Short Communication: Extraction of β -Casein from Goat Milk

S. Lamothe, G. Robitaille, D. St-Gelais, and M. Britten¹

Food Research and Development Centre, Agriculture, and Agri-Food Canada, Saint-Hyacinthe, Quebec, Canada J2S 8E3

ABSTRACT

Peptides derived from milk β -casein have potential biological activities, such as antihypertensive and immunostimulating properties. These biological properties increase the demand for the production of specific bioactive peptides. β -Casein can be isolated directly from renneted skim milk, based on the preferential solubilization of β -casein at low temperature. This study was conducted to compare the recovery and purity of β -casein extracted from goat and cow milks. Rennet casein was prepared from both milks, heat treated, and dispersed in demineralized water at various temperatures. β -Casein recovery in the soluble phase increased with decreasing incubation temperature. Concentration of β -casein was 43% higher in goat milk than in cow milk, which had a direct effect on β -casein recovery. Furthermore, β -casein was extracted more efficiently from goat rennet casein. As a result, the extraction yield of β -casein was 53% higher in goat milk than in cow milk. The purity of β -casein extracted from both milks reached approximately 90% after incubation at 0°C.

Key words: β -casein, goat, cow, milk

Casein is the main protein component of milk, constituting approximately 80% of the total milk protein fraction. Extensive research in vitro and on animal models has suggested that peptides derived from CN are not only nutrients, but also a source of low molecular weight peptides having potential biological activity. These peptides are generated and become active after digestion by proteolytic enzymes or during the fermentation and maturation processes of cheese and yoghurt (Korhonen and Pihlanto, 2006). Major CN-derived opioid peptides are fragments from bovine β -CN, called β -casomorphins because of their morphin-like behavior (Korhonen and Pihlanto, 2006). Antihypertensive and immunostimulating peptides can also be generated from bovine β -CN as well as from caprine β -CN (Minervini et al., 2003; Geerlings et al., 2006; Silva et al., 2006). These

numerous biological activities increase the demand for fractionated and isolated CN as a source for the production of specific bioactive peptides. Molecular structures are important for peptide properties, and any variations in AA sequence and posttranslational modifications, such as phosphorylation or glycosylation, can affect bioactivity.

There are at least 19 polymorphic sites between caprine and bovine β -CN (GenBank database), and caprine β -CN possesses an additional phosphorylation site (Neveu et al., 2002). This opens the possibility of using CN originating from different species to generate new and modified bioactive peptides. For instance, the sequences of some antihypertensive casokinins from bovine β -CN (Silva et al., 2006) are modified or absent in caprine β -CN, and a casokinin identified in caprine β -CN was not found in the bovine counterpart (Gómez-Ruiz et al., 2002). Further research is required to evaluate the biological activities of all these peptides and their potential as food supplements.

Several approaches have been proposed to produce high-quality food-grade β -CN. Microfiltration based on the selective solubility of β -CN at low temperature (Murphy and Fox, 1991; Van Hekken and Holsinger, 2000) is not suitable for the production of large quantities because of problems associated with low yield and temperature control during microfiltration. Recently, Huppertz et al. (2006) developed a large-scale procedure for the isolation of β -CN directly from renneted milk, based on the preferential solubilization of β -CN at low temperature. The purity of the β -CN fraction was greater than 80% and could be used without a further purification step. As a by-product, rennet curd can be used directly in food preparations, such as process cheese. The aim of this work was to prepare β -CN from cow and goat milks with this method to compare the recovery and purity of β -CN extracts obtained from both species.

β -Casein was extracted according to the experimental procedure proposed by Huppertz et al. (2006). Bulk cow (Parmalat, St-Hyacinthe, Quebec, Canada) and goat (Laiterie Tournevent, Drummondville, Quebec, Canada) milks were skimmed, pasteurized at 65°C for 30 min, and cooled to 30°C. For both milks, pH was adjusted to 6.4 with 1 N HCl, and milk was allowed to

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¹Corresponding author: brittenm@agr.gc.ca

Table 1. Protein composition of cow and goat skim milks

Item	Cow	Goat
Total protein, %	3.36 $\pm$ 0.05	3.34 $\pm$ 0.03
CN, %	2.63 $\pm$ 0.05 ^a	2.52 $\pm$ 0.03 ^b
κ -CN, %	10.90 $\pm$ 0.25 ^a	9.02 $\pm$ 0.53 ^b
α_{s2} -CN, %	7.51 $\pm$ 0.20 ^b	20.93 $\pm$ 0.91 ^a
α_{s1} -CN, %	47.25 $\pm$ 0.79 ^a	18.89 $\pm$ 0.70 ^b
β -CN, %	34.34 $\pm$ 0.37 ^b	51.16 $\pm$ 0.12 ^a

^{a,b}Means within a row with different superscripts are different ($P < 0.05$).

equilibrate for 1 h at 30°C. Rennet (double-strength rennet, Maxiren, Chr. Hansen's Laboratory, Ontario, Canada) and CaCl_2 (Sigma, St. Louis, MO) stock solution (32% wt/wt) were added to milk (1 L) at respective concentrations of 0.1 and 0.26 mL/L. Milk was held at 30°C for 15 min under low agitation, allowing CN precipitation. This was followed by 15 min of incubation at 30°C without agitation to increase the particle size. The precipitate was filtered through Whatman no. 40 filter paper, dispersed in demineralized water at 70°C, and held at this temperature for 5 min to inactivate the chymosin. The mixture was filtered and the supernatant was discarded. The precipitate was minced with a 500-mL food processor (Mini-Prep Plus, DLC-2A model, Cuisinart, East Windsor, NJ) for 1 min at maximum speed, suspended in demineralized water (at 30°C) to a volume corresponding to half of the original milk sample, and separated into 4 equal parts. The suspensions were held at 0, 4, 8, and 16°C for 16 h. The supernatants were filtered through Whatman no. 40 filter paper. Total nitrogen, non-CN nitrogen, and NPN were determined according to International Dairy Federation standard methods (1993) and used for CN determination in the original skim milks and supernatants. Relative proportions of each CN fraction in milk and in the supernatants were assessed by HPLC (Jaubert and Martin, 1992). The proportion of β -CN over total CN in the supernatants was taken as the purity of the extract. To determine the recovery of β -CN, the concentration of β -CN in the supernatant after incubation (corrected for the volume that corresponded to half of the original milk sample) was divided by the β -CN content of the original milk and expressed as a percentage. The preparation of bovine and caprine rennet CN and the solubilization of β -CN at various temperatures was repeated 3 times. Compositional data on the original milks and β -CN extracts were subjected to ANOVA (SAS Institute Inc., Cary, NC).

Table 1 presents the protein composition of original cow and goat skim milks. The CN content was slightly higher in cow skim milk ($P < 0.05$). The relative amount of the individual CN in goat and cow milks reported here was consistent with previous studies (Farrell et

Figure 1. Influence of incubation temperature on β -CN recovery from cow (■) and goat (▲) milk.

al., 2004; St-Gelais et al., 2005). β -Casein represented the major protein component of goat milk. The concentration of β -CN in goat milk was 43% higher than in cow milk.

The effect of temperature on β -CN recovery in cow and caprine extracts is shown in Figure 1. Milk origin and rennet CN incubation temperature had significant effects on β -CN yield ($P < 0.0001$). β -Casein recovery in cow and goat extracts increased up to 5- and 8-fold, respectively, as the incubation temperature decreased from 16 to 0°C.

The total amount of β -CN obtained at 0°C was 2 times higher for goat milk, with a production of 0.185 g of β -CN/100 mL of milk, compared with 0.083 g/100 mL of milk for cow milk. This shows the efficiency of the method applied to goat milk. The difference in the amounts recovered can be explained by a greater concentration of β -CN in the CN fraction of goat milk, by the higher solubility of caprine β -CN at low temperature (O'Connor and Fox, 1973; Raynal and Remeuf,

Figure 2. Influence of incubation temperature on the purity of β -CN extracts from cow (■) and goat (▲) milk.

Figure 3. High-performance liquid chromatographic profiles of β -CN extracted from renneted goat skim milk at 0 (—) and 16°C (-----).

2002), and by the weaker curds made from goat milk compared with those from cow milk (Ambrosoli et al., 1988), which may enhance β -CN diffusion in the medium.

In the present work, β -CN recovery from cow milk was about half the value reported by Huppertz et al. (2006) for similar conditions. The difference could be attributed to the method used to break the curd. As mentioned by Huppertz et al. (2006), the dissociation of β -CN is hindered by the curd matrix. To improve β -CN dissociation, they used a mortar and pestle to pulverize the curd particles and increase the surface area, thus facilitating β -CN diffusion in the medium. In the present study, a food processor was used to chop the precipitate, which resulted in larger particles and, as a consequence, decreased the extraction yield. Increasing the incubation time to 24 h and performing a second extraction at 0°C could be considered to improve the yield. The use of a colloidal mill might be an option to decrease particle size further and could be more appropriate for the large-scale isolation of β -CN.

As shown in Figure 2, the purity of β -CN was slightly higher in cow extracts than in goat extracts ($P < 0.05$). The purity significantly decreased when the incubation temperature was raised ($P < 0.0001$), probably because of a higher residual plasmin activity, which is responsible for CN degradation (Bastian and Brown, 1996; Trujillo et al., 1997). High-performance liquid chromatog-

raphy analysis of the supernatants clearly showed that the proportion of impurities increased with increasing incubation temperature (Figure 3). Results presented in this communication showed a high extraction yield of caprine β -CN by solubilization of β -CN from rennet CN at low temperature.

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