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# **FERMENTED MILK**



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# Fermented Milk



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## P.6 - THE STRUCTURE OF CASEIN IN HEATED SUSPENSIONS AFFECTS THE ACID GELATION OF MILK

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### ABSTRACT

In the purpose of improving texture properties of yogurt, milk composition was modified first by substitution of 12% of micellar casein by Na- or Ca-caseinate and by a native phosphocaseinate as control (Pcas) and second by fractionation into a small and a large micelle fractions using centrifugation. Analyses of suspensions include total (TN), non casein (NCN) and non-protein nitrogen (NPN), before and after heat treatment at 90°C for 10 min, mineral partition between colloidal and soluble phase and particle diameter. Bacterial acidification at 42°C was followed by the dynamic viscoelastic method. Whey protein (WP) amount was the same in the 3 substituted suspensions, but the Na-caseinate suspension contained less calcium than the others. The higher moduli and  $\tan \delta$  were obtained for Na-, Ca-caseinate and Pcas in decreasing order, meaning a more viscous character in a firmer gel with a lower level of structuration. The smaller micelles led to a lower modulus, though it had the higher concentration of WP and though the smaller micellar fraction contains more k-casein, which could enhance the formation of heat-induced complexes between casein and WP. Gelation of the larger micelles was observed at a higher pH value (pH 5.5) than for the smaller micelles (pH 5.3).

### 1 Purpose of study

The purpose of the study is to increase the understanding the acid gel construction in order to improve texture properties of acid gels such as yogurt by modification of milk composition. Two hypotheses were tested. First, we investigated the hypothesis that the level of structure or the content of minerals in the casein micelle will play a role during the micelle disintegration caused by acidification of milk. In that case 12% of the micellar casein were replaced by Na- or

Ca-caseinate and by a native phosphocaseinate as control (Pcas). Second, the effect of the micelle size was studied because small micelles are known to contain more  $\kappa$ -casein and could probably lead to more heat-induced complexes between  $\kappa$ -casein and  $\beta$ -Lactoglobulin. Casein micelles were fractionated into a small and a large micelle fraction using centrifugation. Milk suspensions were heated and acidified by a yogurt starter.

## 2 Methods and materials

### Milk suspensions

#### Substitution of micellar casein

A milk sample was reconstituted as a control at 31 g.kg<sup>-1</sup> from low heat skim milk powder (Ingredia, Arras, France) and stored for 1 h. In the other suspensions, 12 % of the casein content were substituted by caseinates, by dissolving a lower content of milk powder into MilliQ water and adding casein to reach the same total casein content as in milk from a sodium and a calcium caseinate (Nacas, Cacas, Armor Protéines, 35460, France) and from a native phosphocaseinate powder (Pcas). The latter contains casein micelles in a rather native state and was prepared at the INRA-LRTL by microfiltration and diafiltration of milk on a 1.4  $\mu$ m membrane and spray drying [1, 2].

#### Small and large micelles

A suspension of casein micelles was prepared by dissolving the phosphocaseinate powder in water at 93.75 g.kg<sup>-1</sup> casein. The suspension was fractionated in a pellet obtained at 10 000 g for 30 min at 20°C representing the fraction of large micelles and a supernatant obtained by centrifugation at 30 000 g for 30 min at 20°C, representing the small micelles. A suspension of Pcas was also prepared and was not fractionated. The 3 fractions were freeze-dried. Suspensions were prepared from the 3 powders at 31.25 g.kg<sup>-1</sup> in a milk microfiltrate prepared on a 1  $\mu$ m membrane.

#### Heat treatment and analyses

Suspensions were heated at 90°C for 10 min in a stainless steel tube immersed in a water bath. Immediately after heating, milk was cooled into a water bath at 0°C.

Total (TN), non casein (NCN) and non protein nitrogen content (NPN) of milk were performed with the Kjeldahl method, using 6.38, 6.25 and 6.19 as converting factors, respectively [3]. The casein and the whey protein (WP) content were determined as TN-NCN and NCN-NPN, respectively.

Mean particle size was estimated on the unheated and heated suspensions of small and large micelles by a dynamic light-scattering method with a Malvern

Zeta sizer 3000 HS (Malvern, France) on a 1:1000 dilution of suspensions into Dalgleish buffer [4] at 20°C as in Michalski et al. [5].

Suspensions were examined after fractionation by ultrafiltration on membranes of 25 kg.mol<sup>-1</sup> molecular weight cut-off (Centriflo CF 25, Amicon Grace, Epéron, France) for the determination of soluble minerals. Ultrafiltration in the centrifuge was carried out at 20°C at 750 g for 45 min. Total and soluble Ca and Na concentrations were determined by atomic absorption spectrometry using a Varian equipment (Les Ulis, France) on milk and milk permeate, respectively, according to Le Graët and Brulé [6].

Each suspension was equilibrated at 42°C for 15 min and was inoculated with non-ropy frozen concentrated cultures of *S. thermophilus* and *L. bulgaricus* (Danone, 92350, France).

The kinetics of acidification were registered using a Cinac system (Ysebaert, Frépillon, France) [7]. The elastic (G') and viscous (G'') moduli and the loss tangent, tan  $\delta$ , were followed at 42°C as a function of time in the AR1000 rheometer (TA instruments, St Quentin-en-Yvelines, France), in the oscillatory mode with a coaxial cylinder geometry at a frequency of 1 Hz and a strain of 2 %. Suspensions were placed in the cup and covered with a thin layer of paraffin. At least 2 gelations per system were performed.

## 3 Results

### Substitution of micellar casein

Table 1 gives the composition of the 3 suspensions. The WP and the casein contents in heated suspensions are given in percentage of the amount in the non-heated sample. The WP content decreased and that of casein increased after the heat treatment because of the loss of solubility of WP at acidic pH after denaturation and because of the complexation with casein. This value in heated milk is around 25 % with 5.6 g.kg<sup>-1</sup> WP (results not shown). A slightly higher residual

Table 1: Composition of suspensions with 12 % of micellar casein substituted with sodium caseinate (Nacas), calcium caseinate (Cacas) and native phosphocaseinate (Pcas).

	Any suspension (g.kg <sup>-1</sup> )		
	Nacas	Cacas	Pcas
Ca <sub>tot</sub> (mg.kg <sup>-1</sup> )	1179	1283	1394
Ca <sub>sol</sub> (mg.kg <sup>-1</sup> )	290	304	300
Na <sub>tot</sub> (mg.kg <sup>-1</sup> )	621	544	526
	In percent of that in non heated milk		
Casein	110	109	110
Whey proteins	28.7	30.4	31.8

concentration of WP appeared in heated suspensions compared to milk, probably due to a lower concentration of WP in unheated suspension, due to the substitution of a small part of milk by caseinate. The 3 heated suspensions had the same level of residual WP (29-32 %) and caseins (110 %).

The  $G'$  values of the suspensions were lower than the one of the control milk with the same casein concentration (Figure 1 see p.379), but the suspensions had lower WP contents and lower total protein content than the control milk. The higher  $G'$  value was obtained for the substitution with sodium caseinate, calcium caseinate and phosphocaseinate, in the decreasing order. The  $\tan \delta$  values were higher than that of milk during the gelation, but the general shape with a local maximum observed in numerous studies [8, 9, 10, 11] was maintained. As the acidification kinetics were not modified by the substitution (results not shown), there were no significant changes in the pH at gelation between milk and between the suspensions. The maximum value of  $\tan \delta$  was higher for the Nacas, Cacas and Pcas in the decreasing order, meaning a more viscous characteristic of the acid gel at the local maximum of the  $\tan \delta$  with the less structured casein or the lower amount in calcium or phosphate.

#### Small and large micelles

Table 2 gives the composition of the suspensions with small or large micelles and the non-fractionated sample. The concentration of casein was slightly higher in the suspension with large micelles and the WP content was lower in the suspension with large micelles. The casein suspension used for fractionation was a micelle suspension with very low concentrations of WP (less than 4% of TN [12]). Reconstitution of suspensions from freeze-dried pellet or supernatant introduced different contents in aqueous phase and consequently different concentrations of WP. Anyway, the residual concentration of WP in the heated suspension was higher in the suspension containing the small micelles, which indicates a lower denaturation or complexation, with the higher whey protein concentration.

The highest  $G'$  was obtained for the suspension with large micelles, with unfractionated micelles and with small micelles, in the decreasing order (Figure 2

Table 2: Composition of suspensions with small or large casein micelles and the control (non-fractionated sample).

	Large	Control (Pcas)	Small
pH	6.67	6.65	6.64
Casein (g.kg-1)	27.3	26.3	26.9
WP (g.kg-1)(non heated)	5.91	5.74	6.55
WP (% of non heated milk)	36.4	37.4	39.8
Micelle diameter (nm) (non heated)	228	237	191
Micelle diameter (nm)(heated)	250	218	202

see p.380). The higher complexation of WP in suspensions with large micelles was accompanied by the higher  $G'$  values. The values of  $\tan \delta$  were in the same range for the 3 suspensions, but it appeared that the large micelles had a higher pH at gelation (pH 5.5) than the small ones (pH 5.3). Due to a poor reproducibility of the experiment with the small micelles, the difference was not significant (F test with  $P>0.4$ ).

#### 4 Discussion

From the substituted milk, it appears that the lower the level of structure, the higher the content of sodium, the lower the colloidal calcium amount, the higher the elastic modulus of the acid gel and the more liquid-like structure of the gel. The micellar casein substitution, in the applied restrictive range, did not change heat-induced whey protein complexation.

The whey protein concentration was ~10 % higher in suspensions of small micelles, but the heat-induced complexation was ~10 % lower. The greater complexation was related to the higher  $G'$  values of acid gels and the higher pH at gelation. The first hypothesis is that when the micelle is covered with WP, the whole particles behave as the coat of denatured WP and gelation took place at a higher pH value, due to the higher isoelectric pH value of WP. The ratio of casein on WP was ~ 4.1 and 4.6, for small and large micelles, respectively. But, as reported by the following studies [13, 14, 15], addition of  $\beta$ -Lactoglobulin does not necessarily lead to a higher complexation of WP on casein micelles. In a second hypothesis, material that coagulates at pH 5.3-5.5 was mainly composed of soluble aggregates of WP alone or with soluble  $\kappa$ -casein. The casein micelle coagulated at pH 5 as in unheated milk. Anyway, the analytical method used in this study to follow the loss of solubility of WP was rather global and improvement of the identification of heat-induced complexes is needed for a better understanding of the effect of heat treatment of milk on its gelation properties with yogurt starters.

#### 5 Conclusion

This work underlines that changing the structure of the casein micelle, either by modification of internal interactions between casein molecules or outside interactions between casein and WP led to changes in the rheological properties of acid gels.

Lowering the level of structure or the content of colloidal calcium or phosphate and increasing the complexation of WP is probably the way to reach higher elastic modulus, more "liquid" characteristics of the gel and a higher pH at gelation.

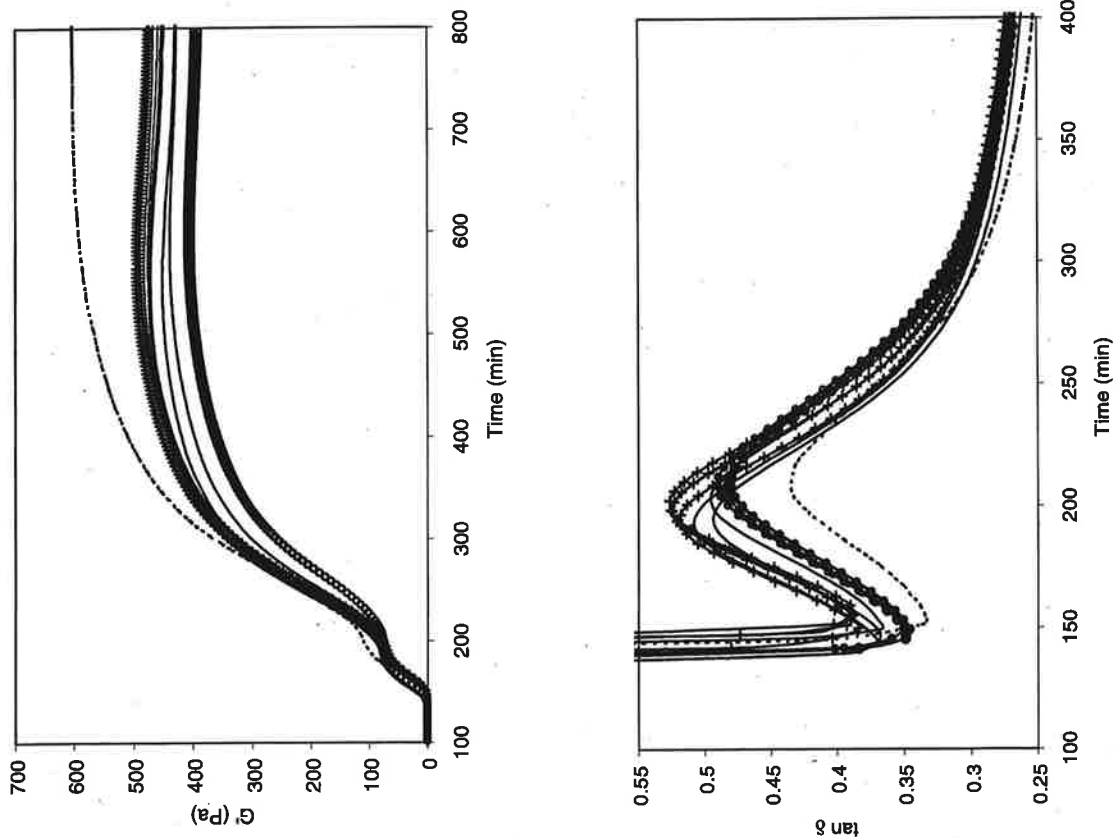


Figure 1: Kinetics of acid gelation at 42°C with a yogurt starter of milk (---) and with the 3 suspensions substituted with sodium caseinate (Nacas, — + —), calcium caseinate (Cacas, —) and native phosphocaseinate (Pcas, — ○ —).

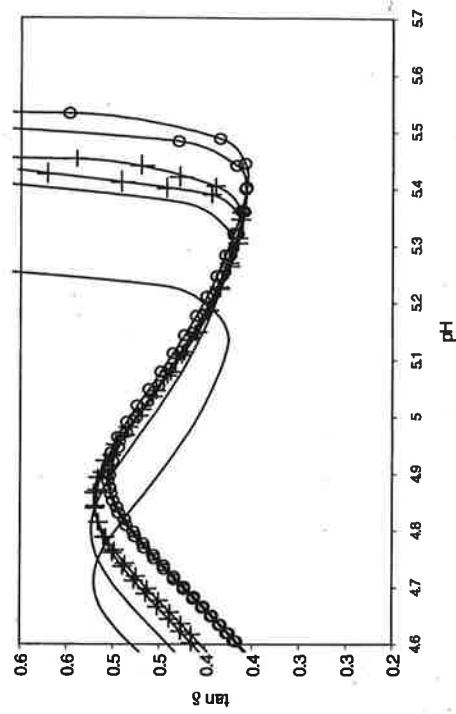
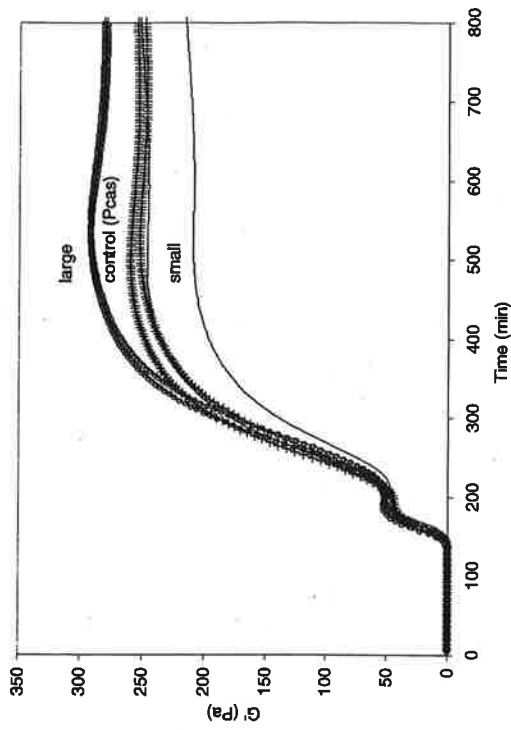


Figure 2: Kinetics of acid gelation at 42°C with a yogurt starter of the 3 suspensions with the native phosphocaseinate non fractionated as control (Pcas) (— + —), small (—) and large micelles (— ○ —).

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