Goat's Milk Stability during Heat Treatment: Effect of pH and Phosphates

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Goat's milk stability during heat treatments is lower than that of cow's milk. We have studied the influence of two factors, pH and phosphates addition, on goat's milk protein denaturation, final pH, and stability of samples submitted to direct or indirect UHT (ultrahigh treatment). Samples at pH 6.7 heated at 140 °C and samples at pH 6.9 or higher and heated at 145 °C using the direct UHT method coagulated. Significant differences in α-lactalbumin and β-lactoglobulin denaturation were found between milk samples adjusted to different pHs and submitted to different temperatures. No coagulation was found in samples with added phosphates. Significant differences in whey protein denaturation where found on milk samples with 0.3 or 0.5 g/L phosphates, and a significant increase in α-lactalbumin denaturation was found between the different temperatures assayed. Our results show that the changes in the pH or the addition of phosphates had little effect in the whey protein denaturation, but it affected the heat stability of goat's milk. Samples heated at 135−150 °C using a UHT indirect method showed, in general, a higher stability for the milk samples with added phosphates than those adjusted to alkaline pH.

Keywords: *pH; phosphates; goat's milk; heat stability*

INTRODUCTION

Milk production from dairy goats constitutes an economic activity of increasing importance. When goat's milk is obtained following proper milking practices, no differences in the bacteriological qualities of cow's and goat's milk are found (Calisay *et al.*, 1983). As for cow's milk, goat's milk must be submitted to thermal treatment before further utilization as fluid milk, manufacturing yogurt and cheese, etc. Although the consumption of liquid goat's milk is small, the potential market is large since goat's milk is a good substitute for cow's milk when bovine milk proteins bring about allergic responses in consumers (Zadow *et al.*, 1983).

There appears to be little information in the literature concerning the stability of goat's milk to UHT (ultrahigh treatment) processing. However, a number of studies on the stability of cow's milk toward UHT processing have been reported (Newstead *et al.*, 1975; Darling, 1980; Sweetsur and Muir, 1980), as well as information about factors affecting the heat stability of cow's milk such as pH (Rose, 1961; Darling, 1980), ionic calcium (Puri *et al.*, 1969; Nieuwenhuise *et al.*, 1988), colloidal calcium phosphate (Nieuwenhuijse *et al.*, 1988), whey proteins (Koning *et al.*, 1974; Kelly, 1982), or caseins (Abdulina and Kovalengo, 1971; Koning, 1974; Miyabe and Tomoi, 1985).

One of the most important factors on the stability of milk is the pH. Zadow *et al.* (1983) showed that extensive sedimentation occurred if the pH of the milk was below 6.6; above pH 6.7 very little sedimentation occurred, but at pH 6.4 UHT processing of cow's milk results in rapid sedimentation of more than 90% of the casein and 40% of the whey proteins in milk. Sweetsur and Muir (1980) reassessed the effect of stabilizers on the heat stability of cow's milk, showing that their primary effect was a consequence of their influence on the pH of milk. It is suggested that Na_2HPO_4, Na_3PO_4, Na_3 citrate, and $NaHCO_3$ should be used if the natural pH of milk falls on the acid side of the heat stability maximum, while NaH_2PO_4 or $CaCl_2$ should be used if the natural pH is alkaline to the maximum, with orthophosphates being generally the most effective stabilizers. Newstead *et al.* (1975) and Dalgleish *et al.* (1987) have also recommended the use of stabilizing salts rather than inorganic acids for pH adjustment of milk.

The UHT treatment of goat's milk under the conditions in which cow's milk is treated is not possible, since goat's milk heat stability is lower than that of cow's milk. Zadow *et al.* (1983) observed that if the initial pH of goat's milk subjected to heat treatment at 140 °C for 3 s is below 6.9, milk sedimentation occurs in the product. They reported that the problem can be controlled by either adjustment of the pH of the milk to about 7.0 or by addition of 2 g/L disodium phosphate to the milk before processing. Fox and Hoynes (1976) found that at the pH of maximum stability goat's milk, although quite variable, had stabilities in the same range as cow's milk.

Although there is little information about the effect of the stabilizing agents on heat stability of goat's milk, some authors have reported the stabilizing effect of phosphate addition on buffalo's milk. Prasad and Balanchandran (1987) working with different stabilizers found that the best results were obtained adding Na_2HPO_4. Puri *et al.* (1969) and Sindhu (1985) reported that the addition of phosphates to buffalo's milk markedly increased the heat stability.

In spite of the possible importance of protein denaturation on the heat stability of goat's milk, there are few results on the changes of whey proteins during heating. Ramos (1978) reported 2% or 3% of denatured protein after heat treatment at 65 °C for 30 min. Khandelwal and Gupta (1980) found that whey proteins were denatured at higher than 60 °C and β-lactoglobulin was completely denatured at 80 °C. Calvo *et al.* (1989) found only a 20% denaturation of β-lactoglobulin and a

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3% denaturation of α-lactalbumin during heating at 90 °C for 30 s.

The aim of this paper was to study the heat stability of goat's milk with different amounts of added phosphates or different values of pH. The changes in proteins during the heat treatments were analyzed.

MATERIALS AND METHODS

Samples. Raw, bulk goat's milk from different herds from the central region of Spain were used. Milk pH was adjusted to pH 6.7−7.2 by addition of 1 M NaOH; 10 g of commercial phosphates Turrixin ST (Giulini Chemie GmbH, Ludwigshafen/Rhein, Germany) containing NaH_2PO_4, Na_2HPO_4, Na_3PO_4, and the presence of total heavy metals as Pb maximum 20 ppm was dissolved in 1 L of raw goat's milk. This solution was added to raw goat's milk until a final concentration of 0−0.9 g/L. All the experiments were performed with five different goat's milk samples from the same source.

Heat Treatments. Direct and indirect UHT treatments were performed. A UHT direct laboratory plant (Armfield, U.K.) was used for direct treatments. Milk (100 mL) was heated at 135−145 °C for 20 s.

Indirect UHT treatments were performed by heating milk (1 mL) at 135−150 °C for 3−15 s in a silicon oil bath in sealed glass-capillary thin-walled capillaries (1 m × 1.1 mm i.d.). After the heating period, samples were immediately cooled in a ice−water bath and kept at 7 °C until analysis.

Whey Protein Analysis. The whey proteins not precipitated were obtained by acidifying milk to pH 4.6 with 2 M HCl and centrifugation. Soluble whey proteins were analyzed by gel permeation. The samples were fractionated by gel permeation FPLC at 20 °C on a Superdex 75 HR 10/30 column (30 cm × 10 mm) (Pharmacia, Spain) in Tris-HCl buffer (pH 7.0, 100 mM Tris, 0.5 M NaCl) at a flow rate of 0.5 mL/min. The absorbance of the eluate was monitored at 280 nm, and a total volume of 26 mL of buffer was passed through the column to ensure complete elution of absorbing material. The level of denaturation of each protein was calculated as the decrease in area of each peak, expressed as percentage of the area of the corresponding peak from raw milk.

Statistical Analysis. Analysis of variance was applied using the BMDP2V program (1981) with a CDC Ciber 188/855 computer, to test the influence of the pH of the milk, the concentration of phosphates, and the intensity of the heat treatment on whey protein denaturation and final pH values in milk samples.

RESULTS

Influence of pH and Phosphates Addition on the Heat Stability of Goat's Milk Submitted to Direct UHT Treatment. To study the effect of pH during heating of goat's milk, five different samples were adjusted to pH 6.7−7.2 and then heated at 135, 140, or 145 °C for 20 s. As can be observed in Table 1, milk samples at pH 6.7 heated at 140 °C and samples at pH 6.9 heated at 145 °C coagulated.

The percentage of denatured α-lactalbumin in samples heated at different temperatures and pH values and the analysis of variance are shown in Table 1. The percentage of denatured α-lactalbumin in samples heated at 135 °C was significantly different ($P < 0.05$) between those with pH up to 6.9 and those at pH 7.0 or 7.2. The stability decreased with increasing whey protein denaturation, and we found a increase in whey protein denaturation at pH 7.0 and 7.2 (Table 1). Significant influences ($P < 0.05$) of the pH on denaturation of α-lactalbumin were found on samples heated 145 °C: denaturation decreased with increasing pH. Some samples adjusted to the same pH values showed a significant increase ($P < 0.05$) in whey protein denaturation with an increase in heating.

Table 1. Effects of Heating Goat's Milk Samples at 135−145 °C for 20 s in a Direct Heating Plant at Different Initial pH Values on the Percentage of Whey Protein Denaturation

whey protein	initial pH of milk	% of denatured whey protein 135 °C	140 °C	145 °C
α-lactalbumin	6.7	17.5^{a} (1.90)[a]	coagulated	coagulated
	6.8	18.3^{Aa} (0.78)	35.4^{Ba} (5.55)	coagulated
	6.9	20.5^{Aac} (4.09)	37.9^{Ba} (3.85)	coagulated
	7.0	24.1^{Abc} (3.79)	41.2^{Ba} (5.00)	46.3^{Ba} (2.55)
	7.2	23.4^{Abc} (1.13)	36.4^{Ba} (2.28)	38.0^{Bb} (2.91)
β-lactoglobulin	6.7	70.5^{d} (3.81)	coagulated	coagulated
	6.8	75.6^{Ad} (2.98)	80.5^{Ad} (2.10)	coagulated
	6.9	74.1^{Ad} (6.33)	81.6^{Ad} (4.22)	coagulated
	7.0	72.2^{Ad} (1.53)	82.1^{Bd} (3.57)	89.6^{Cd} (3.09)
	7.2	72.4^{Ad} (2.93)	81.9^{Bd} (3.29)	83.8^{Be} (1.72)

[a] Mean (standard deviation), $n = 5$. Means labeled a−c within columns without a common superscript are significantly different ($P < 0.05$) for α-lactalbumin determination. Means labeled d or e within columns without a common superscript are significantly different ($P < 0.05$) for β-lactoglogulin determination. Means labeled A−C within rows without a common superscript are significantly different ($P < 0.05$).

Table 2. Influence of Goat's Milk Samples Adjusted at pH 6.7−7.2 and Heated at 135, 140, and 145 °C for 20 s on the Final pH Value

initial pH	pH value (deviation from initial pH) 135 °C	140 °C	145 °C
6.7	−0.140 (0.015)[a]	coagulated	coagulated
6.8	0.028^{A} (0.010)	0.037^{A} (0.009)	coagulated
6.9	0.054^{A} (0.444)	-0.036^{A} (0.118)	coagulated
7.0	0.182^{A} (0.134)	0.098^{B} (0.030)	0.060^{C} (0.008)
7.2	0.058^{A} (0.013)	-0.005^{B} (0.019)	0.038^{A} (0.043)

[a] Mean (standard deviation), $n = 5$. Means labeled A−C within rows without a common superscript are significantly different ($P < 0.05$).

No significant changes in β-lactoglobulin denaturation was observed between milk samples heated at 135 or 140 °C, but there was a significant ($P < 0.05$) decrease in β-lactoglobulin denaturation of milk at pH 7.2 and heated at 145 °C. The analysis of variance also shows that at pH 7.0 or 7.2 there were significant ($P < 0.05$) differences between samples heated at 135 or 140 °C; however, the differences were not significant ($P < 0.05$) on samples heated at 140 or 145 °C.

Table 2 shows the pH of goat's milk after heating. Slight differences on the final pH value were found, and significant differences ($P < 0.05$) between temperatures only were observed on samples at pH 7.0 or 7.2.

The denaturation of whey proteins was also affected by phosphates addition. Milk samples with 0.3 g/L phosphates did not coagulate during heat treatment at 145 °C. The mean of α-lactalbumin and β-lactoglobulin denaturation in samples with different added phosphates concentration and the analysis of variance are shown in Table 3. A significant ($P < 0.05$) influence of heating in α-lactalbumin denaturation was found on samples with 0.5 g/L added phophates: the denaturation increased with increasing heating temperature.

β-Lactoglobulin was more denatured than α-lactalbumin. The denaturation of this protein was slightly

Table 3. Effect of Added Phosphates on Whey Protein Denaturation in Goat's Milk (pH 6.7) Heated at 135, 140, and 145 °C for 20 s in a Direct Heating Plant

	denatured whey proteins (%)			
	α-lactalbumin		β-lactoglobulin	
temperature (°C)	0.3[a]	0.5	0.3	0.5
135	21.7[Aa] (3.96)[b]	6.75[Ba] (0.62)	69.42[F] (4.72)	67.20[Ff] (3.52)
140	24.87[Aa] (3.96)	10.16[Bb] (1.62)	70.01[Ff] (4.09)	72.48[Fg] (2.81)
145	26.37[Aa] (3.05)	18.74[Bc] (3.29)	73.23[Ff] (5.18)	73.15[Fg] (2.20)

[a] Phosphates concentration (NaH_2PO_4, Na_2HPO_4, Na_3PO_4) (g/L). [b] Mean (standard deviation), $n = 5$. Means labeled a−c within columns without a common superscript are significantly different ($P < 0.05$) for α-lactalbumin determinations. Means labeled f−g within columns without a common superscript are significantly different ($P < 0.05$) for β-lactoglobulin determinations. Means labeled A or B within rows without a common superscript are significantly different ($P < 0.05$) for α-lactalbumin determinations. Means labeled F within rows without a common superscript are significantly different ($P < 0.05$) for β-lactoglobulin determinations.

affected by phosphates concentration at the three temperatures assayed. A slight influence of temperature in β-lactoglobulin denaturation was found.

No significant changes in pH were found on heating milk with 0.3 or 0.5 g/L phosphates. The samples with added phosphates showed a mean value of the pH of 6.93, a standard deviation of 0.052, a standard error of 0.006, a maximun value of 7.03, and minimum value of 6.81. Whey proteins from samples with added phosphates were less denatured than those with the pH adjusted; see Tables 1 and 3.

Influence of pH and Phosphates Addition on the Heat Stability of Goat's Milk Submitted to Indirect UHT Treatment. To study the effect of pH and phosphates on indirect UHT treatment, capillaries were heated to 135, 140, 145, and 150 °C for 3−15 s and immediately cooled. The coagulation was observed on the cooled capillaries. Five different milk samples were heated at the different temperatures and times. The results are shown in Figures 1 and 2. The heat treatments at 135 °C up to 15 s showed similar results in all the samples (panels A and B). However, for samples heated at 140 or 145 °C, a lower number of samples with added phosphates coagulated than with the pH adjusted. This effect was higher on samples heated longer. Only slight differences between the two compared treatments of the milk were found on samples heated at 150 °C.

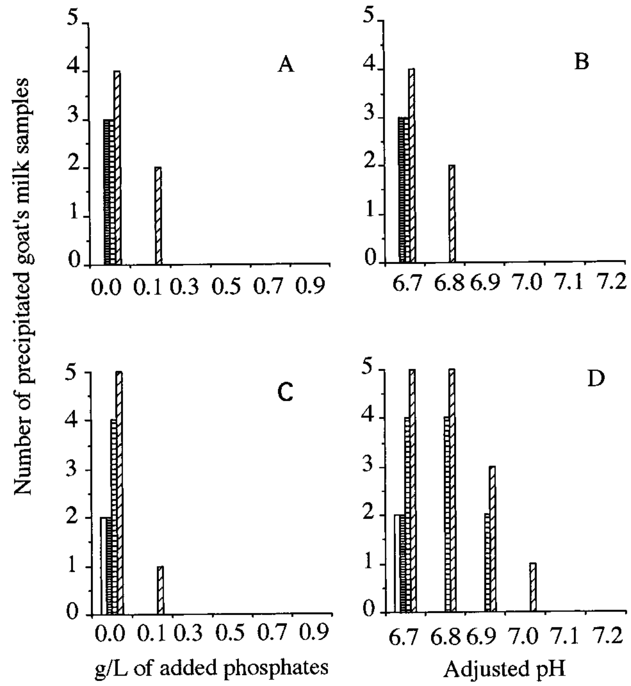

Figure 1. Number of samples precipitated after heat treatment of goat's mik at 135 °C (A and B) or 140 °C (C and D) for 3 (solid bar), 5 (open bar), 7 (densely striped bar), 10 (striped bar), or 15 (slashed bar) s. Milk samples A and C contained added phosphates until a final concentration of 0.0, 0.1, 0.3, 0.5, 0.7, and 0.9 g/L, and milk samples B and D were adjusted to pH 6.7, 6.8, 6.9, 7.0, and 7.2.

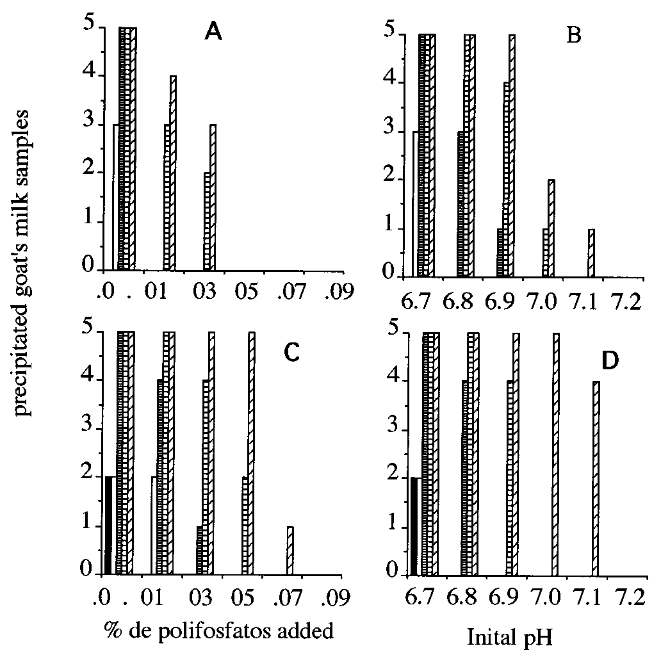

Figure 2. Number of samples precipitated after heat treatment of goat's mik at 145 °C (A and B) or 150 °C (C and D) for 3 (solid bar), 5 (open bar), 7 (densely striped bar), 10 (striped bar), or 15 (slashed bar) s. Milk samples A and C contained added phosphates until a final concentration of 0.0, 0.1, 0.3, 0.5, 0.7, and 0.9 g/L, and milk samples B and D were adjusted to pH 6.7, 6.8, 6.9, 7.0, and 7.2.

DISCUSSION

There are few results on the effect of pH on the heat stability of goat's milk. Fox and Hoynes (1976) reported that at a pH of maximum stability ewe's and goat's milks, although quite variable, had the same range as bovine milks. However, Zadow *et al.* (1983) indicated that goat's milk at a given pH has a reduced stability toward UHT processing compared with cow's milk. Fox and Hoynes (1976) and Zadow *et al.* (1983) reported similar results to those found by us with goat's milk submitted to indirect UHT treatments: the heat stability of goat's milk increased with increased pH value.

The influence of whey proteins on heat stability of cow's milk has also been studied. Rose (1961) showed that the addition of increasing amounts of β-lactoglobulin decreased stability in the alkaline pH values; other authors (Koning *et al.*, 1974; Fox and Hoynes, 1976) have suggested that the β-lactoglobulin−κ-casein complex influences the shape of the heat stability/pH curve. Fox and Hearn (1978) found that the heat stability of milk can be modified by other proteins such as α-lactalbumin.

As can be observed in the results β-lactoglobulin was more denatured on heating goat's milk than α-lactalbumin was, as observed in cow's milk (Larson and Rolleri, 1955). On the other hand, our results show that the changes in the pH or the concentration of phosphates added had a slight influence on the whey protein denaturation. These results could indicate that the influence of pH and phosphates on heat stability is not due to the changes on the whey protein denaturation; these factors could act on the salt balance, which influences the thermal stability.

It is known that one factor that affects the heat stability of milk is the ionic calcium concentration; the heat stability increases by the addition of calcium-sequestering agents in the milk. Zadow *et al.* (1983) found that at a given pH the goat's milk had a substantially higher ionic calcium content than cow's milk. The same authors found that the addition of a 0.2% (w/w) solution of NaH_2PO_4 to raw goat's milk reduced the ionic calcium content. We found that the addition of a mixture of NaH_2PO_4, Na_2HPO_4, and Na_3PO_4 did not change the pH of the raw milk; however, the addition of this mixture increased the heat stability of goat's milk. This increase of the stability could be due to changes on the ionic calcium and the equilibrium of the micellar calcium phosphate. The phosphates could also influence the casein structure. Abduline and Kovalenko (1971) reported an electrostatic interaction of the phosphates with casein and the formation of a complex between the phosphates and caseins during heating.

LITERATURE CITED

Abdulina, T. N.; Kovalenko, M. S. Study of the interaction of milk casein and phosphates. *Izv. Vyssh. Uchebn. Zaved. Pishch. Tekhnol.* **1971,** *4,* 24−26.

Calisay, O. G.; Barraquio, V. L.; Velasco, N. B. Acceptability and keeping quality of laboratory pasteurized goat's milk. *Philippine Agric.* **1983,** *66,* 75−83.

Calvo, M. M.; Amigo, L.; Olano, A.; Martín, P. J.; Ramos, M. Effect of thermal treatments on the determination of bovine milk add to ovine or caprine milk. *Food Chem.* **1989,** *32,* 99−108.

Dalgleish, D. G.; Pouliot, Y.; Paquin, P. Studies on the heat stability of milk. I Behaviour of divalent cations and phosphate in milks heated in a stainless steel system. *J. Dairy Res.* **1987,** *54,* 29−37.

Darling, D. F. Heat stability of milk. *J. Dairy Res.* **1980,** *47,* 199−210.

Fox, P. F.; Hoynes, M. C. T. Heat stability characteristics of ovine, caprine and equine milks. *J. Dairy Res.* **1976**, *43,* 433−442.

Fox, P. F.; Hearn, C. M. Heat stability of milk: influence of denaturable proteins on pH sensitivity. *J. Dairy Res.* **1978,** *45,* 159−172.

Kelly, P. M. Effect of ion-exchange removal of whey protein on the heat stability of skim milk. *Ir. J. Food Sci. Technol.* **1982**, *6,* 91−96.

Khandelwal, O. P.; Gupta, M. P. Thermal denaturation of goat milk whey proteins. *Indian J. Dairy Sci.* **1980**, *33,* 13−16.

Konning, P. J. de; Kooops, J.; Rooijen, P. J. Van. Some features of the heat stability of concentrated milk. III. Seasonal effects on the amounts of casein, individual whey proteins and NPN and their relation to various in heat stability. *Neth. Milk Dairy J.* **1974,** *28,* 186−202.

Larson, B. L.; Rolleri, G. D. Heat denaturation of specific serum proteins in milk. *J. Dairy Sci.* **1955,** *38,* 351−360.

Miyabe, T.; Tomoi, K. Heat stability of bovine milk: the relationship between calcium salts and gamma-casein A in casein micelles. *J. Agric. Chem. Soc. Jpn.* **1985**, *59,* 519−522.

Newstead, D. F.; Sanderson, W. B.; Baucke, A. G. The effects of heat treatment and pH on the heat stability of recombined evaporated milk. *N. Z. J. Dairy Sci. Technol.* **1975**, *10,* 113−118.

Nieuwenhuijse, J. A.; Timmermans, W.; Walstra, P. Calcium and phosphate partitions during the manufacture of sterilized concentrated milk and their relation to the heat stability. *Neth. Milk Dairy J.* **1988**, *42,* 387−421.

Prasad, C.; Balachandran, R. Effect of chemical additives on pH and heat stability of buffalo milk at different levels of concentration. *N. Z. J. Dairy Sci. Technol.* **1987,** *22,* 123−130.

Puri, B. R.; Arora, K.; Toteja, K. K. Studies in stability of protein dispersions in milk. V. Effect of altering calcium content, replacing calcium by other cations in caseinate complex and other factors on heat stability of milk. *Indian J. Dairy Sci.* **1969,** *22,* 85−91.

Ramos, M. Effects of heating on the nitrogen fractions of ewe's, goat's and cow's milk. Proceedings of the XX International Dairy Congress, Paris, 1978; pp 626−627.

Rose, D. J. Factors affecting the pH-sensitivity of the heat stability of milk from individual cows. *J. Dairy Sci.* **1961,** *44,* 1405−1413.

Sindhu, J. S. Influence of sodium phosphate on the heat stability of buffalo milk and its concentrate. *J. Food Process. Preserv.* **1985,** *9,* 57−64.

Sweetsur, A. W. M.; Muir, D. D. The use of permitted additives and heat-treatment to optimize the heat stability of skim milk and concentrated skim milk. *J. Soc. Dairy Technol.* **1980,** *33,* 101−105.

Walstra, P.; Jenness, J. *Dairy Chemistry and Physic. Ed.*; Acribia S.A.: Zaragoza, Spain, 1987.

Zadow, J. G.; Hardham, J. F.; Kocak, H. R.; Mayes, J. J. The stability of goat's milk to UHT processing. *Austr. J. Dairy Technol.* **1983,** *38,* 20−23.

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