Original article

Effect of prematuration conditions on the proteolytic and rheological properties of cheesemilk

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Abstract — Prematuration of cheesemilk involves the addition of cultures of lactic acid bacteria to milk and incubation at temperatures ranging from 8 to 16 ºC for periods up to 24 h, without extensive acidification. Prematuration temperature, individually or in combination with starter type or time, had a significant effect on pH, rennet clotting time (RCT) and the concentration of free amino acids in milk. However, no major differences were evident between the 2% TCA-insoluble or -soluble fractions of prematured milk as seen by urea-PAGE or by RP-HPLC, respectively. Prematuration starters had little effect on the growth of mesophilic or thermophilic cheesemaking starters or on the rate at which pH decreased during incubation. Furthermore, they did not effect the growth of typical indigenous microflora. Although RCT decreased with decreasing pH, results show that the slight decrease in pH which normally occurs during prematuration would result in only a small decrease in RCT. Little difference was evident between chemically (i.e., 4% lactic acid) and biologically (i.e., prematuration) acidified samples with regard to RCT, indicating that any decrease in RCT observed in this study was caused by the effect of pH alone and not by any biological effect of prematuration.

prematuration / milk / lactic acid bacteria / proteolysis / rennet coagulation time

Résumé — Effet des conditions de prématuration sur les propriétés protéolytiques et rhéologiques du lait de fabrication. La prématuration du lait de fabrication implique l'addition de cultures de bactéries lactiques au lait et l'incubation à des températures allant de 8 à 16 ºC pendant des temps allant jusqu'à 24 h, sans acidification importante. La température de prématuration, seule ou en combinaison avec le type de levain ou le temps, a un effet significatif sur le pH, le temps de prise à la présure et la concentration en acides aminés libres dans le lait. Cependant, aucune différence majeure n'était évidente entre les fractions insoluble ou soluble dans le TCA à 12 % du lait prématuré comme montré par électrophorèse urée-PAGE et chromatographie RP-HPLC, respectivement. Les levains de prématuration avaient peu d'effet sur la croissance des levains de fromagerie mésophiles ou thermo-

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philes, ou sur la vitesse à laquelle le pH diminuait au cours de l'incubation. De plus, ils n'affectaient pas la croissance de la microflore sauvage typique. Bien que le temps de prise diminuait quand le pH baissait, les résultats ont montré que la légère baisse du pH qui a lieu normalement durant la prématuration devrait produire seulement une petite diminution du temps de prise. Peu de différences apparaissaient entre les échantillons acidifiés chimiquement (i.e., 4 % d'acide lactique) ou biologiquement (i.e., prématuration) en ce qui concerne le temps de prise, indiquant que toute diminution du temps de prise observé dans cette étude était dû à l'effet du pH seul et non à un effet biologique de la prématuration.

prématuration / lait / bactérie lactique / protéolyse / temps de prise à la présure

1. INTRODUCTION

Acidification of milk, and subsequently curd, is the first biochemical phenomenon of the transformation of milk into cheese. Acidification is due to the metabolic activity of lactic acid bacteria in lactose fermentation, which results in the formation of lactic acid and production of energy necessary for their growth. Lactic acid bacteria have been used for their acidifying activity and their antagonistic properties against other microflora (strongly proteolytic bacteria, spoilage organisms and some pathogens), and have been shown to play a significant role during ripening of cheese [4, 10, 13].

Prematuration of cheesemilk, using a lactic starter culture or the indigenous flora of the milk, is a common practice in the manufacture of many soft (e.g., Camembert) and some hard cheese varieties (e.g., Grana). Prematuration of milk prior to soft cheese manufacture has been practiced in France for many years. Even with the increased use of pasteurisation, milk is still prematured with mesophilic or thermophilic lactic acid bacteria. Typically, prematuration involves the addition of lactic acid bacteria (single or mixed strain cultures) to milk and incubation at temperatures ranging from 8 to 16 ºC for periods up to 24 h, without extensive acidification. During this time, the pH of the milk generally decreases by 0.1 units and coagulation time is shorter [1, 11]. Alternatively, milk is acidified to ~pH 4.3 by the prematuration starter before a small proportion (1–3%) is blended back into the bulk milk.

Relatively few studies have investigated changes in milk during prematuration or the influence of prematuration on the quality of the resultant cheese. Hence, there appear to be few references in the literature concerning the influence of prematuration on the biochemistry of cheese. Bonassi [6] found that cheese manufactured from prematured milk had poorer body, texture and colour than control cheese although flavour scores were similar for both cheeses. Bianchi Salvatori [3, 4] reported that acidification directs the rheological processes of lactic acid coagulation, influencing syneresis, proteolysis and body characteristics.

Lactic acid bacteria may have several potential roles to play during prematuration, including (1) slow acidification of milk which may assist curd formation during cheesemaking and improve the properties of the resultant rennet coagulum, (2) control the growth of adventitious microorganisms in the milk and/or (3) stimulation of the growth of the cheesemaking starter by increasing the level of available peptides/free amino acids. Experiments performed during the course of this study were designed to determine the precise role

of the prematuration starter and the effect of the prematuration process on the proteolytic and rheological properties of milk.

2. MATERIALS AND METHODS

2.1. Cultures

Four cultures for prematuration (coded A, B, C and D for the purposes of this study) were supplied by SOREDAB (La Tremblaye, Paris, France). They were un-defined mixed strain mesophilic lactic acid cultures that are used commercially in France for prematuration prior to cheesemaking. The cultures were stored at –80 ºC and were initially propagated (0.002%, v/v) in 10% (w/v) reconstituted low-low heat skim milk powder (LLHSM; INRA-LTRL, Rennes, France) [14] at 22 ºC × 15 h for all experiments.

Mixed strain mesophilic and thermophilic cultures (one of each), that are used in France as commercial cheese- making starters, were supplied by SOREDAB and were maintained at –80 ºC until re-quired.

2.2. Experimental conditions

2.2.1. Individual and/or combined effect(s) of prematuration starter and time and temperature of prematuration on the biochemical and rheological properties of milk

Aliquots of LLHSM (10%, w/v), which had been tempered at 10 or 16 ºC, were in-oculated with culture A or B (0.4%, v/v) and incubated under experimental conditions described in Table I. The prematured milk samples were then held on ice for ≤ 4 h while they were being analysed. Samples were fractionated with 4% TCA (see

Table I. Description of prematuration condi-tions (i.e., prematuration starter, time and tem-perature) under which samples 1–8 were incubated.

Sample	Description of treatment
1	culture A at 10 ºC for 11 h
2	culture A at 16 ºC for 11 h
3	culture A at 10 ºC for 17 h
4	culture A at 16 ºC for 17 h
5	culture B at 10 ºC for 11 h
6	culture B at 16 ºC for 11 h
7	culture B at 10 ºC for 17 h
8	culture B at 16 ºC for 17 h

Sect. 2.6.) and the insoluble and soluble fractions were analysed by urea-PAGE (see Sect. 2.7.) and RP-HPLC (see Sect. 2.8.), respectively. The concentrations of individ-ual free amino acids in 12% TCA-soluble fractions from the prematured samples were determined. The coagulation proper-ties of the prematured samples were studied by recording rennet coagulation times (RCT; see Sect. 2.4.). The experiment was performed in duplicate. The results were subjected to analysis of variance (ANOVA) according to a 3 factor × 2 level (2^3) facto-rial in a complete block design which al-lowed the main effects of trial and each of the three factors as well as the two- and three-way interactive effects of these fac-tors to be determined. Analyses were per-formed using the SPSS statistical package (SPSS Inc., Chicago, Illinois 60606, USA).

2.2.2. Effect of pasteurisation on prematuration starter cell numbers

Milk (9.1%, w/w, LLHSM) was prematured under extreme conditions (i.e., 18 ºC × 24 h) with starter A, B, C or D. After prematuration, the milk was pasteurised at 72 ºC × 15 s. Samples were taken before and after prematuration and after pasteuris-ation for total bacterial counts. The experi-ment was performed in duplicate.

2.2.3. Effect of prematuration on the growth of the indigenous microflora of raw milk

Raw milk (Dairygold-CMP, Cork, Ireland) was prematured without (control) or with prematuration cultures B or C at 12 °C for 24 h. Changes in pH were monitored during the incubation period. Samples were taken at intervals, under aseptic conditions, and analysed for various types of indigenous bacteria (see Sect. 2.3.). The experiment was performed in duplicate.

2.2.4. Effect of prematuration on the growth of cheesemaking starters

Milk (9.1%, w/w, LLHSM; autoclaved at 115 °C × 10 min) was prematured without (control) or with starter culture A, B, C or D at 12 °C for 24 h. The prematured samples were then pasteurised (72 °C × 15 s) and inoculated with either a mixed-strain mesophilic or thermophilic cheesemaking starter at a level of 0.025 U·L⁻¹ or 1% (v/v), respectively. One unit (U) is the amount of starter cells which cause a decrease of at least 0.8 pH units of milk after 5 h at 35 °C. The samples were then incubated according to either a mesophilic or thermophilic temperature gradient, as outlined in Table II. These temperature gradients are similar to those used during industrial cheesemaking. Samples were taken, under aseptic conditions, before and after pasteurisation and at intervals during the incubation period to measure changes in pH and estimate total bacterial count. The experiment was performed in duplicate.

2.2.5. Effects of biological or chemical acidification on the rheological properties of milk

Samples of LLHSM (9.1%, w/w) were prematured without (one control and 3 samples) or with prematuration starter cultures, A, B or C (0.4%, v/v, inoculum) at 12 °C for 16 h. The samples were then pasteurised at 72 °C × 15 s and allowed to cool to room temperature. The pH of each of the 3 samples (i.e., those incubated without starter culture) was adjusted to that of each of the prematured samples (i.e., A, B or C) using ~4% lactic acid, allowed to stand for approx. 20 min and the pH re-adjusted, if necessary. The time taken for rennet to coagulate each of the milk samples (i.e., control, biologically- and chemically-acidified samples) was determined according to the method outlined in Section 2.4. The experiment was repeated using cultures A and B or lactic acid as acidulant and the rheological properties of rennet gels prepared from these samples were measured, as described in Section 2.5.

2.3. Bacteriological analysis

Total bacterial count was enumerated on LM17 agar after incubation at 30 °C for 3 days; lactobacilli were enumerated on Rogosa agar (Oxoid) after incubation at 30 °C for 5 days; coliforms were enumerated on Violet Red Bile Agar (VRBA)

Table II. Temperature profiles of mesophilic and thermophilic cheesemaking processes.

Mesophilic process		Thermophilic process	
Time (h)	Temperature (°C)	Time (h)	Temperature (°C)
0.0	33	0.0	42
2.0	32	1.0	30
14.5	24	18.0	18
24.0	19		

(Oxoid) after incubation at 30 °C for 24 h; enterococci were enumerated on Kanamycin Aesculin Agar (KAA) (Oxoid) after incubation at 37 °C for 2–3 days; staphylococci were enumerated on Baird Parker Agar Base (Oxoid) after incubation at 37 °C for 1–2 days; pseudomonads were enumerated on Pseudomonas Agar Base (Oxoid) after incubation at 30 °C for 2 days.

2.4. Rennet coagulation time (RCT)

RCT was determined according to a modification of the IDF method [8], outlined as follows. Prematured milk sample (2 mL aliquots) was pipetted into a screw-capped test tube and mixed with 20 µL of 1 N CaCl$_2$ before it was tempered at 30 °C for 1 h. Rennet (520 mg·L^{-1}; supplied by SOREDAB, La Tremblaye, Paris, France), diluted 1:250 (v/v) with distilled water, was added (200 µL) and the test tube was inverted twice before being placed in an oscillating renneting bath heated to 30 °C. The time taken for the milk sample to coagulate was recorded. Each determination was performed in triplicate.

2.5. Rheological properties

The development of gel strength and structure was measured using a controlled strain rate Carri-Med CLS^2/100 rheometer (TA Instruments, Surrey, England) in the dynamic mode. The C25 concentric cylinder measuring system was used, which consisted of a fixed bob and rotating cup. Prematured milk samples (10.8 mL) were tempered at 32 °C for 1 h. Rennet (520 mg·L^{-1}), diluted 1:40 with distilled water, was then added to each sample (1%, v/v), stirred several times with a glass rod and poured into the rheometer cup. Paraffin oil (BDH, Poole, Dorset, UK) was added to the surface of the rheometer cup to avoid evaporation. The run began exactly 60 s after addition of rennet and lasted 90 min, during which time G' (storage modulus), G'' (loss modulus) and tanδ were measured

continuously. The experiment was performed in duplicate.

2.6. Nitrogen fractionation

2 or 12% trichloroacetic acid (TCA)-soluble and -insoluble fractions were prepared by mixing equal volumes of sample with 4 or 24% TCA-solution and standing them at room temperature for 1 h, with occasional stirring. The mixtures were then filtered through Whatman No. 541 filter paper. 2% TCA-soluble fractions were centrifuged at 10 000 $g \times 30$ min, filtered through 0.45 µm cellulose acetate filters and analysed by HPLC while the insoluble fractions were freeze-dried and analysed by urea-polyacrylamide gel electrophoresis (urea-PAGE). The concentration of individual free amino acids was determined in the 12% TCA-soluble fractions.

2.7. Urea-polyacrylamide gel electrophoresis (urea-PAGE)

Urea-PAGE (12.5% C, 4% T, pH 8.9) of 2% TCA-insoluble fractions was performed using a Protean II xi vertical slab-gel unit (Bio-Rad Laboratories Ltd., Watford, UK) according to the method of Andrews [2], with modifications. The gels were stained directly by the method of Blakesley and Boezi [5] with Coomassie Brilliant Blue G250.

2.8. Reversed phase high performance liquid chromatography (RP-HPLC)

2% TCA-soluble fractions were analysed by RP-HPLC using an automated Shimadzu system (Shimadzu Corp., Kyoto, Japan), incorporating an LC-9A solvent delivery system with an FCV-9AL flow control valve, a DGU-2A degassing unit and an SIL-9A autoinjector. Wide-pore Nucleosil C$_8$ (5 µm particles, 300 Å pore size) guard (4.6 mm × 4.5 cm) and analytical (4.6 mm × 25 cm) columns (Capital HPLC Ltd.,

Broxburn, West Lothian, UK) were used and detection was by means of an SPD-6A spectrophotometric detector (Shimadzu) at 214 nm. The system was interfaced with a personal computer using Varian Star Workshop software (Varian Associates Inc., Walnut Creek, CA, USA). The chromatographic conditions were: *solvent A* – 0.1% trifluoroacetic acid (v/v, TFA, sequential grade, Sigma, St. Louis, MO, USA) in deionized, HPLC-grade water; *solvent B* – 0.1% TFA (v/v) in acetonitrile (HPLC grade, Rathburn Chemicals Ltd., Walkerburn, Scotland). Samples (4 mg·mL^{-1}) were dissolved in solvent A and filtered through 0.45 μm cellulose acetate filters (Sartorius GmbH, Gottingen, Germany). Filtrate (20 μL) was applied to the column and eluted at a flow rate of 0.75 mL·min^{-1} using the following gradient: 100% A for 5 min followed by a gradient from 0 to 50% B over 55 min, elution at 50% B for 6 min, a further gradient from 50 to 60% B over 4 min and finally 60% solvent B for 3 min.

2.9. Free amino acid analysis

Concentrations of individual free amino acids were determined using a Beckman model 6300 Amino Acid Analyser (Beckman Instruments Ltd., High Wycombe, UK) equipped with a Beckman model P-N 338052 Na$^+$ cation exchange column (12 cm × 0.4 cm), as described by Fenelon et al. [9].

3. RESULTS AND DISCUSSION

3.1. Individual and/or combined effect(s) of prematuration starter and time and temperature of prematuration on the biochemical and rheological properties of milk

As expected, temperature, individually ($p \leq 0.01$) or in combination with starter type ($p \leq 0.05$) or time ($p \leq 0.01$), had a sig-

nificant effect on the pH of milk at the end of prematuration (Fig. 1a). The pH of milk prematured at 16 °C × 17 h with either culture A or B (Fig. 1a, sample 4 or 8) was considerably lower than the pH of the other samples (Fig. 1a). The RCTs of milks prematured using culture B (Fig. 1b, samples 4–8) were shorter than those of samples incubated at similar temperature × time regimes but prematured using culture A (Fig. 1b, samples 1–4). Temperature, alone or in combination with time, also had a significant ($p \leq 0.01$) influence on RCT (Fig. 1b). These results demonstrate that the choice of incubation conditions as well as the prematuration starter will dictate changes in pH and RCT that occur during prematuration.

Significant differences were found between the control and prematured milk samples with regard to the concentration of at least some of the free amino acids in milk (Fig. 2). Differences in the level of individual amino acids were also evident between the various prematured milk samples (Fig. 2), indicating that the conditions under which milk is prematured will influence changes in the free amino acid content of the milk. However, urea-PAGE of the 2% TCA-insoluble fractions of the milks indicated little hydrolysis of either α_{s1}- or β-casein in any of the samples during prematuration (results not shown). Furthermore, no differences were evident from the RP-HPLC peptide profile of the 2% TCA-soluble fractions of the control milk (i.e., that without starter) and any of the milk samples containing the prematuration starters under the experimental conditions used in this study (results not shown). Results show that the conditions under which milk is prematured will influence at least some of the biochemical and rheological properties of the prematured milk. The biochemical changes which occur during prematuration appear relatively subtle (i.e., changes in the levels of individual free amino acids) and so may not be determined using methods which measure differences
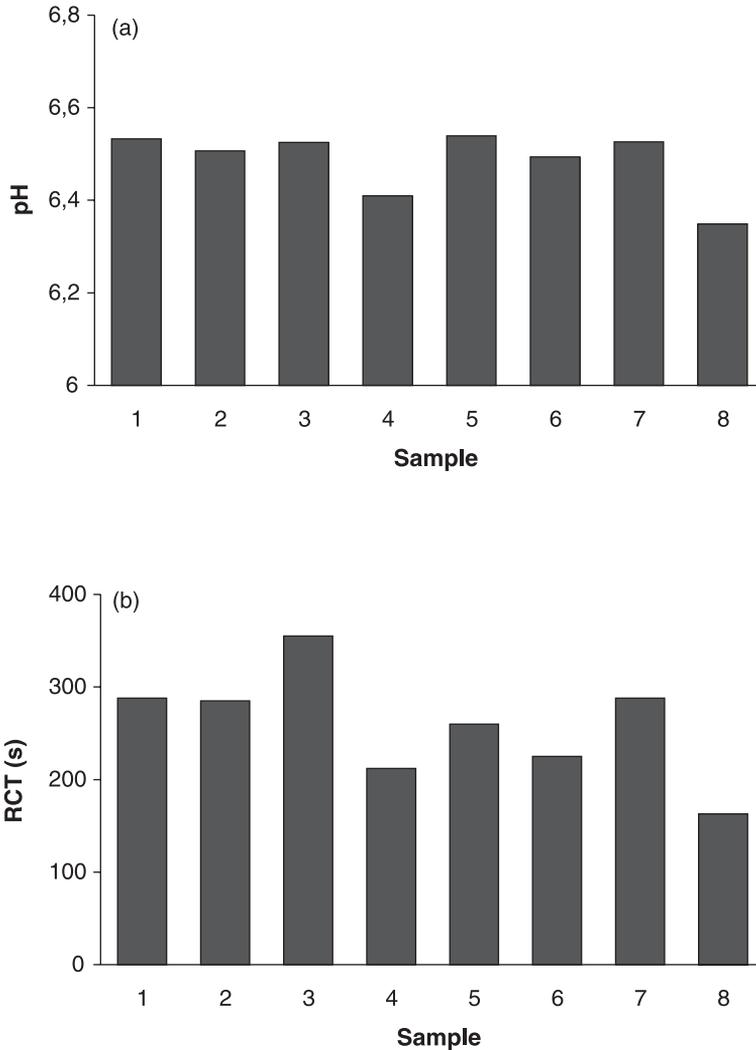
Figure 1. pH (a) and rennet coagulation time (b) of milks incubated under various conditions of prematuration starter type, time and/or temperature (see Tab. I for description of samples 1–8). Results represent means of duplicates.

at the protein or peptide level, such as urea-PAGE and RP-HPLC, respectively.

3.2. Effect of pasteurisation on prematuration starter cell numbers

Prematuration starters grew by between 2 and 3 log cycles and reduced the pH of milk by between ~0.3 and 0.5 pH units during prematuration under extreme conditions, i.e., 18 °C × 24 h (Tab. III). Pasteurisation reduced total bacterial count in all of the prematured milk samples to < 10 cfu·mL^{-1} (Tab. III). These results suggest that the prematuration starter will not survive pasteurisation and, consequently, will not interfere with the activity of the
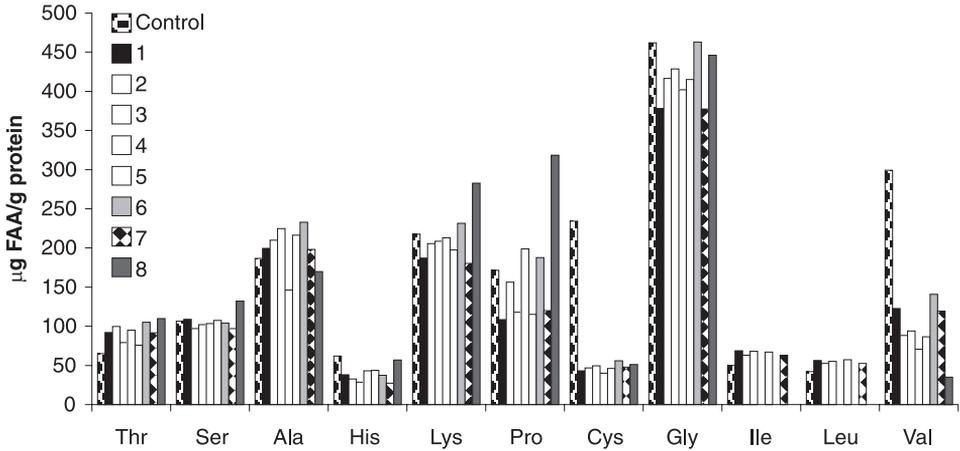
Figure 2. Free amino acid profiles of 12% TCA-soluble fractions from control and milk samples prematured under various conditions (see Tab. I for a description of samples 1–8). Results represent means of duplicates.

Table III. Effect of pasteurization (72 °C × 15 s) on starter cell numbers after incubation under extreme prematuration conditions (18 °C for 24 h).

| Starter type	pH at 0 h	pH at 24 h	I	II	III
A	6.556 ± 0.001	6.278 ± 0.011	3.22×10^4	5.95×10^7	< 10
B	6.556 ± 0.002	6.096 ± 0.018	2.83×10^5	2.77×10^8	< 10
C	6.556 ± 0.000	6.156 ± 0.017	2.53×10^5	3.80×10^7	< 10
D	6.556 ± 0.001	6.124 ± 0.037	5.92×10^5	4.74×10^8	< 10

Results represent mean of duplicates; I = starter cell numbers (cfu·mL^{-1}) at the start of the incubation period; II = starter cell numbers (cfu·mL^{-1}) in the prematured milk samples (i.e., 18 °C × 24 h); III = starter cell numbers (cfu·mL^{-1}) in the prematured milk samples after pasteurization (72 °C × 15 s).

starter during cheesemaking. However, no comments can be made regarding the survival of the enzymes of prematuration starters and any effect their activity may have on the properties of the prematured milk.

3.3. Effect of prematuration on the growth of the indigenous microflora of raw milk

Prematuration starter activity at 12 °C reduced the pH of milk prematured using culture B or C by approximately 0.41 or 0.20 pH units, respectively, reaching a pH value of 6.347 or 6.563 at the end of prematuration (Fig. 3). The pH of the control (i.e., without prematuration starter) decreased only slightly during the later stages of incubation, having a pH of 6.626 at the end of prematuration (Fig. 3). As was expected, the initial total bacterial counts were higher in the milk samples prematured with cultures B or C (2.36×10^6 and 2.15×10^5 cfu·mL^{-1}, respectively) than in the control (3.46×10^4 cfu·mL^{-1}) and increased in all three samples during prematuration reaching levels of 5.90×10^7 (culture B), 5.38×10^7 (culture C) and 4.85×10^7 cfu·mL^{-1} (control), respectively
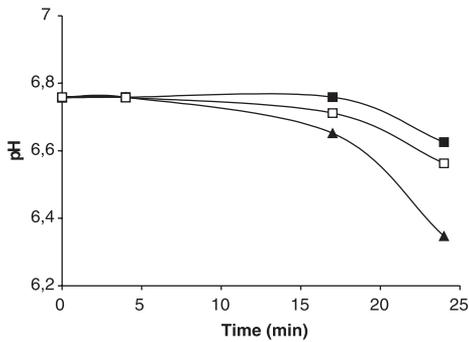
Figure 3. Changes in pH of control (■) and samples prematured with culture B (▲) or C (□) at 12 °C for 24 h. Results represent means of duplicates.

(Fig. 4a). Prematuration had little effect on the growth of the indigenous microflora (i.e., lactobacilli, coliforms, enterococci, staphylococci and pseudomonads) in raw milk containing culture B or C during incubation at 12 °C for 24 h, all of which increased in both the control and the prematured samples by between 1 and 3.5 log cycles during the incubation period (Figs. 4b–4f). These results suggest that prematuration starter activity may have little effect on the growth and survival of microorganisms indigenous to milk.

3.4. Effect of prematuration on the growth of cheesemaking starters

Prematuration starter cultures A, B, C and D, incubated at 12 °C for 24 h, grew by approximately 2.5 log cycles reaching levels of between 10^7 and 10^9 cfu·mL^{-1} and reduced pH by ≤ 0.50 units. Pasteurisation reduced cell numbers to <10 cfu·mL^{-1} in the case of cultures C and D but only reduced those in cultures A and B to 1.27×10^5 and 3.90×10^4 cfu·mL^{-1}, respectively.

The ability of pasteurisation to reduce cell numbers obviously depends on the type of prematuration starter present in the milk and on the conditions under which it grows. The prematured samples were then inoculated with either a mixed-strain mesophilic or thermophilic cheesemaking starter and incubated according to either a mesophilic or thermophilic temperature gradient, as outlined in Table II. These temperature gradients are similar to those used during industrial cheesemaking. The rate of growth of mesophilic or thermophilic cheesemaking starters was similar in all samples (results not shown), indicating that the prematuration conditions used in this study (i.e., 12 °C × 24 h using culture A, B, C or D) had no effect on the growth of these cheesemaking starters or the cell numbers they reached after 24 h incubation. Furthermore, prematuration had little effect on the rate at which pH decreased during simulated cheesemaking or on the final pH of the prematured samples containing mesophilic or thermophilic cheesemaking starters when compared with the control sample (results not shown).

3.5. Effects of biological or chemical acidification on the rheological properties of milk

Prematuration at 12 °C for 16 h using culture A, B or C caused a decrease in pH of 0.043, 0.214 or 0.026 units, respectively. Milk prematured with culture A (pH 6.625) or C (pH 6.642) had only a slightly lower RCT than the control (pH 6.668) while a large difference in RCT was evident between milk prematured with culture B (pH 6.454) and the control (Fig. 5). Little difference was evident between the biologically- (i.e., prematured) and chemically-acidified samples with regard to RCT (Fig. 5), indicating that any decrease in RCT observed in this study was caused by the effect of pH alone and not by any biological effect of prematuration. It has been
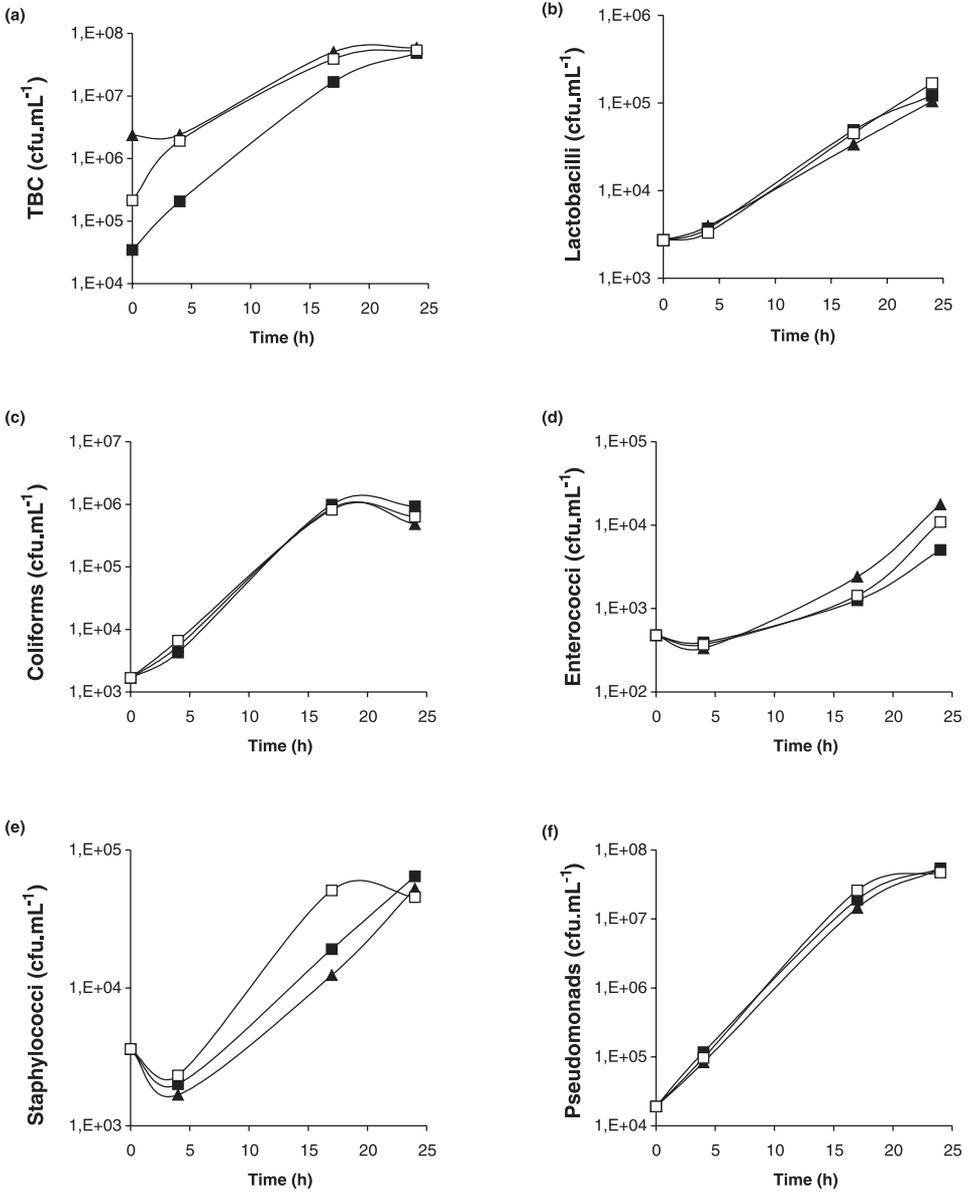
Figure 4. Changes in total bacterial count (a), lactobacilli (b), coliforms (c), enterococci (d), staphylococci (e) and pseudomonads (f) in control (■) and samples prematured with culture B (▲) or C (□) at 12 °C during 24 h incubation period. Results represent means of duplicates.
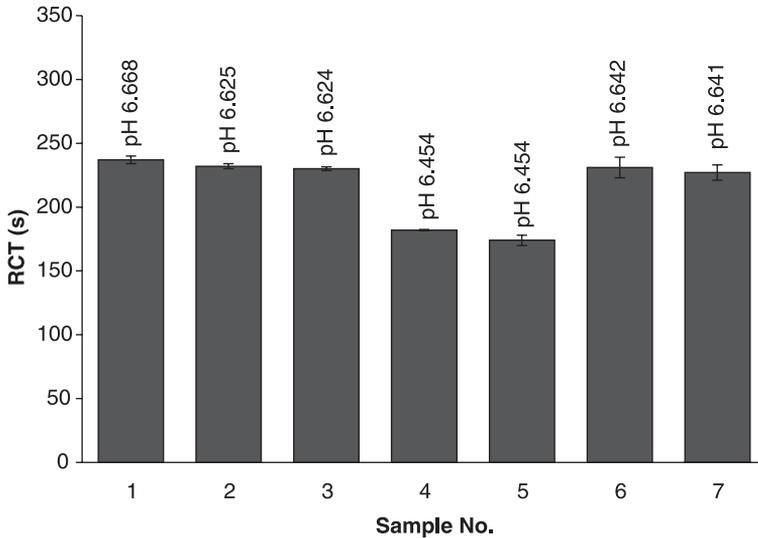
Figure 5. Rennet coagulation times (RCT) of control (1) and milk samples acidified biologically using culture A (2), B (4) or C (6) or acidified chemically using 4% lactic acid to pH 6.624 (3), 6.454 (5) or 6.641 (7).

shown previously that pH has a strong effect on rennet coagulation of milk [7]. Decreasing pH in the range 7.0 to 5.2 causes an increase in both rennet activity [15] and the aggregation of casein [12], resulting in a reduction in rennet coagulation time (RCT).

Figure 6 shows G' as a function of time for gels prepared from LLHSM samples which were either acidified biologically with prematuration culture A or B or chemically to pH values corresponding to those caused by the activity of culture A or B. Decreasing pH in the range 6.740 to 6.525 resulted in a progressive reduction in the time required for the onset of gelation (i.e., the point in time at which G' is ≥ 1 Pa). The rate of aggregation and gel formation, monitored by measuring changes in storage modulus [G'] over time, increased with decreasing pH (Fig. 6). These results are in agreement with the findings of other authors including van Hooydonk et al. [15]

and highlight the importance of pH in relation to gel strength and structure. However, little difference in G' was observed between milks that were biologically- (i.e., prematuration) or chemically- (i.e., ~4% lactic acid) acidified to similar pH values (Fig. 6). Similar profiles were obtained when loss modulus (G") was plotted as a function of time (results not shown). These results confirm the fact that differences found in the rheological properties between control and prematured milk samples are primarily due to the effect of pH and not to any biological effect of prematuration.

4. CONCLUSIONS

Results show that the type of prematuration starter will influence the rate of acidification, the final pH and may increase the free amino acid pool in cheesemilk.
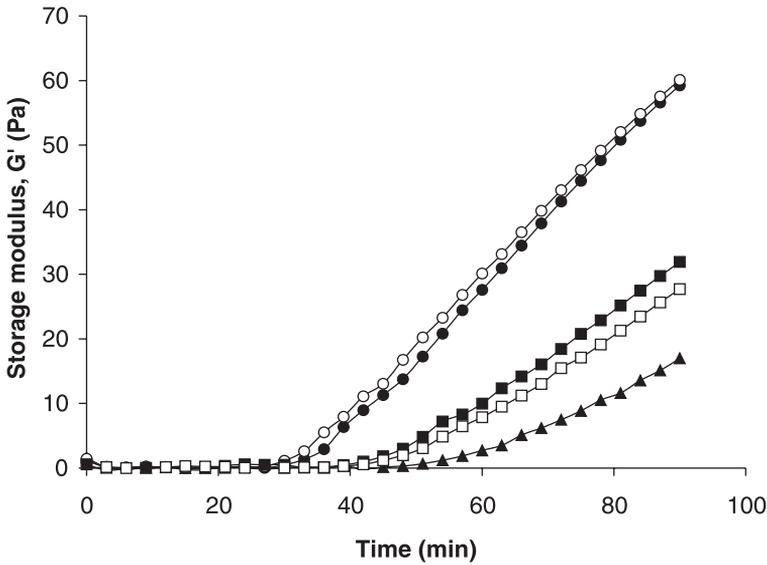
Figure 6. Storage modulus (G') as a function of time for gels prepared from control milk at pH 6.740 (▲), milk biologically acidified with culture A (■) or B (●) or milk chemically acidified to pH 6.730 [corresponding to culture A] (□) or 6.525 [corresponding to culture B] (○).

Prematuration starter activity will have little effect on the growth of the indigenous microflora of raw milk or that of the cheesemaking starter bacteria (mesophilic or thermophilic cultures). Furthermore, the acidifying activity of the starter during cheesemaking appears not be affected by prematuring the milk beforehand. The importance of pH in relation to rennet coagulation time (RCT) and rennet gel strength and structure is demonstrated clearly in these studies. Hence, the small decrease in pH, which normally occurs during prematuration (≤ 0.10 pH units), caused by the activity of the prematuration starter, will influence the rheological properties of the prematured milk. However, the extent to which this drop in pH will affect the rheological properties of soft cheese made from prematured milk, is not clear as yet and is part of ongoing studies.

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