

## Influence of addition of plasmin or mastitic milk to cheesemilk on quality of smear-ripened cheese

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(Received 2 April 2001; accepted 8 August 2001)

**Abstract** – Smear-ripened cheese varieties are characterised by the growth of a smear culture, containing predominantly *Brevibacterium linens*, on the cheese surface during ripening. In such cheese, considerable zonal differences in biochemistry of ripening exist, due to moisture loss from, and growth and metabolic activity of smear microflora at, the cheese surface. In this study, the effects of adding exogenous plasmin or small amounts of mastitic milk to good quality milk on the quality of smear-ripened cheese made subsequently was examined. Addition of plasmin did not influence cheese composition immediately after manufacture, but slightly decreased the rate of moisture loss during cheese ripening. Plasmin activity decreased during the early stages of ripening, but subsequently increased towards the end of ripening, perhaps due to changing pH conditions in the cheese. Addition of plasmin increased rates of primary proteolysis in cheese, as measured by levels of pH 4.6-soluble N and urea-PAGE, although production of later products of proteolysis appeared less affected. Addition of mastitic milk had largely similar effects to addition of exogenous plasmin, which may reflect a high content of plasmin or plasminogen activators in such milk. Overall, changes in milk quality and enzymology appear to influence the quality of smear-ripened cheese.

### Plasmin / cheese / smear-ripened

**Résumé** – Influence de l'addition de la plasmine ou du lait de mammite sur l'affinage des fromages à pâte pressée et croûte lavée. Les fromages à croûte lavée ont leur surface caractérisée par la croissance de bactéries corynéformes, contenant *Brevibacterium linens* de manière prédominante. Dans un tel fromage, la biochimie de l'affinage présente des différences de zone considérables, dues à la perte en humidité de la surface du fromage et à la croissance et l'activité métabolique de la microflores à la surface du fromage. Cette étude traite des effets de l'ajout de plasmine exogène ou de petites

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quantités de lait pathologique (mammite) mélangées à du lait de bonne qualité sur la qualité du fromage à pâte pressée et croûte lavée. L'addition de plasmine n'a pas influencé la composition du fromage immédiatement après fabrication, mais a diminué légèrement le taux de perte en humidité pendant l'affinage. L'activité de la plasmine a diminué pendant les premières étapes de l'affinage, mais a augmenté vers la fin de l'affinage, peut-être suite au changement de pH dans le fromage. Par ailleurs, l'addition de plasmine a augmenté les taux de protéolyse dans le fromage, comme l'ont montré les taux d'azote soluble à pH 4,6 et l'analyse des hydrolysats avec urée-PAGE, même si les produits ultérieurs de la protéolyse se sont révélés moins affectés. En grande partie, l'addition de lait de mammite a eu des effets similaires à l'addition de plasmine exogène, ce qui semble refléter une quantité élevée d'activateurs de plasmine ou de plasminogène dans ce lait. Dans l'ensemble, les changements dans la qualité et l'enzymologie du lait paraissent influencer la qualité du fromage à croûte lavée.

## Plasmine / fromage / pâte pressée à croûte lavée

### 1. INTRODUCTION

The principal proteinase in bovine milk is the alkaline serine proteinase, plasmin (E.C. 3.4.21.7) [4]. Most of the potential plasmin activity in milk is in the form of its inactive precursor, plasminogen, which is converted to active plasmin by a heterogeneous group of plasminogen activators (PA). Plasmin has a pH optimum of 7.5 and readily hydrolyses  $\beta$ -casein and  $\alpha_{s2}$ -casein and, more slowly,  $\alpha_{s1}$ -casein [16].

Plasmin activity influences the quality of many dairy products [4, 19]. Plasmin-mediated hydrolysis of casein influences milk coagulation properties and cheese yield [24]. A number of studies have examined the effect of adding exogenous plasmin to milk on the quality of cheese made from that milk, and have shown that plasmin action is important in cheese ripening. Its role in cheese ripening appears to be mainly in primary proteolysis of caseins (particularly  $\beta$ -casein), which may enhance cheese flavour [13, 14]. Plasmin activity in cheese may also be increased by addition of the plasminogen activator urokinase, with similar effects on cheese ripening [3]. The studies mentioned above all examined the significance of elevated plasmin activity for ripening of Cheddar cheese. However, varietal differences in cheese plasmin activity have been observed, which have been at-

tributed, at least in part, to differences in cooking temperatures used in their manufacture [12].

The somatic cell count of milk (SCC), which increases dramatically in mastitis, is also known to influence the ripening and quality of cheese, due to greatly elevated levels of plasmin and cell lysosome-derived proteolytic enzymes [2, 18, 31]. Milk somatic cells also possess PA activity [33, 34].

During ripening of certain cheese varieties, a smear solution containing a mixed microbial culture, dominated by *Brevibacterium linens*, is applied to the surface of the cheese. The subsequent growth of the smear microflora in smear-ripened cheese results in a significant increase in pH from  $\sim 5.0$  to 6.5–7.0 at the cheese surface during 40–50 d of ripening, and extensive proteolysis in this region, due to secretion of proteolytic enzymes [17]. The manufacture and ripening of smear-ripened cheese varieties were reviewed by Rejs [29].

In hard cheese varieties such as Cheddar, the pH remains relatively constant during ripening. However, in cheese varieties where pH increases during ripening (such as smear-ripened cheese), it may be expected that the relative contribution of alkaline serine proteinases such as plasmin and PA would increase during ripening. In the mould-ripened cheese variety Camembert,

it has been shown that the activity and contribution of plasmin to ripening can vary between surface and core regions due to differences in pH [32].

The objective of this study was to examine the effect of adding exogenous plasmin to milk on the manufacture and ripening of smear-ripened cheese. In parallel experiments, the influence of addition of high SCC milk to creamery milk on the quality of this cheese was also studied.

## 2. MATERIALS AND METHODS

### 2.1. Milk for cheesemaking

Fresh raw creamery bulk milk was obtained from a local supplier on the morning of cheesemaking, standardised to a fat: protein ratio of 1.15:1 and pasteurised at 73 °C for 15 s, before being filled into 4 matched 100 L vats, at a temperature of 30 °C. To two separate vats were added 0.125 mg·L<sup>-1</sup> or 0.25 mg·L<sup>-1</sup> porcine plasmin (Sigma-Aldrich, Tallaght, Dublin 24, Ireland), respectively, while to a third vat was added 10–20% high SCC (> 1 000 000 cells·mL<sup>-1</sup>) milk from mastitic cows (supplied from the experimental herd of the Dairy Production Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland). The average SCC of the creamery milk was 150 000 cells·mL<sup>-1</sup>, and addition of high SCC milk resulted in an increase in SCC to around 300 000 cells·mL<sup>-1</sup>. A final vat, with no additions, acted as a control. This experiment was repeated on three separate occasions.

### 2.2. Cheese manufacture

Milk was inoculated with a bulk starter culture containing thermophilic micro-organisms (TH3 and LB18, Chr. Hansens, Little Island, Cork, both grown overnight in 10% reconstituted skim milk powder and added at a level of 1% each) and allowed to

ripen for 60 min, after which time chymosin (Chymogen, Chr. Hansens) was added at a level of 16.5 mL·100 L<sup>-1</sup>. Calcium chloride (0.02%) was also added and, after stirring for 3 min, the milk was allowed coagulate for 50 min, cut, and allowed heal for 10 min before raising the temperature to 34 °C, with constant stirring, over 30 min by introducing steam into the jacket of the vat. The temperature was maintained at 34 °C for 10 min, and then approximately one-third of the whey was removed and replaced with hot water at 34 °C over a 20 min period. The curds and whey were then drained and filled into perforated moulds (seven cheeses of typical size 21 cm (diameter) × 4.5 cm (height) were produced per vat), which were inverted, placed into warm empty cheese vats, and inverted regularly over 2 h before being allowed to stand overnight. The cheeses were then brined for 8 h at 10 °C in a 23% (w/v) salt brine containing 0.56% calcium chloride.

Immediately after manufacture, cheeses were ripened at 16 °C (relative humidity of 95–98%) for 9 d, turned every 2–3 days, held for 6 d at 12 °C, wrapped in grease-proof paper and ripened for a further 32 d at 8 °C. After brining, and 5 and 8 d after manufacture, cheese were inoculated by dipping into a concentrated smear culture solution containing *Brevibacterium linens* (Laboratorium Visby, Tønder, Denmark).

### 2.3. Analytical techniques

A single cheese from each batch (four experimental treatments, repeated in triplicate, yielding twelve batches of cheese, where each batch represents a vat yielding seven individual cheeses) was taken immediately after manufacture (d 1) and after 2, 5, 10, 15, 28 and 48 d of ripening. At each sampling time, a portion of cheese 9 cm in diameter and 4 cm in height was cut from the centre of the cheese round, and from this sample 1 cm was removed from the top and bottom (denoted as surface sample) and the

central 1 cm thick zone (denoted as core sample). Surface and core samples were analysed separately as follows.

Cheese pH (of a 1:1 cheese: water slurry) and moisture (oven drying at 102 °C) and protein levels (macro-Kjeldhal) were measured at each sampling point, as described previously [23]. Fat and salt contents were measured at d 1 and d 48, by the Gerber method and the potentiometric method of Fox [15], respectively. pH 4.6-Soluble extracts were prepared from cheese at each sampling point and sub-fractionated to give ethanol-soluble N and phosphotungstic acid-soluble N fractions, as described by Ardö and Polychroniadou [1]. Nitrogen levels in all three extracts were measured by Kjeldahl and expressed as % of the total N in the cheese at that time. Cheese samples were also analysed by urea-PAGE electrophoresis [25].

Plasmin activity in cheese samples was measured by the method of Richardson and Pearce [30] using *N*-succinyl-alanyl-phenyl-alanyl-lysyl-7-amido-4-methyl coumarin as substrate, and expressed as plasmin units/g cheese (where 1 plasmin unit is the activity necessary to release 1 nmol of 7-amido-4-methyl coumarin from the substrate per min at pH 7.5 and 25 °C). Cheese samples for plasmin analysis were prepared for assay by dispersing 10 g grated cheese in 90 mL 2% trisodium citrate at 37 °C for

15 min, followed by stomaching for 5 min and centrifugation at 27 000 *g* for 10 min at 4 °C to recover supernatants, which were used in the plasmin assay.

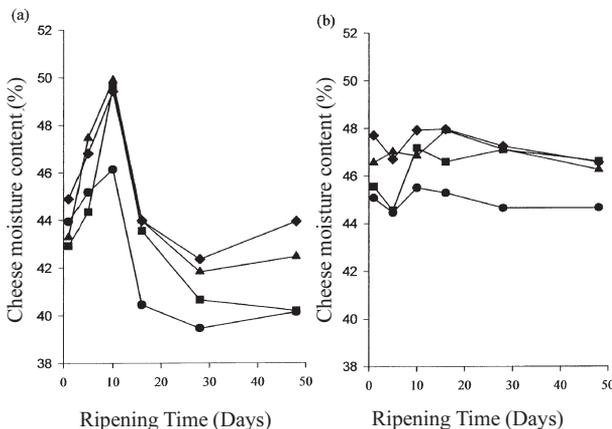
## 2.4. Statistical analysis

The effects of the four different experimental treatments (control, low and high level of plasmin addition and addition of mastitic milk) in the two distinct regions of the cheese (surface and core), at six different sampling points during ripening were investigated using a split-plot design. Treatment, cheese region and ripening time were the main plot, sub-plot and sub-sub-plot factors, respectively, and there were three replicates. These data were analysed using the statistical analysis package Genstat 5 [28]. The values used in figures are means of triplicate trials.

## 3. RESULTS

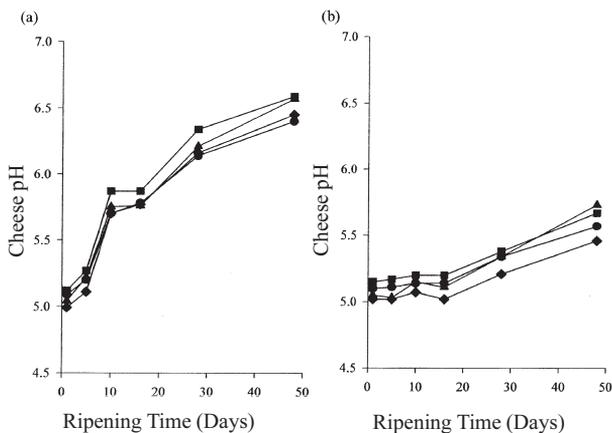
### 3.1. Composition of cheese

The moisture content of experimental smear-ripened cheese was significantly affected by region within the cheese ( $P < 0.001$ , being higher at the cheese core), and by ripening time ( $P < 0.001$ ), presumably due to loss of moisture from the



**Figure 1.** The effect of addition of exogenous plasmin and mastitic milk on the moisture content of the (a) surface and (b) core regions of smear-ripened cheese during ripening. Cheese was made from control milk (●), milk with 0.125 mg·L<sup>-1</sup> exogenous plasmin (■), milk with 0.25 mg·L<sup>-1</sup> exogenous plasmin (▲), and milk with added mastitic milk (◆).

**Figure 2.** The effect of addition of exogenous plasmin and mastitic milk on the pH of the (a) surface and (b) core regions of smear-ripened cheese during ripening. Cheese was made from control milk (●), milk with 0.125 mg·L<sup>-1</sup> exogenous plasmin (■), milk with 0.25 mg·L<sup>-1</sup> exogenous plasmin (▲), and milk with added mastitic milk (◆).



surface of the unpackaged cheese during ripening, as previously reported for smear-ripened cheese varieties [27, 29]. Changes in moisture content of cheese during ripening are shown in Figure 1. Initial moisture levels (43–48%) were similar to those reported for Saint-Paulin cheese by Kawabata et al. [20]. The difference in rates of moisture loss from the surface and core regions led to a statistically significant interaction between cheese region and ripening time ( $P < 0.001$ ). Although experimental treatment (addition of plasmin or mastitic milk) did not significantly affect cheese moisture content, numerical differences between treatments were observed.

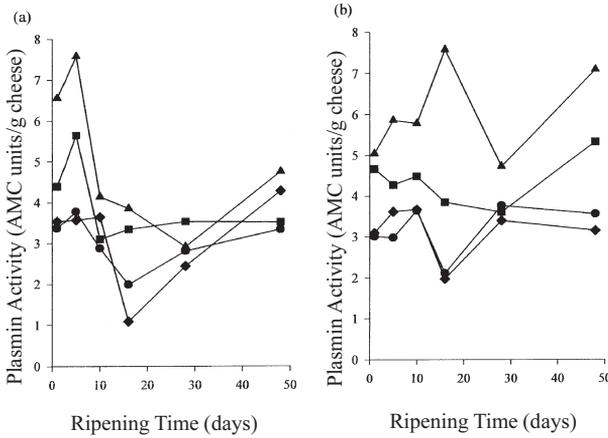
Cheese pH increased during ripening ( $P < 0.001$ ), and was higher at the surface of the cheese than at the core ( $P < 0.001$ ) (Fig. 2), with a significant interaction again being found between these factors ( $P < 0.001$ ). This is presumably due to the growth and metabolic activity of the surface microflora which becomes established in the cheese during ripening [8, 27]. However, little difference in cheese pH was evident between experimental treatments.

Contents of fat, protein and salt in cheese, measured at d 1 and d 48, were significantly affected by cheese region, ripening time and the interaction between these factors (all  $P < 0.01$ ; data not shown), reflecting the loss of moisture from the

cheese surface exerting a concentration effect on other cheese constituents in this region. However, there were no significant effects of experimental treatments on these parameters.

### 3.2. Plasmin activity in cheese during ripening

Plasmin activity in cheese was significantly affected by experimental treatment ( $P < 0.05$ ). At d 1 of ripening, activities were similar in control cheese and cheese made from milk to which mastitic milk had been added, but addition of 0.125 and 0.25 mg·L<sup>-1</sup> exogenous plasmin increased activity proportionately at both the surface and core of the cheese, as would be expected (Fig. 3). Plasmin activity was also significantly affected by stage of ripening ( $P < 0.05$ ), although the trend here was not very clear, with an initial decrease in plasmin activity (up to around 26 d of ripening) followed by an increase towards the end of ripening. Activities in surface and core regions of cheese were similar, but the ripening trend was less marked in the core region. At the end of ripening (d 48) plasmin activities at the cheese surface remained higher in cheeses with added plasmin or mastitic milk than in the control, although at the core of the cheese plasmin



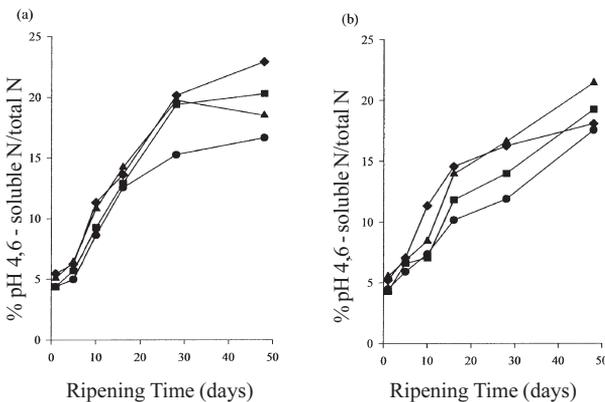
**Figure 3.** The effect of addition of exogenous plasmin and mastitic milk on plasmin activity in the (a) surface and (b) core regions of smear-ripened cheese during ripening. Cheese was made from control milk (●), milk with 0.125 mg·L<sup>-1</sup> exogenous plasmin (■), milk with 0.25 mg·L<sup>-1</sup> exogenous plasmin (▲), and milk with added mastitic milk (◆).

activity was lower in cheese made from milk mixed with mastitic milk.

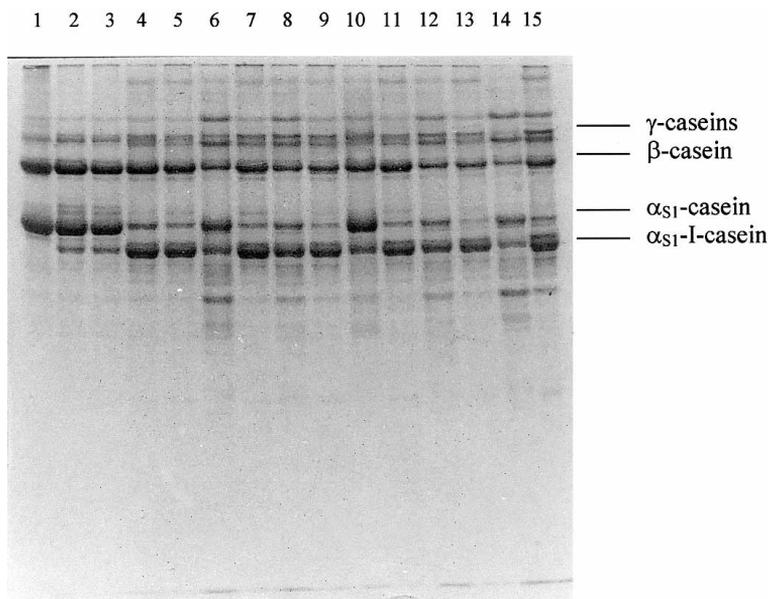
Plasminogen-derived activity in cheese was significantly affected by experimental treatment ( $P < 0.01$ ) and cheese region (mean value was higher in the core region than at the cheese surface;  $P < 0.05$ ) (not shown). In general, plasminogen-derived activities were highest in control cheese and lowest in cheese receiving the higher level of exogenous plasmin. At the surface of the cheese, the increase in plasmin activity in the later stages of ripening was paralleled by a decrease in plasminogen-derived activity.

### 3.3. Proteolysis in cheese during ripening

Levels of pH 4.6-soluble N (SN) increased significantly in all cheese batches during ripening ( $P < 0.001$ ; Fig. 4). Levels were, on average, higher at the cheese surface than the cheese core ( $P < 0.05$ ), and there was a significant interaction between cheese region and stage of ripening ( $P < 0.001$ ), reflecting the fact that increases in pH 4.6-SN were more rapid at the surface. Experimental treatment also significantly affected levels of pH 4.6-SN during ripening ( $P < 0.01$ ), with higher levels being found in cheese made from milk



**Figure 4.** The effect of addition of exogenous plasmin and mastitic milk on levels of pH 4.6-soluble N in the (a) surface and (b) core regions of smear-ripened cheese during ripening. Cheese was made from control milk (●), milk with 0.125 mg·L<sup>-1</sup> exogenous plasmin (■), milk with 0.25 mg·L<sup>-1</sup> exogenous plasmin (▲), and milk with added mastitic milk (◆).



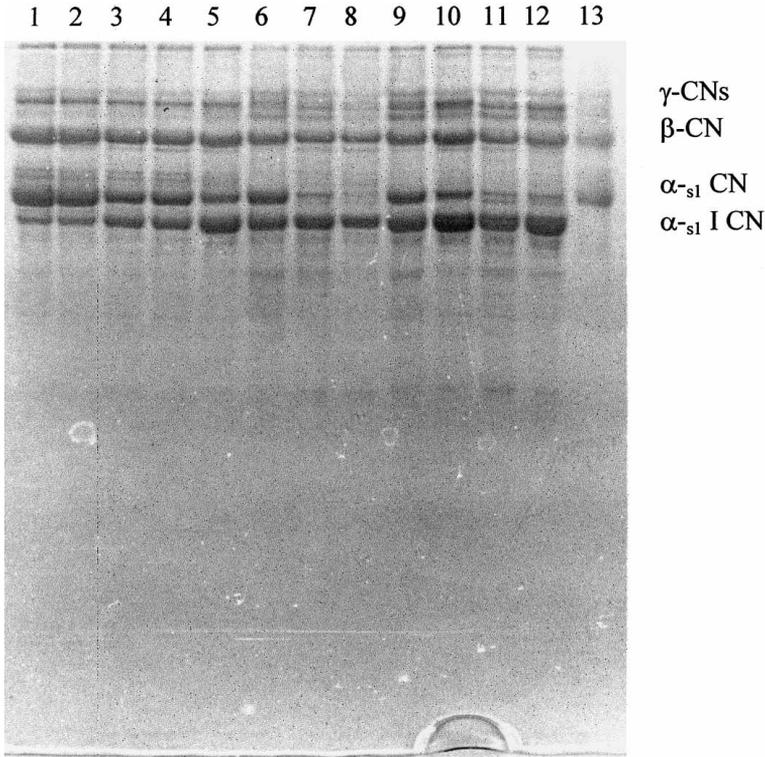
**Figure 5.** Urea-PAGE electrophoretograms (pH 8.9, 12.5% T, 4% C) of smear-ripened cheese made from milk with addition of two levels of exogenous plasmin. Lane 1, sodium caseinate. Lane 2, control cheese surface sample, d 1; lane 3, control cheese core sample, d 1; lane 4, control cheese surface sample, d 16; lane 5, control cheese core sample, d 16; lane 6, cheese made from milk with  $0.125 \text{ mg}\cdot\text{L}^{-1}$  exogenous plasmin, surface sample, d 16; lane 7, cheese made from milk with  $0.125 \text{ mg}\cdot\text{L}^{-1}$  exogenous plasmin, core sample, d 16; lane 8, cheese made from milk with  $0.25 \text{ mg}\cdot\text{L}^{-1}$  exogenous plasmin, surface sample, d 16; lane 9, cheese made from milk with  $0.25 \text{ mg}\cdot\text{L}^{-1}$  exogenous plasmin, core sample, d 16; lanes 10-15, as lanes 4-9, but at d 48 of ripening.

which had received either level of plasmin addition or mastitic milk than in the control cheese.

Neither levels of ethanol-soluble N nor phosphotungstic acid-soluble N were significantly affected by experimental treatment, although in both cases the trend towards more rapid proteolysis in experimental cheeses than in control cheese was apparent (not shown). Both parameters increased significantly during ripening ( $P < 0.001$ ), and were higher at the cheese surface than in the core ( $P < 0.001$ ), and in both cases there was a significant interaction between ripening time and cheese region ( $P < 0.001$ ).

Urea-PAGE electrophoretograms of control cheese and cheese receiving the two

levels of exogenous plasmin are shown in Figure 5. At d 16 of ripening, there was higher production of  $\gamma$ -caseins and more residual intact  $\alpha_{S1}$ -casein at the cheese surface (lane 4) than at the core (lane 5), presumably reflecting the differences in pH between these regions, which will favour the activities of plasmin and chymosin, respectively. In cheese made from milk to which had been added  $0.125 \text{ mg}\cdot\text{L}^{-1}$  plasmin, the same difference was apparent between surface and core regions, but the levels of  $\gamma$ -caseins, indicative of plasmin action, were noticeably higher than in the relevant control cheese samples. A correlation between increasing plasmin addition to cheesemilk and degradation of  $\beta$ -casein to  $\gamma$ -caseins has been previously reported



**Figure 6.** Urea-PAGE electrophoretograms (pH 8.9, 12.5% T, 4% C) of smear-ripened cheese made from milk with added mastitic milk. Lane 1, control cheese' surface sample, d 1; lane 2, control cheese' core sample, d 1; lane 3, cheese made from milk mixed with mastitic milk, surface sample, d 1; lane 4, cheese made from milk mixed with mastitic milk, core sample, d 1; lane 5, control cheese' surface sample, d 28; lane 6, control cheese core sample, d 28; lane 7, cheese made from milk mixed with mastitic milk, surface sample, d 28; lane 8, cheese made from milk mixed with mastitic milk, core sample, d 28; lane 9, control cheese' surface sample, d 48; lane 10, control cheese' core sample, d 48; lane 11, cheese made from milk mixed with mastitic milk, surface sample, d 48; lane 12, cheese made from milk mixed with mastitic milk, core sample, d 48; lane 13, sodium caseinate.

[13, 14]. Increased plasmin-derived proteolysis was also clearly apparent in cheese made from milk which had received the higher level of plasmin ( $0.25 \text{ mg}\cdot\text{L}^{-1}$ ). Differences in rates of proteolysis between surface and core regions, and accelerated breakdown of  $\beta$ -casein and production of  $\gamma$ -caseins with increasing plasmin addition, were also clearly visible after 48 d of ripening.

On comparing electrophoretograms of cheese made from milk to which mastitic

milk was added (Fig. 6) to control cheese, it was apparent that the former cheese exhibited more extensive primary proteolysis of caseins during ripening than the latter. In particular, breakdown of  $\alpha_{s1}$ -casein was considerably accelerated in cheese made from mastitic milk after 28 and 48 d of ripening. Breakdown of  $\beta$ -casein was also accelerated following addition of mastitic milk, although production of  $\gamma$ -caseins was not markedly increased.

#### 4. DISCUSSION

Addition of plasmin to cheesemilk had relatively little effect on initial (d 1 of ripening) moisture content at the surface of smear-ripened cheese (Fig. 1a). The cheese dried out considerably during ripening, however, and, by the end of ripening, the highest moisture content was found in cheese made from milk which had received either the higher level of plasmin or the mastitic milk. At the cheese core, a similar pattern of moisture contents relative to milk treatment was apparent at d 1, and remained throughout ripening. It has been previously suggested that the caseinolytic activity of plasmin in milk may result in increased moisture content of cheese [11], although this has been disputed [24]. In general, however, addition of plasmin to cheesemilk immediately before manufacture has not been associated with elevated cheese moisture content [13, 14], at least for Cheddar cheese. In this study, it appears that the action of plasmin may influence water retention in a cheese variety where the moisture content is not constant during ripening (as for Cheddar) but rather decreases due to moisture loss. It is possible that increased proteolysis may result in production of charged amino and carboxyl groups which may retain water more tightly.

High moisture retention was also observed when mastitic milk was added to cheesemilk, which may be linked to previous reports of high moisture content of Cheddar cheese made from high SCC milk [2]. When the experimental strategy applied here was used previously for Cheddar cheese, addition of relatively low amounts of mastitic milk to good quality milk before cheesemaking was associated with increasing moisture content of the cheese [18].

The activity of plasmin in cheese changed in a complex manner over the course of cheese ripening. While initial (d 1) plasmin levels in cheese reflected experimental treatments, plasmin activity at

the cheese surface decreased during the first 20 d or so of ripening, before increasing in the latter stages. Plasmin activity generally increases slightly during ripening of Cheddar [3] and Danbo [6] cheese, while a slight decrease during ripening of Saint-Paulin cheese has been observed [5]. The reason for the initial decrease in activity at the surface in the current trial is unclear, although it may be linked to the increase in cheese moisture content observed over the same period. The increase in plasmin activity during later stages of ripening, in particular at the cheese surface, is probably linked to the increasing pH in this region favouring the action of PA. Although no studies have directly reported the pH optimum of PA, it is generally accepted that they have alkaline pH optima: for instance, assays for PA are generally performed at alkaline pH values [22, 34].

In terms of relative contribution to proteolysis, plasmin is an alkaline protease with a pH optimum around 7.5 [4], and thus increasing pH should generally favour the activity of plasmin during the later stages of ripening. Thus, in general, the changing conditions during ripening should move from an initial environment which is not optimal for plasmin activity (pH~5.0, as common for many varieties) to conditions far more conducive to plasmin action. Concomitantly, the increasing cheese pH will move further from the optimum conditions for chymosin activity (pH optimum~4.0), the other major agent of primary proteolysis of casein during cheese ripening. These effects should be more pronounced at the cheese surface than in the core region, which corresponds closely to the patterns of proteolysis observed. Activities of both plasmin and chymosin during cheese ripening, and the effects of varying pH conditions during ripening and between regions of the cheese, were clearly evident from urea-PAGE electrophoretograms. The difference in rates of proteolysis between surface and core regions suggests that plasmin contributes more significantly to

proteolysis in this cheese variety than chymosin. As well as well-characterised proteolytic pathways known to arise from the respective actions of these two enzymes, such as those leading to production of  $\gamma$ -caseins and  $\alpha_{s1}$ -I-casein, enzymes of *Brevibacterium linens* are likely to have contributed to breakdown of both  $\alpha_{s1}$ - and  $\beta$ -caseins [7, 10]. Extracellular enzymes of *B. linens* have been reported to hydrolyse  $\alpha_{s1}$ -casein more readily than  $\beta$ -casein [10] and to have alkaline pH optima [7], which would suggest that the surface of the cheese would exhibit significant  $\alpha_{s1}$ -casein breakdown during ripening. However, this was not clearly evident, even in control cheese, suggesting that the relative importance of chymosin activity at the core was proportionately higher than that of extracellular proteinases at the surface.

Accelerated production of water-soluble N with addition of plasmin to cheese milk has previously been reported [13, 14]. The elevated levels of proteolysis in cheese made from milk the SCC of which had been increased by mixing with mastitic milk may be due either to a direct contribution of somatic cell lysosomal enzymes to proteolysis, or to activation of plasminogen by cell-associated PA. While few studies have directly examined proteolysis in cheese made from high SCC milk, Klei et al. [21] recently observed faster proteolysis in Cottage cheese made from such milk than control cheese.

Addition of exogenous plasmin clearly accelerated breakdown of  $\beta$ -casein to  $\gamma$ -caseins, as previously observed for Cheddar cheese [13, 14]. Accelerated breakdown of  $\alpha_{s1}$ -casein observed in cheese made from milk mixed with mastitic milk may be due either to increased chymosin activity in the cheese (although cheese pH and moisture contents were similar to those in cheese made from milk with added plasmin, where increased  $\alpha_{s1}$ -casein breakdown was not observed) or to a direct contribution of lysosomal enzymes such as cathepsin D,

which is known to hydrolyse  $\alpha_{s1}$ -casein with a specificity similar to that of chymosin [26]. It has been previously reported that when high SCC milk was added to cheesemilk used for manufacture of Swiss-type cheese, accelerated breakdown of  $\alpha_{s1}$ -casein during ripening relative to control cheese was apparent [9].

## 5. CONCLUSION

Addition of exogenous plasmin, or increasing SCC of milk by inclusion of mastitic milk in the cheese milk, had relatively minor effects of composition of cheese, although rates of loss of moisture from cheese during ripening differed between experimental treatments. Primary proteolysis during cheese ripening, however, was significantly influenced by addition of plasmin or mastitic milk, with both treatments increasing proteolysis of  $\beta$ -casein during the early stages of ripening. Thus, natural variations in milk quality may have implications for the quality of this cheese type, and perhaps other smear-ripened cheese varieties, possibly related to changes in pH during cheese ripening. The magnitude of changes observed was perhaps less than previously reported for similar studies of cheese varieties such as Cheddar. This may be related to the relatively greater changes in environmental conditions in smear-ripened cheese during ripening (such as cheese pH and moisture content), which give rise to differences in rates of proteolysis between cheese regions and at different stages of ripening of greater magnitude than those effected by variations in milk quality.

## ACKNOWLEDGEMENTS

This research was partly funded by grant aid under the Food Sub-Programme of the Operation Programme for Industrial Development,

which is administered by the Department of Agriculture, Food and Rural Development and supported by National and EU funds. The authors wish to acknowledge the technical assistance of D. Waldron at University College Cork.

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