate.
3. Non protein nitrogen (NPN) (Barbano, Lynch, & Fleming, 1991). NPN was determined by precipitation of the protein using 12% trichloroacetic acid, applying the Kjeldahl method to the filtrate.
4. Proteose-peptone protein + non-protein nitrogen (PPN + NPN) (Casado Cimiano, 2001). PPN and NPN were measured by precipitating the globulin and albumen in the serum obtained from

the NCN (paragraph 2), applying the Kjeldahl method to the filtrate.

5. Globulin nitrogen (GN) (Casado Cimiano, 2001). GN was measured by treating the NCN with magnesium sulphate and applying the Kjeldahl method to the precipitate.
6. Nitrogen protein-3.
7. Serum protein: 2–3.

Calculations: multiply % nitrogen by 6.38 to calculate % total protein and multiply globulin by 6.40 for the casein content.

2.5. Statistical analysis

Analysis of variance was used to establish significant differences amongst samples as a function of their origin (cow/goat). The Statgraphics Plus Statistical 4.1 package (Statgraphics Plus & Statistical Graphics Corp., 1999) was used for the analyses.

3. Discussion and results

Amongst the commercial milks, the nitrogen fraction was higher in goat versus cow milk. Table 2 shows the levels and percentages of the different nitrogen fractions in the commercial milks and raw goat's milk.

The protein composition of goat milk varies amongst breeds (Marti, 1997). Studies of raw milk (no heat treatment) in Spain have reported total protein values of 3.46–3.70 g/100 g (Quiles Sotillo, 1994), 3.11–3.91 g/100 g for Granada (Andalusia) breeds, 3.49–3.73 g/100 g for Granada-Murcia breeds, 3.58 g/100 g for Verata goat milk (Gonzalez-Crespo, Lozano, & Serrano, 1996) and 3.52–3.99% for goats from Extremadura (Gonzalez Crespo & Roa Ojalvo, 2001). Peláez Puerto, Fresno Baquero, Díaz Romero, and Darías Martín (2004), using an automatic measurement method (Milkoscan), reported protein values of 3.85–3.96% in goat milk from the island of Tenerife in a study that considered the seasonal variability, with values reaching more than 4% in some cases during the summer. In the present study, the mean total protein value was 3.67 g/100 g, with values ranging from 3.36–4.16%.

Some authors have reported a very similar total protein composition between cow and goat milk. Thus, both types of milk were found to have a protein value of 3.3 g/100 ml by Spreer (1991) and by Walstra et al. (1999) and a value of 3.22 g/100 g by Luquet (1991). In contrast, Ordóñez Pereda et al. (1998) described significantly lower values in cow milk than goat milk (3.1–3.9 g/100 g vs. 4.2 g/100 g).

In the present study, appreciably higher total protein values were obtained in commercial (3.27 g/100 g) and raw (3.67 g/100 g) goat milk than in commercial cow milk (3.14 g/100 g), and it should be taken into account that the commercial milks were semi-skimmed (higher relative protein concentration).

Spanish food composition tables published by CESNID (2004), Mataix Verdú (2003) and Moreiras, Carbajal, Cabrera, and Cuadrado (2007) give a single value of 3.4% for total protein in whole goat milk, without differentiating between raw and heat processed milk. This is a lower value than observed in any of the

Table 1
Repeatability of the analytical method.

Component (g/100 g)	Raw goat milk				Commercial cow milk			
	n	Mean	σ_{n-1}	r (repeatability)	n	Mean	σ_{n-1}	r (repeatability)
Total nitrogen	10	0.5549	8.10×10^{-3}	0.0047	10	0.5356	9.97×10^{-3}	0.0058
Non-casein nitrogen	10	0.0848	6.25×10^{-3}	0.0036	10	0.0747	3.97×10^{-3}	0.0023
Non-protein nitrogen	10	0.0397	8.15×10^{-3}	0.0047	10	0.0397	9.68×10^{-3}	0.0056
Proteose-peptone protein + non-protein nitrogen)	10	0.0502	9.35×10^{-3}	0.0054	10	0.0400	9.70×10^{-3}	0.0056
Globulin nitrogen	10	0.0200	9.90×10^{-3}	0.0057	10	0.0100	10.02×10^{-3}	0.0058

Table 2

Results.

Component (g/100 g)	Commercial goat milk		Commercial cow milk		Raw goat milk	
	$X \pm \sigma_{n-1}$	(%)	$X \pm \sigma_{n-1}$	(%)	$X \pm \sigma_{n-1}$	RSD (%)
Total nitrogen	0.513 $\pm$ 0.036		0.492 $\pm$ 0.023		0.576 $\pm$ 0.039	
Total protein ($\times 6.38$)	3.270	100	3.140	100	3.670	100
Non-casein nitrogen	0.118 $\pm$ 0.026		0.106 $\pm$ 0.032		0.138 $\pm$ 0.029	
Non-protein nitrogen	0.035 $\pm$ 0.013	6.8 ^a	0.027 $\pm$ 0.076	5.5 ^a	0.040 $\pm$ 0.014	6.9 ^a
Protein nitrogen	0.478	93.2 ^a	0.465	94.5 ^a	0.536	93.1 ^a
True protein ($\times 6.38$)	3.050		2.967		3.420	
Casein ($\times 6.40$)	2.530	82.9 ^b	2.470	83.4 ^b	2.800	81.9 ^b
Serum protein	0.520	17.1 ^b	0.490	16.6 ^b	0.620	18.1 ^b
Globulin ($\times 6.40$)	0.096 $\pm$ 0.047	18.5 ^c	0.064 $\pm$ 0.057	13.1 ^c	0.130 $\pm$ 0.059	21.0 ^c
Protease peptones ($\times 6.38$)	0.077 $\pm$ 0.074	14.8 ^c	0.076 $\pm$ 0.074	15.5 ^c	0.087 $\pm$ 0.086	14.0 ^c

^a With respect to total nitrogen.^b With respect to true protein.^c With respect to serum protein.

present raw goat milk samples. No value for goat milk is given in the official tables of the Spanish Ministry of Agriculture (MAPA, 1999). Tables from Novartis Consumer Health S.A. (Jiménez Cruz, Cervera Ral, Bacardí, 2002) describe whole goat milk (4.5% fat content) as having a mean total protein value of 3.9%, higher than the mean value found in the present study. Tables from the Royal Society of Chemistry (McCance, 2006) include values for goat milk derivatives but not for the whole milk. The small Souci-Fachmann-Kraut. *El pequeño*. (1999) gives a value for whole goat milk (3.9% fat) of 3.7 g/100 g, similar to the present finding, whilst the French INRA tables (Feinberg, Favier, & Ireland-Ripert, 1991) report a lower value in raw whole milk of 3.4 g/100 g.

There have been no published reports on skimmed, semi-skimmed or sterilized goat milk, which is the type of milk commercially sold. It is necessary to include these data in food composition tables, as proposed in the present study.

A review of data on UHT-treated semi-skimmed cow milk showed values of 3.44% in Tables of the Spanish Agriculture Ministry tables (MAPA, 1999), 3.5% in those published by Mataix Verdú (2003), and 3.4% in tables produced by Novartis Consumer Health S.A. (Jiménez Cruz et al., 2002) and Souci-Fachmann-Kraut (1999). These values are all higher than the present findings in commercial milk of similar characteristics and even higher than those reported by the milk producers. These findings suggest that commercially available milk may be low in proteins, which can also be caused by the use of industrial techniques for obtaining protein concentrates to prepare cheeses and yoghurts. This possibility should be taken into account in the construction of new food composition tables.

Higher absolute values and percentages of NPN were obtained in sterilized goat milk (0.035 g/100 g = 6.8% total nitrogen) and raw goat milk (0.04 g/100 g = 6.9% total nitrogen) than in cow milk (0.027 g/100 g = 5.5% total nitrogen). These results are in agreement with the findings by Luquet (1991) and Trujillo, Guamis, and Carretero (1997) of higher NPN in goat than cow milk. Non-protein nitrogen is nutritionally important, especially given its elevated presence in human milk (up to 25% of total nitrogen).

The percentage of casein (with respect to the total protein content) found in the commercial cow milk (83.4%) was higher than reports of 80% in the literature (Baro Rodríguez et al. (2005) and Walstra et al. (1999)) and slightly higher than the percentages found in commercial (82.9%) and raw (81.9%) goat milk. There was no significant difference in casein levels between raw and commercial goat milk, indicating that the sterilization of raw goat milk has no significant influence on the casein content (Fontecha Alonso et al., 2004).

Serum protein values were similar between commercial goat milk (0.52 g/100 g) and cow milk (0.49 g/100 g), in agreement with previous reports (Walstra et al., 1999). Serum protein levels were

significantly ($p < 0.05$) higher in raw goat milk (0.62 g/100 g) than in commercial goat milk (0.52 g/100 g), indicating that the sterilization of goat milk significantly reduces its serum protein content. Account should be taken of the high total protein content of raw crude goat milk and the elevated concentration in commercial semi-skimmed milks.

PPN and GN concentrations were significantly higher in raw goat milk than in commercial cow and goat milk.

Given the beneficial nutritional properties of goat milk (elevated concentration of proteins with high nutritional value) and its possible use in the prevention of cow-milk allergies in non-sensitized children (Lara Villoslada, 2005; Sanz Cevallos, 2007), its increased consumption by the general population can be recommended.

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