Lait 82 (2002) 673–681

DOI: 10.1051/lait:2002041


**Original article**

# Heat-induced coagulation of goat milk: modification of the environment of the casein micelles by membrane processes

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(Received 27 November 2001; accepted 12 February 2002)

**Abstract** – Unlike bovine milk, goat milk is characterised by a very low heat stability at its natural pH (i.e., 6.6–6.7). In the present work, combined membrane processes were used to evaluate how modifying the environment of the casein micelles affects the heat stability of milk. It was shown that the increase of 1.4 times of the caseins: whey proteins ratio, either through a reduction of whey proteins content or selective concentration of caseins, increased the heat coagulation temperature from 135 °C to 141 °C. Further improvement of the stability was obtained by concomitant dilution of both whey proteins and soluble low molecular weight components. From these results, we propose that the destabilising effect of whey proteins at the natural pH is linked to their ability to sensitise casein micelles to heat-induced precipitation of calcium phosphate.

**Goat milk / heat stability / membrane processing / casein:whey proteins ratio**

**Résumé** – **La modification de l'environnement des caséines micellaires par technologies à membranes améliore la stabilité thermique du lait de chèvre.** La faible stabilité thermique au pH naturel (pH 6,6–6,7) constitue une des particularités des laits de chèvre. Nous avons utilisé une cascade de procédés de filtration sur membranes afin d'étudier l'effet de la modification de l'environnement des caséines micellaires sur la stabilité thermique du lait caprin. L'augmentation du rapport caséines:protéines sériques soit par extraction sélective des protéines sériques soit par concentration sélective des caséines conduit à une augmentation de la température de coagulation qui passe de 135 °C pour le lait d'origine à 141 °C pour les mélanges reconstitués. La réduction concomitante des concentrations en protéines sériques et en constituants de la phase aqueuse accentue l'augmentation de la stabilité thermique, confirmant le rôle des composés de faibles masses moléculaires. Par analogie avec les travaux réalisés sur le lait de vache, le rôle des protéines sériques dans la déstabilisation thermique serait lié à leur capacité à sensibiliser les micelles de caséines à la précipitation phospho-calcique.

**Lait de chèvre / stabilité thermique / procédé à membranes / rapport caséine:protéines sériques**

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## 1. INTRODUCTION

Intensive investigations have been carried out to understand the relationship between the physico-chemical composition and the sensitivity of bovine milk to UHT treatment. The main compositional factors that control the heat stability of milk are: pH, urea, ionic calcium content, caseins: whey protein interactions and lactose. The effect of pH is predominant since it acts directly on the nature, the interactions and/or the equilibrium between all other factors [13, 16].

The most widely advanced mechanism for the heat-induced, pH-dependent behaviour of bovine milk is related to the calcium-mediated precipitation of κ-casein-depleted micelles. Such a mechanism is under the control of whey proteins (i.e., β-lactoglobulin): at the pH of maximum stability, β-Lg exhibits a stabilising effect by reducing the heat-induced dissociation of κ-casein, while in contrast, at the pH of minimum stability, it enhances such dissociation and, consequently, the resulting κ-casein-depleted micelles aggregate and precipitate through a calcium-mediated mechanism [13, 16]. More recently, O'Connell and Fox [11] presented a modified view of the above mechanism. They stated that the stabilising and destabilising effects of β-Lg are related to its calcium-chelating properties at the pH of maximum stability and to its ability to sensitise casein micelles to heat-induced precipitation of calcium phosphate at the pH of minimum stability, respectively. Whatever the mechanism involved, it seems clear that the behaviour of milk under heating is governed by myriad inter-dependent reactions involving the main milk components.

In the case of caprine milk, heat-induced destabilisation is even more pronounced, since the reports available indicate that caprine milk at its natural pH (i.e., pH 6.6) has lower heat stability than bovine milk [1, 3, 17]. The ionic form of calcium has been clearly identified as a factor responsible for this low heat stability and the use of calcium chelating agents, such as phosphates, is nowadays commonly used as a way of producing UHT caprine milks [6, 7]. However, the use of additives is not entirely satisfactory since it tends to influence the consumer's acceptance.

As part of a larger study on the heat instability of goat milk and with the prospect of elaborating a new UHT process, the objective of the present work was to study how modification of the relative proportion of the proteins and their environment through integrated membrane processes affects the heat-induced destabilisation of goat milk.

## 2. MATERIALS AND METHODS

### 2.1. Milk samples

Bulk fresh goat milk samples, obtained from Triballat (Noyal sur Vilaine, France) were skimmed at 45 °C and used immediately for filtration experiments. The natural pH of the milk studied was pH 6.68 ± 0.2.

### 2.2. Fractionation experiments

Various reconstituted milks (Mix) were prepared by mixing permeates and retentates from two integrated membrane processes. The first process (preparation of Mixes 1 to 4) involved the fractionation of skimmed milk through membranes with increasing molecular weight cut-off (MWCO) (step 1), followed by filtration of the resultant permeate (PMF or PUF) through a 1 $kg \cdot mol^{-1}$ membrane (step 2) (Fig. 1). Reconstitution was performed by diluting the retentates from step 1 with the permeate from step 2.

The second process of fractionation (preparation of mixes called mixes 5 to 16) aimed to concentrate casein micelles before mixing, as shown in Figure 2. Skimmed milk was concentrated by microfiltration,

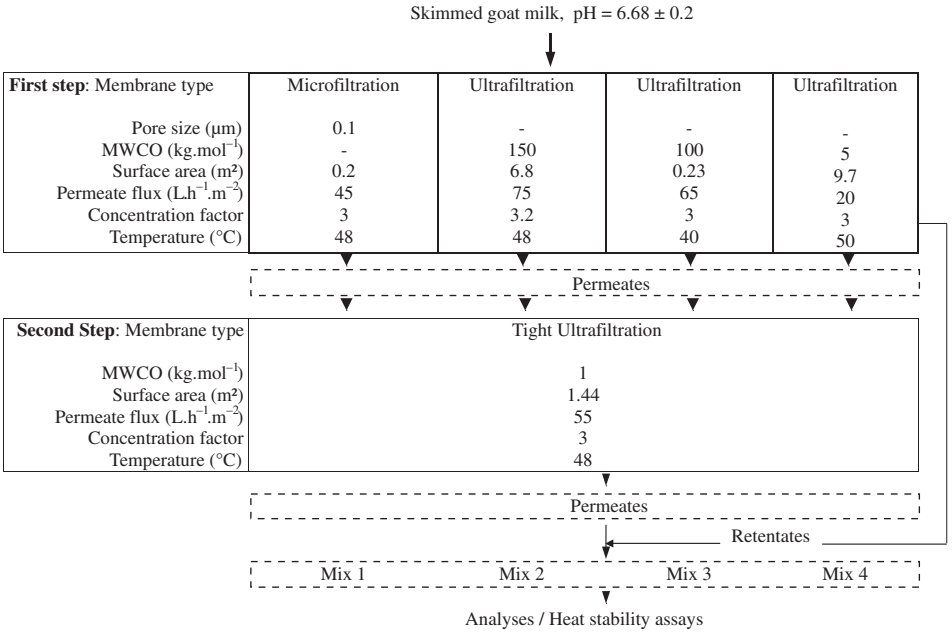


**Figure 1.** Schematic outline of the membrane process used to reduce the whey protein content of goat milk. Reconstitution of modified milk was performed at constant casein concentration.

followed by ultrafiltration and dilution of the retentate to the desired casein concentration with PMF, PMF + PUF, PMF + RUF or water. Five membranes were combined in the present study: ceramic multitubular membranes Membralox® 0.1 µm and 1 kg·mol$^{-1}$ (Exekia, Tarbes, France); polymeric spiral wound membrane 5 kg·mol$^{-1}$ (Koch membrane systems, Wilmington, MA, USA); spiral wound membrane 100 kg·mol$^{-1}$ (Millipore Corporation, Bedford, MA, USA); and Carbosep monotubular membrane 150 kg·mol$^{-1}$ (Orelis, Miribel, France).

### 2.3. Heat stability assay

The heat stability of the goat milk samples was determined according to the protocol reported by Remeuf [12]. Samples were sealed in glass capillary tubes and gradually heat-treated in an oil bath at temperatures in the range 130 to 145 °C, at increments of 2 °C·min$^{-1}$. The heat coagulation temperature (HCT) was defined as the maximum temperature, T, at which the sample was stable for 1 min +1 °C. This heat treatment was performed at the natural pH without adjustment. No significant change in pH was observed after cooling. Three replicates were performed for each reconstituted sample.

### 2.3. Analyses

The dry matter was determined by weight difference after drying of samples for 7 h at 102 °C. Total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined by the Kjeldhal method. The casein content was determined by precipitation at pH 4.2 (the isoelectric point of caprine caseins). From these results, total protein, casein and whey protein levels were deduced as follows:

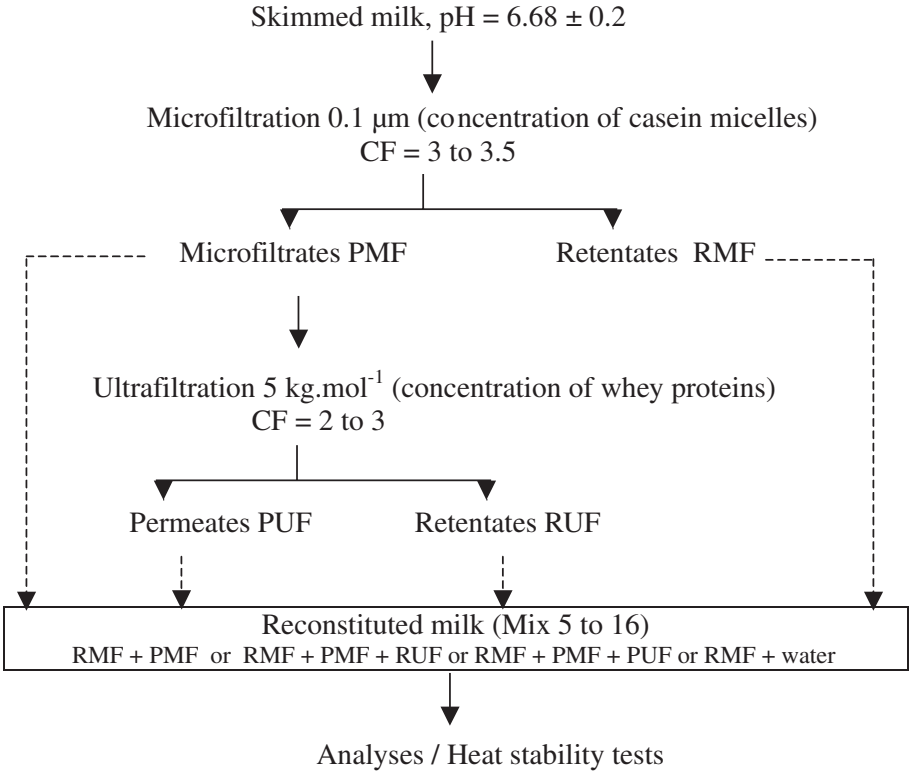


**Figure 2.** Schematic outline of the combined process used to prepare modified milk with either high micellar casein or high and low whey protein contents.

total protein = (TN – NPN) × 6.38; casein = (TN– NCN) × 6.38; whey protein = (NCN – NPN) × 6.38. The experiments were duplicated and the results presented as the mean of these duplicates. Reverse-phase HPLC was carried out on milk and reconstituted samples, as described by Jaubert and Martin [5].

## 3. RESULTS

### 3.1. Heat stability of mixtures with decreasing whey proteins content

In the first experiments, the effect of various membranes with decreasing MWCO or pore size on the heat coagulation temperature (HCT) of reconstituted milk was evaluated. All these membranes totally retained the casein micelles. Sample reconstitution was performed by mixing, at a constant casein content, the first-step filtration retentates with the second-step filtration permeates (Fig. 1). The second filtration step, performed with a tight UF membrane, retained all whey proteins (WPs). As shown in Table I, a relationship was found between membrane pore size and the heat stability of reconstituted samples; this relation was attributed to the level of retention of WP: the lower the retention level, the higher the coagulation temperature. Also, the HCT of the skimmed milk was not modified when the membrane used retained about 95% of WP (Mix 1).

### 3.2. Heat stability of mixtures with various concentrations of casein micelles and whey proteins

To determine the effect of casein and WP concentrations on the heat stability, various mixes were prepared. The measured pH values after mixing ranged from 6.63 to 6.73 (Tab. II). However, since the observed variation in pH was limited and not correlated to the HCT of the mixes, the heat treatment experiments were performed without adjustment of the pH.

The results summarised in Table II indicate that the HCT of the mixes depended strongly on the concentration of both casein and WP and consequently on the casein: WP ratio. The latter was modified either by increasing the concentration of casein micelles or by reducing the whey protein content. The first group of samples (mixtures 5 to 8) showed that at a constant casein content, close to that in milk, reducing the WP content increased the HCT. Similarly, as shown by the result of the second experimental group (mixtures 9 to 13), the HCT was improved by increasing the casein to WP ratio by differential concentration of both protein groups. Hence, concomitantly increasing the casein content and the casein to WP ratio seemed to be the most efficient way to improve the heat stability, since samples with a high casein content and a casein: WP ratio higher than 6.5 exhibited the highest heat stability (HCT > 140 °C). For all these heat-stable samples, the casein to WP ratio was at least 1.4 times higher than in natural milk. The crucial role of WP content for the HCT, even at a high casein concentration, was confirmed by adding concentrated whey proteins. Increasing the WP concentration from 6.3 to 7.5 $g \cdot L^{-1}$ impaired the HCT which decreased from 141 °C to 139 °C, respectively (Tab. II, mixtures 13 and 14). The observed effect could not be linked to a specific whey protein since RP-HPLC analysis of prepared mixtures showed that the natural proportion of the major whey proteins, i.e., β-lactoglobulin and α-lactalbumin, was not modified significantly (results not shown).

The dependence of HCT on the casein to WP ratio is further illustrated in Figure 3. As a general trend, the higher the casein to WP ratio, the higher the heat stability.

The heat stability was even higher (CT ≥ 46 °C) when the reconstitution of samples with a high casein to WP ratio was performed in water instead of permeates (mixtures 15 and 16, Tab. II and Fig. 3). Since samples with a reduced WP content (diluted with UF permeate) did not show such high stability, the observed behaviour

**Table I.** Heat coagulation temperature (HCT) of reconstituted mixtures with decreasing whey protein content prepared by mixing various retentates with the filtrate obtained by a second-step filtration on a 1 $kg \cdot mol^{-1}$ membrane (total retention of WP). The observed pH change after mixing was less than 0.08 pH units.

| Membrane type | | UF 5 $kg \cdot mol^{-1}$ | UF 100 $kg \cdot mol^{-1}$ | UF 150 $kg \cdot mol^{-1}$ | MF 0.1 μm |
|---|---|---|---|---|---|
| Sample code | SM[1] (control) | Mix 1 | Mix 2 | Mix 3 | Mix 4 |
| WP retention level (%)[2] | | 95 | 73 | 50 | 15 |
| Heat coagulation temperature (°C) | 135 | 135 | 137 | 139 | 141 |

[1] SM = skim milk (control).
[2] The retention rate (R) determined during the filtration experiments is defined as R = [1–Cp/Cr] × 100 (Cp and Cr: concentration of WP in the permeate and retentate, respectively).

**Table II.** Nitrogen composition and heat coagulation temperature (HCT) of skim milk and experimental reconstituted mixtures.

| Sample Code | Designation | pH | Total nitrogen ($g \cdot L^{-1}$) | Caseins ($g \cdot L^{-1}$) | Whey proteins ($g \cdot L^{-1}$) | Caseins/whey proteins ratio | Coagulation temperature (°C) |
|---|---|---|---|---|---|---|---|
| SM | Skimmed milk | 6.68 | 31.8 | 25.0 | 5.2 | 4.8 | 135 |
| Mix 5 | RMF diluted to 1 with (0.75 PMF + 0.25 PUF) | 6.67 | 31.7 | 25.2 | 4.4 | 5.8 | 137 |
| Mix 6 | RMF diluted to 1 with (0.5 PMF + 0.5 PUF) | 6.68 | 31.7 | 25.7 | 3.7 | 6.9 | 137 |
| Mix 7 | RMF diluted to 1 with (0.25 PMF + 0.75 PUF) | 6.69 | 31.7 | 26.4 | 3.1 | 8.6 | 139 |
| Mix 8 | RMF diluted to 1 with (0 PMF + 100 PUF) | 6.69 | 31.5 | 26.6 | 2.4 | 11.0 | 139 |
| Mix 9 | RMF diluted to 1.1 with PMF | 6.73 | 41.5 | 33.5 | 5.6 | 6.0 | 137 |
| Mix 10 | RMF diluted to 1.2 with PMF | 6.71 | 44.2 | 36.2 | 5.7 | 6.4 | 139 |
| Mix 11 | RMF diluted to 1.3 with PMF | 6.71 | 47.5 | 39.3 | 5.9 | 6.7 | 141 |
| Mix 12 | RMF diluted to 1.4 with PMF | 6.73 | 50.6 | 42.2 | 6.0 | 7.0 | 141 |
| Mix 13 | RMF diluted to 1.5 with PMF | 6.63 | 53.9 | 44.9 | 6.3 | 7.1 | 141 |
| Mix 14 | RMF diluted to 1.5 with (0.5 PMF + 0.5 RUF) | 6.73 | 54.0 | 44.9 | 7.5 | 6.1 | 139 |
| Mix 15 | RMF diluted to 1.1 with water | 6.73 | 37.9 | 33.5 | 3.2 | 10.5 | ≥146 |
| Mix 16 | RMF diluted to 1.5 with water | 6.67 | 50.6 | 44.9 | 4.3 | 10.5 | ≥146 |

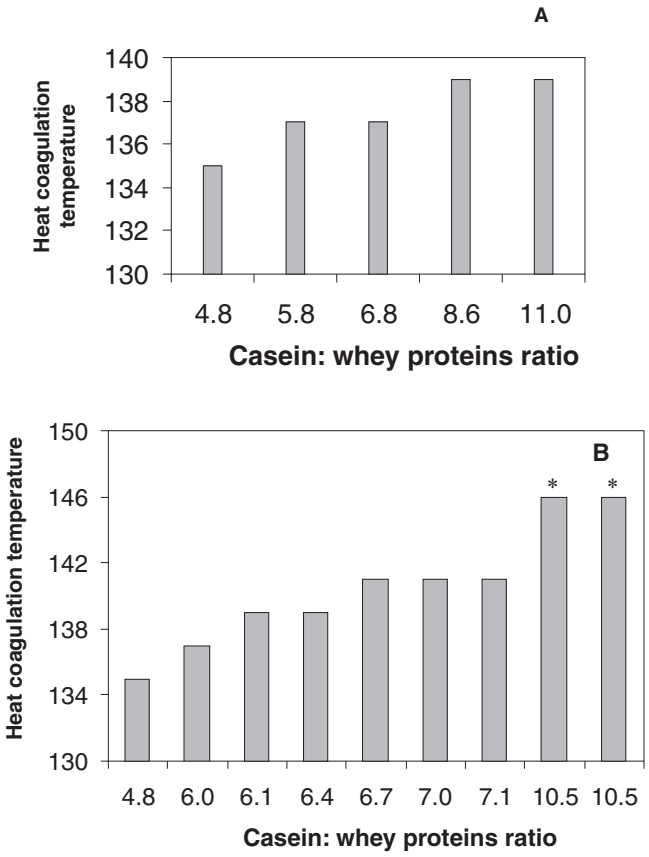


**Figure 3.** Dependence of the heat coagulation temperature of reconstituted goat milk on the micellar casein to whey proteins ratio. (A): effect of reducing the level of whey proteins at a constant casein content (25.5 $g \cdot L^{-1}$). (B): effect of increasing casein concentration from 33.5 to 45 $g \cdot L^{-1}$.
* Dilution of the retentates in water (see Tab. II, Mix 15 and 16).

of the water-diluted samples was attributed to a combined effect between low WP content and dilution of the soluble low molecular weight components such as non-protein nitrogen, lactose and minerals.

## 4. DISCUSSION

The results presented in this paper show that the heat stability of goat milk could be improved by increasing the casein to whey protein ratio by the use of membrane processes. Improvement of HCT can be achieved either by reducing the whey protein content or by selectively increasing the casein content.

The effect of whey proteins, mainly β-lactoglobulin, on the heat stability of bovine milk versus pH has been reported in several studies. Newstead et al. [10] showed that the heat stability of skim milk with a low whey protein concentration was higher than that of those with a normal or

high whey protein content. The observed effect was shown to be specific and strongly pH-dependent [10, 14]. Using purified β-Lg, Tessier and Ross [14] and O'Connell and Fox [11] demonstrated that β-Lg has an opposite effect on the heat stability of milk, respectively enhancing and reducing stability at pH 6.7 and 6.9. Further, adding β-Lg to a type B milk (which shows continuous heat stability over pH) results in the development of type A behaviour (milk with a maximum and a minimum heat stability as a function of pH value).

The mechanism of the β-Lg-induced stability at pH 6.6–6.7 was related either to the ability of the protein to reduce the dissociation of κ-casein from the micelles [16] or to its calcium-chelating properties [11]. In the case of goat milk, the role of whey proteins and the mechanism by which they act at pH 6.6 seem to be different. Firstly, the poor heat stability of this milk was not improved by adding whey proteins (4 $g \cdot L^{-1}$) at the natural pH, i.e., 6.6 [8]. Secondly, the results of the present work clearly show that the heat stability of goat milk is improved by reducing the whey protein content. Consequently, the effect of whey proteins on the heat stability of goat milk at pH 6.6 is linked to their observed destabilising effect in bovine milk at pH 6.9. In this case, the destabilising mechanism was proposed to be due to either the ability of β-Lg to enhance the dissociation of κ-casein from the micelles [13, 16] or to increase their hydrophobicity [11]. According to Anema and Stanley [1], an increase in κ-casein and β-Lg in the soluble phase was favoured only at pH values > 7. The latter explanation seems to prevail in the case of goat milk at pH 6.6–6.7. Reducing the whey protein content reduced the heat-sensitive denatured whey proteins-κ-casein complex. This explanation is partly supported by the results of our recent study which showed the formation of a heat-induced covalent complex between β-Lg and casein micelles from goat milk at pH 6.7 [4].

Another factor that affects the heat stability of milk is the mineral equilibrium between colloidal and soluble phases which depends on the casein concentration [2]. This is illustrated by comparing the HCT of mixtures with a similar casein:WP ratio but different casein content, e.g., mixture 6 versus mixture 13. The higher stability of mixture 13 (with a higher casein content) can be related to a lower tendency to protein aggregation due to the lower amount of calcium bound per g of casein. Furthermore, the most heat-stable samples in this study were obtained by simultaneously reducing the content of both whey proteins (relative to caseins) and soluble low molecular weight components (mixtures 15 and 16). These results emphasise, as expected, that the heat stability of milk is governed by several factors in a cumulative, if not a synergistic, manner. The role of some of them has been already reported [9, 15, 17]. Although the involvement of other components was not demonstrated here, our experiments suggest that the destabilising effect of whey proteins through sensitising the casein micelles to calcium-mediated precipitation, as suggested in the case of bovine milk [11], is a highly probable mechanism.

## ABBREVIATIONS USED

β-Lg: β-lactoglobulin; HCT: Heat coagulation temperature; HS: Heat stability; PMF: Microfiltration permeate; RMF: Microfiltration retentate; RUF: Ultrafiltration retentate; PUF: Ultrafiltration permeate; WP: Whey proteins.

## ACKNOWLEDGEMENT

We thank G. Henry and E. Le Put for performing chromatographic and physico-chemical analyses. This work was supported by a grant from the French Ministry of Agriculture and Fisheries in the program "Aliment-Qualité-Sécurité" TQ 42, Décision No 99.P.0391.

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