Effects of phosphates and citrates on sediment formation in UHT goats' milk

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Sediment formation was investigated during UHT treatment of goats' milk, subjected to indirect treatment at 140 °C for 2 s, with upstream homogenisation. Stabilisers evaluated were sodium hexametaphosphate (SHMP), trisodium citrate (TSC), disodium hydrogen orthophosphate (DSHP), and sodium dihydrogen orthophosphate (SDHP). With no added stabiliser, goats' milk produced a heavy sediment on UHT treatment. Addition of SDHP reduced pH, had little effect on ionic calcium and did not substantially reduce sediment. However, addition of SHMP, DSHP and TSC each reduced ionic calcium, increased ethanol stability and reduced sediment. Following stabiliser additions, there was a good correlation between ethanol stability and ionic calcium ($R^2=0.85$) but not between ethanol stability and pH ($R^2=0.08$). Overall, reducing ionic calcium reduced the amount of sediment formed for all these three stabilisers, although there was no single trend line between sediment formation and ionic calcium concentration. Sediment formation was not well correlated with pH for TSC or for SHMP, but it was for DSHP, making it the only stabiliser where sediment formation correlated well both with ionic calcium and pH, which might account for its effectiveness at higher ionic calcium levels. Sediment was much reduced when the temperature was reduced from 140 °C to 125 °C and 114 °C. There were no further changes in sediment on storage for two weeks. Analysis of the sediment showed that it was predominantly fat and protein, with a mass ratio ranging between 1·43:1 and 1·67:1. Its mineral content was usually less than 5% of dry weight. The maximum amounts of P and Ca were found to be 2·32% and 1·63%, respectively.

Keywords: UHT, goats' milk, ionic calcium, ethanol stability, phosphates, citrates, stabilisers, sediment.

Most UHT milk contains a slight amount of sediment, which is not usually sufficient to be a problem (Burton, 1988). Several investigators have found that sediment increases with severity of the treatment and is more severe in direct rather than in indirect UHT processed milk (Perkin et al. 1973; Ramsey & Swartzel, 1984). However, on some occasions a more voluminous sediment appears, for example with goats' milk and with cows' milk which has been supplemented with calcium. Zadow (1978) found that little sediment was formed in UHT cows' milk if pH was kept above 6·62, below this value sedimentation increased rapidly. In contrast sedimentation was severe in goats' milk when the pH was below 6·9. Similar trends were observed for concentrated skim milk. Milk was stable above pH 6·55 but below this value, severe sedimentation occurred (Zadow & Hardham, 1981).

Different stabilisers have been investigated to reduce sediment to acceptable levels and to reduce heat induced thickening and coagulation in concentrated milk. Sweetsur & Muir (1980) proposed that disodium hydrogen phosphate (DSHP), trisodium phosphate, trisodium citrate (TSC) and sodium bicarbonate should be used if the natural pH falls on the acid side of the heat stability maximum, while sodium dihydrogen phosphate (SDHP) or calcium chloride should be used if the natural pH is alkaline to the maximum, with orthophosphates being generally the most effective stabilisers. Mittal et al. (1990) evaluated sodium hexametaphosphate (SHMP) addition to UHT recombined milk and found that this was effective at retarding gelation. No sediment was found in UHT milk, with or without SHMP.

Goats' milk is much more susceptible to sediment formation on UHT treatment (Zadow et al. 1983). Its ionic calcium level has been reported to be higher compared with cows' milk. Addition of 0·05% $CaCl_2$ further increased ionic calcium from 3·2 to 4·5 mm and further

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increased sediment. Addition of DSHP reduced ionic calcium and reduced sediment drastically. Montilla & Calvo (1997) observed that a mixture of NaH_2PO_4, Na_2HPO_4 and Na_3PO_4 did not change the pH of the raw goats' milk and increased its heat stability. Use of mixed phosphates was found to be more effective than pH adjustment by means of NaOH.

The alcohol (ethanol) stability test has been used as a simple indicator of cows' milk freshness and suitability for UHT processing (Shew, 1981), and cows' milk should be stable in 74% alcohol to be suitable for UHT treatment. Goats' milk has been found to have a much lower ethanol stability than cows' milk (White & Davies, 1958; Horne & Parker, 1982; Guo et al. 1998). Sediment formation is still reported to be problem during UHT goats' milk production, even with addition of DSHP (Sutton, 2004).

This paper investigates the effects of different stabilisers and the role of pH and ionic calcium on the voluminous sediments arising from goats' milk, using a stable ion electrode system described by Lin et al. (2006).

Materials and Methods

Milk samples and processing conditions

Raw goats' milk was supplied by Delamere Dairies (Yew Tree Farm, Knutsford, Cheshire, UK) and Willowbrook Farm, Garford, Abingdon, UK. It was subjected to indirect UHT treatment using an APV Junior plate heat exchanger, described and characterised in terms of its temperature/time heating profiles and corresponding B* and C* by Browning et al. (2001). Four batches of raw goats' milk were processed using the same conditions of 140 °C for 2 s. Upstream homogenization during the heat treatment was at a pressure of 180 bar. Four different stabilisers were added in different concentrations, namely SDHP, SHMP, TSC and SDHP; the latter was only used in the first two trials. The samples were collected in sterile pots (250 ml; Bibby Sterilin Ltd, UK) in a laminar air-flow cabinet. In trial 4, raw goats' milk was processed at 125 °C and 114 °C, to observe the effects of temperature on sediment formation.

Milk Analyses

Analyses were performed on raw milk and UHT milk samples. Protein, fat, solids-not-fat and lactose contents were measured using a DairyLab II Analyser (Multispec Limited, York, UK) and were the average of two readings.

The pH of the milk samples was measured using a Sentron 3001 pH meter, which was calibrated prior to the analyses with standard buffer solutions of pH 4·0 and 7·0.

Ionic calcium was measured using a Ciba Corning 634 ISE Ca^{2+}/pH Analyser (Lin et al. 2006). The instrument was calibrated in its millivolt ouput mode with solutions of 1·0, 1·5, 2·0, 2·5, and 3·0 mм-Ca^{2+} daily, prior to use. There

was a linear relationship between log (ionic calcium) and mV output, with correlation coefficients greater than 0·99.

Ethanol stability was determined by mixing equal volumes (2 ml) of milk and a range of ethanol solutions, and examining for the presence of clots when poured into a Petri dish. Depending upon the formation of clots, ethanol solutions of increasing or decreasing concentration were used and the highest concentration of ethanol which did not cause coagulation was defined as the ethanol stability. All the above analyses were done at room temperature.

Sediment was measured by a centrifugation method. The milk was well shaken in its container. Approximately 40 ml was accurately weighed and poured into a calibrated tube and centrifuged (Centaur 2 at 4200 rpm for 15 min), corresponding to 2760 *g* maximum. After removing the supernatant, the sediment volume was then measured (expressed as ml/100 ml). The sediment wet weight was measured and the sediment was then oven-dried at 102 °C to constant weight to determine its dry weight (g/100 ml). This method was also used on a selection of commercial cows' milk and goats' milk products.

After removal of the sediment, the supernatant was examined for protein, fat and lactose, to determine losses into the sediment. Ash content was measured using the AOAC method (1995), except that dried sediment was used instead of the dried milk. Results were expressed as percentage of ash in sediment dry weight.

The concentration of total phosphorus (P) in milk was determined according to British Standard Methods (BS 1741-12: 1992). This method was adapted to determine the total P content of the milk sediment, which was dry-ashed.

Total Ca concentration of the sediment from the milk samples was determined by the method of Murthy & Rhea (1966) employing a Pye Unicam SP9 Flame Emission Spectrophotometer (Pye Unicam Ltd., Cambridge, UK).

Results

Sediment was estimated within 24 h of UHT production. In raw milk without stabiliser, over 5% sediment (dwb) was produced in three of the four batches, which is equivalent to over 40% of the total milk solids (Table 1). Sediment in the 4th batch was lower, but still substantial at 3·5%. However, on this occasion, the temperature fell within two minutes from 140 to 135 °C, suggesting heavy fouling of the heat exchanger. This lower temperature may have accounted for the reduced sediment. There was considerable variation in ionic calcium for the four raw goat's milk samples, ranging from 1·94 to 2·80 mм, whilst ethanol stability ranged between 50 and 64% (Table 1). All these milks were below the threshold value of 74%, recommended for UHT treatment (Shew, 1981). Sediment appeared after standing for less than 1 h and the milk had an unacceptable chalky mouthfeel. Sediment after one

Table 1. Ethanol Stability, pH and calcium ion concentration before and after UHT treatment and sediment formation for all four trials with goats' milk and the effects of selected stabilisers

Sample† (g/100 ml)	Before UHT treatment			After UHT treatment			
	Ethanol stability	pH	Ca^{++}(mM)	ethanol stability	pH	Ca^{++}(mM)	Sediment (dwb) g/100 mL
Trial 1							
Control	50	6·56	2·80		6·52	2·81	5·5
0·1 SHMP	70	6·59	1·61	70	6·48	1·52	4·8
0·2 SHMP	84	6·61	0·98	70	6·43	0·87	3·6
0·2 TSC	98	6·63	0·98	74	6·46	0·91	0·3
0·1 DSHP	55	6·65	2·19	45	6·62	2·11	4·9
0·2 DSHP	68	6·7	1·66	68	6·67	1·59	3·8
0·1 SDHP	55	6·29	2·61		6·24	2·83	6·3
Trial 2							
Control	50	6·6	2·44	45	6·56	2·43	5·4
0·1 SHMP				74	6·53	1·36	1·9
0·2 SHMP				80	6·49	0·85	0·13
0·1 TSC				70	6·71	1·62	2·2
0·2 TSC				74	6·82	1·22	0·15
0·1 DSHP				70	6·64	1·77	3·6
0·2 DSHP				68	6·69	1·42	2·3
0·1 DSHP					6·41	2·39	5·1
0·2 DSHP					6·25	2·45	4·9
Trial 3							
Control	60	6·53	1·94	58	6·53	1·88	6·5
0·1 SHMP	74	6·54	1·25	78	6·57	1·18	3·3
0·2 SHMP	80	6·54	0·89	78	6·61	0·85	0·6
0·3 SHMP	98	6·58	0·65	82	6·45	0·64	0·9
0·1 TSC	72	6·61	1·41	80	6·43	1·37	3·8
0·2 TSC	78	6·70	1·07	78	6·42	1·09	1·2
0·3 TSC	80	6·75	0·90	76	6·62	0·90	0·8
0·1 DSHP	68	6·57	1·68	68	6·73	1·48	5·1
0·2 DSHP	72	6·65	1·29	76	6·83	1·29	0·3
Trial 4							
Control	64	6·75	1·97	72	6·59	1·77	3·5‡
0·1 SHMP	80	6·77	1·28	68	6·49	1·10	5·1
0·2 SHMP	90	6·78	0·85	68	6·42	0·76	3·8
0·3 SHMP	100	6·78	0·68	72	6·41	0·62	2·6
0·1 TSC	72	6·85	1·46	78	6·66	1·36	4·2
0·2 TSC	76	6·95	1·14	76	6·75	1·06	2·7
0·3 TSC	78	7·02	0·95	76	6·83	0·90	2·2
0·1 DSHP	70	6·82	1·64	76	6·63	1·43	1·2
0·2 DSHP	72	6·84	1·50	78	6·64	1·33	0·4
0·3 DSHP	76	6·87	1·37	76	6·67	1·16	0·2

† Stabilisers evaluated were sodium hexametaphosphate (SHMP), trisodium citrate (TSC), disodium hydrogen orthophosphate (DSHP), and sodium dihydrogen orthophosphate (SDHP)

‡ Temperature fell quickly to 135 °C

week and 2 weeks was also measured in the first trial and further changes over this period were minimal, the one exception being milk containing 0·2% SDHP, which increased from 6·05 to 9·48%. Sediment separation by centrifugation permitted measurement of its volume, its wet weight and its dry weight (g/100 g). There was a good agreement between the three methods, although dry weight was felt to be the most useful, as it could be directly related and compared to solids in milk.

SDHP was not effective at reducing sediment: adding it to raw milk reduced pH and had little effect on ionic calcium. Milk containing SDHP caused noticeable fouling during UHT treatment, within minutes of starting the process.

On the other hand, SHMP, DSHP and TSC were all effective; each reduced ionic calcium and increased ethanol stability. The combined results for these three stabilisers for all four trials are presented in Table 1.
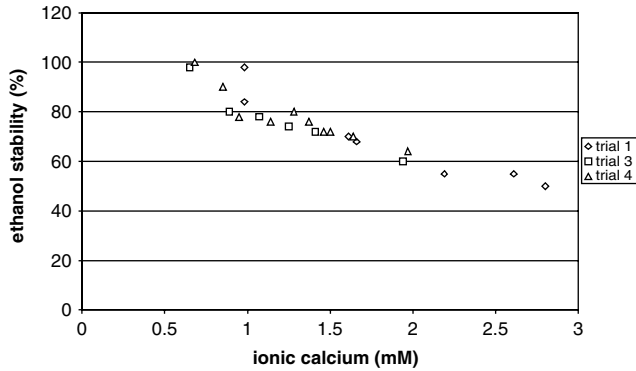
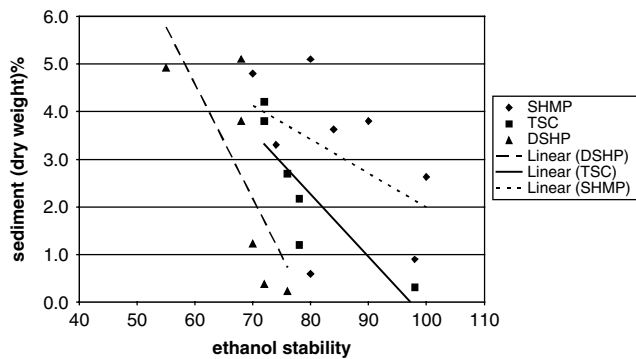**Fig. 1.** Effect of stabiliser addition on ethanol stability.

Fig. 2. Relationship between sediment formation and ethanol stability for different additions of ◆, sodium hexametaphosphate (SHMP); ■, trisodium citrate (TSC) and ▲, disodium hydrogen orthophosphate (DSHP).

Fig. 3. Relationship between sediment formation and ionic calcium for different additions of ◆, sodium hexametaphosphate (SHMP); ■, trisodium citrate (TSC) and ▲, disodium hydrogen orthophosphate (DSHP).
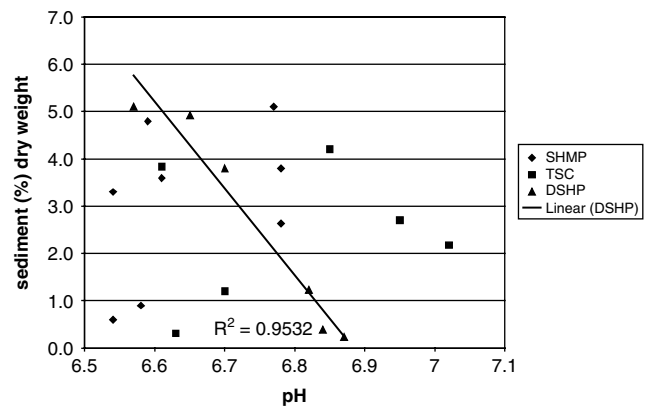
Fig. 4. Relationship between sediment formation and pH for different additions of SHMP, TSC and DSHP.

Ethanol stability, pH and ionic calcium were evaluated before and after UHT treatment, except for trial 2, where results prior to heat treatment are not available. There was a good general correlation between ethanol stability and ionic calcium prior to heat treatment. The correlation coefficient was significant ($R^2 = 0.85$; Fig. 1). Thus, ethanol stability is a useful test for indicating changes in ionic calcium brought about by addition of these stabilisers.

Again, UHT treatment was found to reduce ionic calcium in most cases. However, changes in ethanol stability were less consistent: in 6 cases it was higher and in 18 cases it was similar or lower. This did not appear to be related to the stabiliser type. Figure 2 shows the relationship between ethanol stability and sediment formation for these three stabilisers. The trend shown is that increasing ethanol stability reduced sediment formation for the three stabilisers. The overall correlation coefficient is $R^2 = 0.34$. However, the relationships are offset, and relatively small improvements in ethanol stability are more effective for DSHP ($R^2 = 0.72$) than for TSC ($R^2 = 0.57$) and least effective for SHMP ($R^2 = 0.22$). Thus ethanol stability appears not to be a reliable indicator of sediment formation for SHMP.
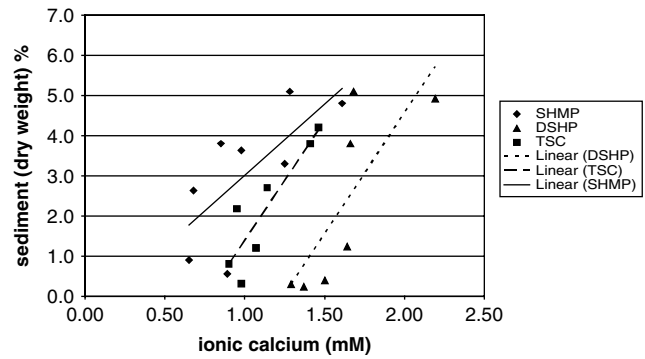
Figure 3 shows how sediment formation was influenced by ionic calcium for all three stabilisers. The overall correlation coefficient is $R^2 = 0.43$. However, a different picture emerges when the individual stabilisers are examined. For each stabiliser, there is a clear reduction in sediment as ionic calcium is reduced. The best correlation is for TSC ($R^2 = 0.80$), whereas SHMP is effective but more erratic in its performance. ($R^2 = 0.50$). When comparing their performance at similar levels of ionic calcium, DSHP is the most effective (Fig. 3) although its R^2 value is 0.62.

Some samples, with low ionic calcium levels, produced more sediment than expected, for example 0.3 % TSC and 0.2 % SHMP. It is possible that reducing the ionic calcium too much may destabilise casein micelles, rendering them more susceptible to heat induced aggregation. Figure 4 shows the relationship between pH and sediment formation, plotted for the three stabilisers. The overall correlation coefficient is $R^2 = 0.17$. It is interesting that there is no effect for TSC and for SHMP, but there is a positive correlation for DSHP ($R^2 = 0.95$), which may help explain

Table 2. Percentage ash, phosphorus (P) and calcium (Ca) in sediment dry weight and fat/protein ratios (2nd trial)

Sample† (g/100 ml)	% ash in sediment dry weight	% P in sediment dry weight	% Ca in sediment dry weight	Fat/protein ratio
No stabiliser	4·81	1·32	1·26	1·63
SHMP (0·1)	10·62	2·32	1·63	1·54
TSC (0·1)	4·07	1·62	1·39	1·67
DSHP (0·2)	4·73	1·80	1·50	1·53
DSHP (0·1)	4·59	1·51	1·39	1·43
SDHP (0·2)	3·41	1·46	1·17	1·45
SDHP (0·1)	3·68	1·02	0·89	1·48

† See Table 1

why it is more effective than TSC and SHMP, when ionic calcium levels are similar. (see Fig. 3). There was also a poor correlation between ethanol stability and pH ($R^2 = 0.08$).

The effect of temperature on sediment was evaluated on goats' milk with no added stabiliser. Sediment formation was above 5% at 140 °C in the first three trials and 3·5% in the fourth trial, although it was observed that the temperature quickly fell to 135 °C. When processing temperature was reduced to 125 °C and 114 °C in the fourth trial (holding time=2 s), sediment formation was much lower, at 0·7% in both cases. This shows that sediment formation is quite temperature dependent.

The sediment from the first two trials was analysed for phosphorus and calcium. In the first trial, phosphorus ranged between 1·22 to 2·03%, being highest for SHMP. Calcium ranged between 0·99 to 1·36%. The ratio of P/Ca ranged from 1·05 to 1·50 and did not show any change one week later. In trial 2, the ash content was also analysed and found to range between 3·4 and 10·6% (dwb), being highest for SHMP (Table 2). P ranged between 1·12 and 2·32% and calcium between 0·89 and 1·63%. The low ash content of the sediment suggested that it was predominantly fat and protein. This was confirmed by the reduction in fat and protein found in UHT milk, following removal of the sediment. Based on the assumption that all the fat and protein lost from milk ended up in the sediment, the ratios of protein to fat were estimated to be between 1·43 and 1·67. Overall, there was a very good correlation between loss of protein and fat and the amount of sediment formed. The R^2 values for the three trials investigated were 0·98, 0·96 and 0·88. Freezing point depression did not change following UHT treatment. This also suggested that lactose and minerals had changed little in the milk as a result of the heat treatment. The type of stabiliser did not appear to grossly affect the chemical composition of the sediment formed, but they may have produced subtle changes in their mineral contents. Sediment formed with SHMP seemed to produce slightly higher amounts of calcium and phosphate.

Sediment (dwb) was measured in raw goats' milk (0·4%), commercial UHT goats' milk (0·29%), pasteurised semi-skim goats' milk (0·19%), semi-skim cows' milk (0·08%) and sterilised semi-skim cows' milk (0·13%) These results suggest that all milk shows a small amount of sediment, which is in agreement with Burton (1988). One question relates to what is an acceptable level of sediment and how much is present before it can be perceived.

Discussion

Sediment in UHT goats' milk is one manifestation of poor heat stability. Its formation appears to involve aggregation of casein micelles, promoted by ionic calcium. In general, addition of TSC, DHSP and SHMP all reduced ionic calcium, increased ethanol stability and reduced sediment. At similar levels of ionic calcium, DSHP was most effective. However, the overall mineral content in the sediment was low. Since homogenization was upstream, the fat globules would be coated with casein micelles and thus be incorporated into the sediment. However, despite containing substantial fat, the sediment was more dense than the serum, and no separate layer of fat was observed. The ratio of phosphorus to calcium found in the deposits (0·96 to 1·50) was higher than that found in milk (approx. 0·78) and in the casein micelle fraction (approx. 0·48). Material responsible for sediment was formed as a result of the heat treatment.

The role of pH is less clear. Walstra & Jenness (1984) illustrated that pH of milk will fall below 6·0 at 140 °C. This will also increase ionic calcium and zeta potential; all these factors combined will influence its heat stability. What is probably of importance is the absolute pH value of the milk at 140 °C, lower values will also lead to higher levels of ionic calcium and have a destabilising influence. This will be influenced by the buffering capacity of the milk, so milk with a higher buffering capacity will be subject to a smaller drop in pH and thus be less susceptible to sediment formation. This type of mechanism has been attributed to the role of urea in improving heat stability (Metwalli & Boekel, 1996) and it may also be the reason for the effectiveness of DSHP as a stabiliser. Since the fall in pH will be less as temperature is reduced, this may be a contributory factor to the lower level of sediment observed at 125 and 114 °C.

The UHT conditions used in this work correspond to an F_0 value of 5·3, C* of 0·39 and B* value of 1·25 (Browning et al. 2001). On occasions, fouling of the heat exchanger was also observed, which was sometimes rapid, indicated by a reduction in the temperature in the holding tube. Thus it is problematic for two reasons, firstly the adverse effects on the sensory characteristics of the milk caused by the presence of the sediment; and secondly the detrimental consequences of fouling, such as shortened processing

runs and additional cleaning costs. Burton (1968) suggested that sediment is produced by the same mechanism which is responsible for fouling of heat exchangers, with sediment representing fouling material which has not become attached to heat exchanger surface. The fact that sediment formation was often accompanied by an observed deterioration in the performance of the heat exchanger would support this. Further support is provided by Prakash et al. (2007), who have investigated fouling behaviour during UHT processing of goats' milk, with and without stabilisers.

In commercial processing, monitoring pH, ethanol stability and ionic calcium routinely would be useful for understanding factors affecting sediment formation and also plant performance and its susceptibility to fouling. This would also help understand the effects of natural variations in milk composition. Milk from individual animals and to a lesser extent bulk milks have been found to show considerable variations in ionic calcium levels (Lin, 2002), although the exact reasons for this have not been established. Montilla & Calvo (1997) suggested that one factor that affects the heat stability of milk is ionic calcium concentration, although they did not measure it. The heat stability of milk increases by the addition of calcium sequestering agents in the form of mixed phosphate salts. Ionic calcium is higher in goats' milk than cows' milk. Fox & Hoynes (1976) and Horne & Parker (1982) attributed the low stability of goats' milk to their different chemical composition and their different casein profiles. Tziboula (1997) demonstrated that the heat stability of caprine milk is dependent on the casein genotype, with milks having a high content in α_{s1}-casein being of lower heat stability than those having low α_{s1}-casein content. The complexity of the casein fraction is highlighted by the fact that since the 1970s, when it was thought that goats' milk contained no α_{s1}-casein, 13 alleles have been identified in the various amounts of α_{s1}-casein in caprine milk (Ng-Kwai-Hang & Grosclaude, 2003). Guo et al. (1998) suggested that the low ethanol stability of goats' milk may be related to the ratio of sodium to potassium. Morgan et al. (2000) showed that caprine milk is more heat stable when it contains less soluble Ca and more P. The level of citrate was found to be an important parameter that governs the ionic calcium level of goats' milk and therefore its stability towards heat treatment. Citrate is 40% lower than in bovine milk.

Thus overall, any factors which change the negative charge on the micelle (e.g. Ca^{2+}, H^+, Na^+, K^+ and Mg^{2+}), or induce changes in ionic calcium activity, such as differences in phosphates and citrates, or the casein fraction components, will influence heat induced sediment formation.

Where ionic calcium measurement is not available, strategies which increase ethanol stability would be useful to reduce sediment. There is scope for further reducing it by downstream homogenisation and by using different homogenisation pressures, but probably not by direct UHT processing (Ramsey & Swartzel, 1984; Perkin et al. 1973).

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