Comparison of heat stability of goat milk subjected to ultra-high temperature and in-container sterilization

B. Y. Chen, A. S. Grandison, and M. J. Lewis¹

Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading, RG6 6AP, United Kingdom

ABSTRACT

Goat milk with and without stabilizing salt was subjected to in-container and UHT sterilization. Heat stability was assessed by measuring the amount of sediment in the milk. Without stabilizing salts, goat milk usually produced less sediment when subjected to in-container sterilization compared with UHT processing. Addition of stabilizing salts up to 12.8 mM resulted in a progressive increase in sediment for in-container sterilization. In contrast, adding stabilizing salts at 6.4 mM initially reduced sediment formation in UHT-treated milk but addition of stabilizing salts at 12.8 mM increased sediment formation. Adding stabilizing salts to goat milk increased pH, decreased ionic calcium, and increased ethanol stability. Adding up to 2 mM calcium chloride increased sediment formation more after UHT treatment than after in-container sterilization. These results suggest that no single mechanism or set of reactions causes milk to produce sediment during heating and that the favored pathway is different for UHT and in-container sterilization processes. Poor heat stability could be induced both by increasing ionic calcium and by decreasing it. Ethanol stability is not a good indicator of heat stability for in-container sterilization, but it may be for UHT sterilization, if milk does not enter the region of poor heat stability found at low concentrations of ionic calcium.

Key words: stabilizing goat milk, sterilization, stabilizing salts, ionic calcium

INTRODUCTION

After cow milk and buffalo milk, goat milk is ranked third in the world in terms of production, and it is particularly important in developing countries (Guo et al., 1998). It has been reported that goat milk shows poor heat stability compared with cow milk. For cow milk, it was found that if pH was kept above 6.62, only a small amount of sediment formed following UHT

processing, but sediment increased dramatically below this pH value (Zadow, 1978). Compared with that in cow milk, sediment production was greater in goat milk when the pH was <6.9, due to the higher concentration of ionic calcium.

Stabilizing salts are added to milk to improve heat stability. According to Sweetsur and Muir (1980), disodium hydrogen phosphate (**DSHP**) and trisodium citrate (**TSC**) should be used if the natural pH falls on the acidic side of the heat stability maximum, whereas sodium dihydrogen phosphate (**SDHP**) or calcium chloride should be used when the natural pH falls on the alkaline side of the heat stability maximum, with phosphate generally being the most effective stabilizing salt. Tessier and Rose (1958) reported that addition of both potassium phosphate (pH 6.7) and sodium citrate (pH 6.8) decreased ionic calcium. Whereas addition of phosphate precipitates calcium, addition of citrate dissolves colloidal calcium. In addition, Montilla and Calvo (1997) found that a mixture of SDHP, DSHP, and trisodium phosphate increased heat stability without changing the pH of raw goat milk. Use of mixed phosphate salts was reported to be more useful in improving heat stability than pH adjustment by NaOH.

According to Shew (1981), ethanol stability (**ES**) provides a simple way of indicating whether cow milk is suitable for UHT processing, with an ethanol stability of 74% being the threshold below which milk is not suitable. However, goat milk has lower ES than cow milk (White and Davies, 1958; Horne and Parker, 1982; Guo et al., 1998) and is more susceptible to fouling and sedimentation during heat treatment. Horne and Muir (1990) proposed that no correlation exists between ES and heat coagulation time (**HCT**) because of differences in their mechanisms of precipitation. Relatively few HCT studies compare milk samples at their natural pH. Most studies aim to understand the factors that influence the pH dependence of these reactions and explain the maximum and minimum values for HCT that are found for type A milk samples (Singh, 2004). However, a different picture emerges when HCT is measured as a function of pH, where a sigmoidal relationship is found, similar to that shown for ES upon addition of calcium and phosphate (Miller and Sommer, 1940). Boumpa

Received March 15, 2011.

Accepted October 14, 2011.

¹Corresponding author: m.j.lewis@reading.ac.uk

et al. (2008) showed that sediment formation could be improved in UHT-treated goat milk by addition of stabilizing salts. However, no reports exist on the effects of stabilizing salts on in-container sterilization of goat milk, although intuitively one might expect stabilizing salts to improve heat stability.

Therefore, this research was designed to investigate whether ES is a good indicator of heat stability for goat milk subjected to in-container sterilization and UHT treatment.

MATERIALS AND METHODS

Disodium hydrogen phosphate and TSC were added to goat milk at concentrations of 3.2, 6.4, 9.6, and 12.8 mM. These milk samples were then subjected to indirect UHT and in-container sterilization to evaluate their heat stability. Three replicated trials were undertaken using different batches of milk. Three further trials were performed that involved addition of DSHP and TSC (6.4 and 12.8 mM) and calcium chloride ranging from 0.5 to 2.0 mM, together with measurement of average casein micelle size before and after heat treatment.

Milk Samples and Processing Conditions

Pasteurized semi-skimmed goat milk was used for these trials (Delamere Dairies, Knutsford, Cheshire, UK). The milk contained 1.5% fat and was homogenized during the pasteurization process. Samples were subjected to indirect UHT treatment using a tubular UHT plant (UHTAC Cooktube génération 2, Cooktube, Fareins, France) with a capacity of about 30 L/h, described by Omoarukhe et al. (2010). In total, 3 batches of goat milk with different concentrations of stabilizing salts (TSC and DSHP) only and 3 batches with stabilizing salts and calcium chloride were processed using the same conditions of 140°C for 5.6 s over a period from October to March. The samples were collected in sterile pots (250 mL; Bibby Sterilin Ltd., Newport, UK) in a laminar airflow cabinet.

For in-container sterilization, milk samples were placed in baby food cans (125-mL capacity), sealed, and sterilized under static conditions at 120°C for 20 min. The come-up time was 5 min and samples were cooled as quickly as possible at the end of the holding period. All UHT and in-container sterilized samples were stored at 4°C before analysis.

Milk Analysis

Analyses were performed on goat milk before heat treatment and following indirect UHT and in-container

sterilization. Ionic calcium concentration, pH, percentage of dry sediment, and ES were all measured in triplicate at room temperature (20°C).

Ionic calcium was measured using a Ciba Corning 634 ISE Ca^{2+} /pH analyzer (Lin et al. 2006). The instrument was calibrated in the millivolt output mode with solutions of 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, and 3.00 mM Ca^{2+} daily before use. A linear relationship between log (ionic calcium) and mV output was observed, with correlation coefficients >0.99. The pH of the milk samples was measured using a Sentron 3001 pH meter (Sentron, Roden, the Netherlands), which was calibrated with standard buffer solutions of pH 4.0 and 7.0.

Ethanol stability was determined by mixing equal volumes (1 mL) of milk and a range of ethanol solutions, and examining for the presence of clots when poured into a test tube. Ethanol solutions of increasing concentrations were used and the highest concentration of ethanol that did not cause coagulation was defined as the ES.

Dry Sediment

Sediment was measured by a centrifugation method. The milk was well shaken, and approximately 48 mL was accurately weighed and poured into a calibrated tube and centrifuged (Centaur 2, DJB Lab Care, Newport Pagnell, UK) at $2,760 \times g$ for 30 min. After the supernatant was removed, the sediment was oven-dried at 105°C to constant weight to determine its percentage dry weight.

Casein Micelle Size

The average casein micelle size was measured by using the Zeta Master particle size analyzer (Malvern Instruments, Malvern, UK). Milk samples (0.125 mL) were diluted to 25 mL with deionized water. The measurements were done in duplicate for all milk samples. Furthermore, for each of the 2 duplicates, 12 to 16 sub-measurements were performed and the output number was a mean average of the 12 to 16 measurements.

The casein micelle size of goat milk samples was measured before and after addition of stabilizing salts and small additions of calcium chloride (0.5 to 2.0 mM).

Statistical Analysis

Statistical analysis of all data used SPSS 16.0 software (SPSS Inc., Chicago, IL). One-way ANOVA was used to determine whether samples with different amounts of stabilizing salts were significantly different.

RESULTS AND DISCUSSION

Effects of Addition of TSC and DSHP on Properties Related to Heat Stability

Table 1 shows the effects of adding DSHP and TSC on the pH, ionic calcium concentration, and ES of goat milk before and after indirect UHT and in-container sterilization. Overall, addition of DSHP and TSC had similar effects on these properties: they increased pH and ES, but reduced Ca^{2+} concentrations. Goat milk without any added stabilizing salts produced considerably less sediment after in-container sterilization compared with that after UHT processing (Figures 1 and 2).

Effects of UHT Treatment

Following UHT treatment, the amount of sediment was reduced as the concentration of stabilizing salts was increased up to 6.4 mM for TSC and DSHP. However, for both salts, the amount of sediment then increased when addition was increased above 9.6 mM (Figures 1 and 2). Sediment in UHT milk is one manifestation of poor heat stability. According to Boumpta et al. (2008), sediment is formed by aggregation of casein micelles and its formation is prevented in goat milk by a moderate reduction of Ca^{2+} concentration, which will increase the negative charge and make casein micelles less susceptible to aggregation.

The pH decreased by about 0.05 pH units after indirect UHT (Table 1). The UHT treatment also decreased

Figure 1. The relationship between dry sediment formation (% dry weight basis, %dwb) and disodium hydrogen phosphate (DSHP) concentration following UHT and in-container sterilization for 3 fully replicated trials. Error bars are standard deviations.

Table 1. The effect of addition of disodium hydrogen phosphate (DSHP) and trisodium citrate (TSC) on pH, ionic calcium concentration, and ethanol stability (ES) before and after indirect UHT and in-container sterilization treatment¹

Addition and concentration (mM)	pH	Ca ²⁺ (mM)	ES (%)	Indirect UHT		In-container sterilization		dpH (UHT) ²	dpH (Ster) ³
				pH	Ca ²⁺	pH	Ca ²⁺		
DSHP									
0	6.70 ± 0.10 ^a	1.76 ± 0.23 ^a	62 ± 13.53 ^a	6.65 ± 0.11 ^a	1.57 ± 0.26 ^a	6.40 ± 0.07 ^a	1.39 ± 0.32 ^a	−0.05 ± 0.06	−0.31 ± 0.05
3.2	6.77 ± 0.12 ^{ab}	1.24 ± 0.17 ^b	79 ± 2.00 ^b	6.73 ± 0.16 ^a	1.16 ± 0.15 ^b	6.42 ± 0.07 ^a	0.95 ± 0.18 ^b	−0.04 ± 0.10	−0.35 ± 0.06
6.4	6.82 ± 0.11 ^{ab}	0.99 ± 0.18 ^{bc}	84 ± 4.36 ^b	6.78 ± 0.17 ^a	0.89 ± 0.16 ^b	6.45 ± 0.07 ^a	0.62 ± 0.14 ^{bc}	−0.04 ± 0.11	−0.38 ± 0.06
9.6	6.89 ± 0.07 ^{ab}	0.77 ± 0.15 ^{cd}	85 ± 4.16 ^b	6.84 ± 0.13 ^a	0.70 ± 0.12 ^b	6.48 ± 0.05 ^a	0.42 ± 0.15 ^c	−0.05 ± 0.08	−0.41 ± 0.03
12.8	6.93 ± 0.10 ^b	0.65 ± 0.17 ^d	87 ± 4.36 ^b	6.90 ± 0.15 ^a	0.59 ± 0.15 ^b	6.51 ± 0.07 ^a	0.29 ± 0.13 ^c	−0.04 ± 0.10	−0.42 ± 0.04
TSC									
0	6.71 ± 0.09 ^a	2.04 ± 0.41 ^a	56 ± 7.64 ^a	6.65 ± 0.11 ^a	1.85 ± 0.42 ^a	6.43 ± 0.03 ^a	1.62 ± 0.26 ^a	−0.07 ± 0.04	−0.28 ± 0.07
3.2	6.81 ± 0.08 ^{ab}	1.47 ± 0.32 ^b	77 ± 0.58 ^b	6.74 ± 0.11 ^{ab}	1.37 ± 0.26 ^b	6.53 ± 0.04 ^b	1.14 ± 0.13 ^b	−0.07 ± 0.03	−0.28 ± 0.06
6.4	6.90 ± 0.09 ^{bc}	1.11 ± 0.16 ^{bc}	85 ± 1.15 ^c	6.83 ± 0.11 ^{abc}	1.03 ± 0.15 ^{bc}	6.60 ± 0.06 ^{bc}	0.84 ± 0.07 ^c	−0.07 ± 0.03	−0.30 ± 0.06
9.6	6.98 ± 0.10 ^{bc}	0.91 ± 0.12 ^c	87 ± 0.58 ^c	6.91 ± 0.11 ^{bc}	0.83 ± 0.05 ^c	6.65 ± 0.07 ^c	0.65 ± 0.04 ^{cd}	−0.07 ± 0.03	−0.33 ± 0.06
12.8	7.05 ± 0.09 ^c	0.75 ± 0.07 ^c	91 ± 3.06 ^c	6.99 ± 0.15 ^c	0.69 ± 0.02 ^c	6.69 ± 0.07 ^c	0.51 ± 0.02 ^d	−0.05 ± 0.07	−0.35 ± 0.05

^{a-d}Means ($\pm$ SD) within a column and within each property followed by the same letter are not significantly different ($P > 0.05$).

¹Results were the average of 3 replicated trials.

²dpH (UHT) = the difference in pH between the before heat treatment and after indirect UHT treatment.

³dpH (Ster) = the difference in pH between the before heat treatment and after in-container sterilization treatment.

Figure 2. The relationship between dry sediment formation and trisodium citrate (TSC) concentration following UHT and in-container sterilization for 3 fully replicated trials. Error bars are standard deviations.

Ca^{2+} in goat milk by about 10%, which is in line with the observations of Geerts et al. (1983) for cow milk. Higher additions of stabilizing salts reduced Ca^{2+} substantially. For example, goat milk with 9.6 mM DSHP had an Ca^{2+} concentration before UHT treatment of 0.77 mM, compared with 1.76 mM without stabilizing salt. Boumpa et al. (2008) observed that, in most cases, addition of stabilizing salt reduced sediment in UHT-treated goat milk, but addition of sodium hexametaphosphate caused an increase in sediment at its highest concentration, corresponding to the greatest decrease in Ca^{2+} . The authors did not explain this finding.

As stabilizing salt concentration increased, ES increased (Table 1). The lower Ca^{2+} and higher pH both contribute toward this increase in ES. This suggests that ES is a good predictor of heat stability with addition of stabilizing salts up to 6.4 mM, where its value is 84%, but above this value it may no longer be a good predictor. Therefore, one practical suggestion for ensuring that sediment in UHT-treated goat milk is minimized is to add only sufficient stabilizing salt to increase the ES value to no more than 84%. During these UHT experiments, the temperature in the holding tube fluctuated by no more than 2°C and no sudden increases occurred in back pressure in the equipment, giving no indication of fouling, although the processing time for each run was short.

Effects of In-Container Sterilization

Compared with indirect UHT treatment, in-container sterilization showed just one trend: sediment formation increased as the concentration of TSC or

DSHP increased (Figures 1 and 2). The average dry sediment percentage increased from 0.35 to 4.83% for DSHP and from 0.33 to 4.42% for TSC. In addition, for in-container sterilization, addition of stabilizing salt increased sediment formation, even though Ca^{2+} had decreased. This has not been reported in the literature previously for sterilization of goat milk, although most reports have dealt with UHT processing rather than in-container sterilization. However, a similar observation was reported for cow milk that had been sterilized in the container and stored for 6 mo (Tsioulpas et al., 2010). In contrast to UHT processing, in-container sterilization of goat milk caused a much greater decrease in pH, which averaged 0.31 pH units. This situation has also been reported by Fox and McSweeney (2003) and more recently by Tsioulpas et al. (2010) for cow milk.

For in-container sterilization, sediment formation increased as ES increased (Table 1), which has not been previously reported. For UHT processing, increasing ES is reported to improve heat stability (Shew, 1981; Lewis et al., 2011).

Comparison Between TSC and DSHP

Overall, TSC showed effects similar to DSHP in terms of Ca^{2+} , pH, ES, and trends for sediment formation. It can be seen that DSHP was slightly less effective than TSC for reducing sediment in indirect UHT treatment (Figures 1 and 2). Tessier and Rose (1958) reported some differences between these 2 stabilizing salts; phosphate addition precipitated calcium, whereas citrate dissolved colloidal calcium. However, addition of both and phosphate and citrate decreased Ca^{2+} . Udabage et al. (2000) found that addition of calcium and mixtures of SDHP and DSHP increased micellar calcium phosphate and casein, whereas addition of citrate and EDTA had the opposite effects. All these stabilizing salts were reported to reduce Ca^{2+} activity, but no effects on heat stability or ES were investigated. On the other hand, addition of both TSC and DSHP increased sediment formation during in-container sterilization treatment, which suggested that reducing Ca^{2+} too much might result in a decrease in heat stability. Because this was an interesting finding, we investigated it in more detail.

Effects of Heat Treatment on Sediment Formation and Casein Micelle Size Following Additions of Stabilizing Salts and Calcium Chloride

Three additional replicated experiments were performed to measure casein micelle size following additions of TSC and DSHP and minor additions of calcium chloride to goat's milk. Addition of these compounds

tended to increase micelle size slightly (Figure 3). However, for all samples, micelle size further increased substantially on heat treatment. Tsioulpas et al. (2010) found similar results for cow milks, demonstrating that addition of these stabilizing salts increased micelle size only slightly, but the measured micelle size increased considerably after sterilization. It was found that when calcium chloride was added, UHT treatment caused a much greater increase in measured micelle size than in-container sterilization. In contrast, for addition of TSC and DSHP, a greater increase in measured casein micelle size occurred following in-container sterilization compared with UHT processing. The trends regarding how the amount of sediment changed as a result of adding stabilizing salts were similar to those found in the previous experiment (Figure 4). The data confirm the proposition that excessive sediment forms because of a heat-induced increase in the measured casein micelle size. It is well established that heating milk causes denaturation of whey proteins, especially β -lactoglobulin. According to Lewis and Heppell (2000), UHT processing will partially denature whey proteins, from about 40% to >90%. In contrast, after in-container sterilization, whey protein denaturation should be closer to 100%, as measured by the turbidity test (Lewis and Heppell, 2000). However, the significant changes in particle size induced by in-container sterilization are unlikely to be because of the deposition of denatured whey proteins on casein micelles, as discussed by Jeurnink and de Kruif (1995) and Mohammad and Fox (1987). A more likely explanation is that casein micelles, especially smaller ones, aggregate (Ono et al., 1999) or that further association of whey-casein aggregates occurs. If these aggregates are formed through a calcium-mediated mechanism (O'Connell and Fox, 2001), it could also explain the decrease in Ca^{2+} found after in-container sterilization. We did not expect to observe the increase in sediment with increasing addition of stabilizing salt during in-container sterilization. This could be explained by a heat-induced increase in casein micelle size. According to Anema and Li (2003), Corredig and Dalgleish (1996), and Law et al. (1994), in-container sterilization caused a 2- to 3-fold increase in particle size compared with UHT treatment, which resulted in only a slight increase in micelle size. This was different from what has been found in our studies.

General Discussion

Sediment formation provided a robust way of measuring heat stability during in-container sterilization, because standard deviations were small for replicated determinations of the same sample. However, when different milk samples were compared, comparatively high

Figure 3. The relationship between casein micelle size and addition of both stabilizing salts (disodium hydrogen phosphate, DSHP, and trisodium citrate, TSC) and calcium chloride before and after sterilization for 3 fully replicated trials. Error bars are standard deviations.

standard deviations were found for the control milk and some of the higher additions of stabilizing salts. This was thought to be due to differences in heat stability for the different batches of milk used in these experiments, which were conducted from October through March. For example, in 5 out of 6 batches of milk, more sediment was found for the control milk following UHT processing than following in-container sterilization.

Figure 4. The relationship between dry sediment formation and addition of both stabilizing salts (disodium hydrogen phosphate, DSHP, and trisodium citrate, TSC) and calcium chloride after sterilization for 3 fully replicated trials. Error bars are standard deviations.

From the differences observed in trends for sediment in indirect UHT and in-container sterilization, we propose that 2 situations (regions) exist where heat stability is poor. One is at higher levels of Ca^{2+} and the other at lower levels of Ca^{2+} . Our results suggest that the transition thresholds for these regions are different for UHT treatment and for in-container sterilization. When adding stabilizing salts, conditions that correspond to thresholds for entering the lower heat stability region are encountered. In this region, problems were observed sooner for in-container sterilization compared with UHT processing (Figures 1 and 2). This situation arises because of the considerable increase in measured casein micelle size during in-container sterilization. The existence of this lower heat stability region has not been specifically noted previously, because it is not intuitive that reducing Ca^{2+} would decrease heat stability. However, some evidence is found in the literature that this may be the case. For example, Tsioulpas et al. (2010) found that the amount of sediment (measured after 6 mo of storage) increased as Ca^{2+} in the milk before in-container sterilization decreased. A good correlation between sediment formation and measured casein micelle size was found ($R^2 = 0.76$).

We also propose that the heat stability problems encountered at higher concentrations of Ca^{2+} are more likely to be found for UHT treatment than for in-container sterilization. This was shown to be the case for the control milk samples and when adding small amounts of calcium chloride (Figures 3 and 4). However, addition of 6.4 mM stabilizing salts was sufficient to shift the milk into the stable region for UHT processing (sediment decreased) but toward the lower instability region for in-container sterilization (sediment increased). Addition of 12.8 mM stabilizing salts shifted the milk well into the lower instability region for in-container sterilization, but just over the threshold of instability for UHT processing. Omoaruhke et al. (2010) found that cow milk with added calcium was more stable to in-container sterilization than to UHT treatment, which provides further evidence for differences in susceptibility of UHT and in-container sterilization processes in this higher instability region.

One important difference between UHT treatment and in-container sterilization arises from the higher temperatures and greater heating and cooling rates for UHT processes. During both sterilization processes, milk pH will decrease, probably falling well below 6.0 (Walstra and Jenness, 1984). This might be expected to increase Ca^{2+} , but the effect of increasing acidity is more than counteracted by the decrease in calcium phosphate solubility, so Ca^{2+} also decreases as temperature increases (Chandrapala et al., 2010; On-Nom et al., 2010). It is likely that milk will reach a lower pH during

UHT processing than during in-container sterilization, although it is less clear how quickly pH and Ca^{2+} change with temperature. How much the pH will decrease when temperature increases is influenced by the buffering capacity of the milk. Salaun et al. (2005) reviewed the factors affecting buffering capacity but did not discuss how buffering capacity affects milk stability. Therefore, a higher milk buffering capacity would be subject to a smaller decrease in pH and thus, perhaps, would be less susceptible to sediment formation. This type of mechanism has also been attributed to the role of urea in improving heat stability (Metwalli and Boekel, 1996). Although the mechanisms are discussed in terms of Ca^{2+} reduction, it is likely that the processes that give rise to a reduction in Ca^{2+} also alter conditions within the vicinity of the micelle surface, making micelles more susceptible to coagulation. Therefore, the different heat stability mechanisms in indirect UHT and in-container sterilization need further investigation, especially with regard to understanding of the role of pH and Ca^{2+} values that would be found in milk at the sterilization temperatures.

Another difference between these sterilization procedures is in agitation and turbulence. In these trials, a static in-container sterilization procedure was used, so heat was transferred by natural convection, whereas in UHT processing, heat transfer was by forced convection.

Chavez et al. (2002) proposed that the alcohol stability test would be useful to predict the heat stability of milk, as it provided an easy method to evaluate this property. However, in the current study, we showed that the alcohol stability test is not a good method for predicting heat stability for in-container sterilization. The alcohol stability test also needs to be treated with caution for UHT treatment, to avoid moving the milk into the lower heat stability region.

CONCLUSIONS

Heat stability of milk in commercial sterilization processes can be reduced both by increasing and by decreasing Ca^{2+} . Within the region of good heat stability, sterilization increases casein micelle size, but micelle size is prevented from increasing substantially, whereas outside this stable region micelle size does increase substantially. Ethanol stability is useful for predicting heat stability for indirect UHT treatment, although addition of stabilizing salts should not increase ES to >84%. For in-container sterilization processing, sediment formation increased as ES increased, which is counterintuitive. Results suggest that TSC and DSHP are equally effective when added at optimum concentrations, which for these milk samples was found to be about 6.4 mM

for UHT processing; above this optimum, TSC caused less sediment than DSHP.

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