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Original article

Limited ripening of low-fat UF-cheese due to $CaPO_4$ barrier?

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Abstract – The ripening of industrial soft cheeses manufactured using a liquid pre-cheese produced from the ultrafiltration (UF) of milk was observed to be slow in comparison to that of cheese manufactured by a traditional process. Moreover, in the UF-cheeses investigated in this study, which were produced using *Penicillium camemberti* as the surface flora, several surface defects were observed: the texture was 'carton' like and the rind frequently detached from the cheese. To gain a fuller understanding of the development of these surface defects in UF-cheeses, the migration of different minerals and ions, and the study of the rind microstructure by scanning electron microscopy and X-ray mapping were performed. The results suggest that the slower diffusion of lactate, possibly due to the mineral layer at the surface of cheeses, acting as a barrier to its diffusion, may have caused an alteration in the metabolism and growth of the surface mould and may explain the surface defects of these UF-cheeses.

surface-mould cheese / UF-cheese / calcium phosphate / migration / lactate

摘要 – $CaPO_4$ 是否阻碍作用限制了低脂 UF 干酪的成熟。在工业生产中，用超滤截留物 (UF) 生产出软质干酪的成熟时间低于传统方法生产的软质干酪。本文研究了以 *Penicillium camemberti* 作为表面菌落的 UF-卡门贝干酪出现的表面缺陷问题，如表面像硬纸板，外壳破裂和与内部干酪分开等问题。 为了深入地研究 UF-干酪产生表面缺陷的原因，研究了不同矿物盐和离子的迁移特征，以及通过扫描电镜和 X-射线映射图谱研究了干酪外壳的微观结构。 试验结果表明乳酸盐的扩散速度较慢，可能是由于干酪表面的矿化层障碍了乳酸盐的扩散，进而影响了表面霉菌生长和代谢。 这一现象解释了引起 UF-干酪表面缺陷的原因。

表面霉菌干酪 / 超滤干酪 / 磷酸钙 / 迁移 / 乳酸盐

Résumé – L'affinage limité des fromages maigres produits par UF s'expliquerait-il par la présence d'une barrière de phosphate de calcium ? Les fromages à pâte molle industriels produits par ultrafiltration (UF) à partir d'un pré-fromage liquide présentent un affinage retardé par rapport à des fromages produits à l'aide d'un procédé traditionnel. De plus, des fromages UF

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modèles produits avec seulement *Penicillium camemberti* comme flore de surface montrent une texture de carton et la croûte se détache souvent du fromage. La caractérisation des migrations des minéraux et des ions et l'étude de la microstructure de la croûte de ces fromages modèles par microscopie électronique à balayage et micro-analyse X ont été réalisées afin de comprendre l'origine de ces défauts. La diffusion retardée du lactate, peut-être due à la présence d'une couche minérale à la surface des fromages, pourrait être à l'origine de changements de métabolisme et de la croissance de la moisissure et pourrait expliquer la moindre qualité des fromages.

fromage à pâte molle / fromage UF / phosphate de calcium / migration / lactate

1. INTRODUCTION

The use of ultrafiltration (UF) technology in cheese-making has many potential benefits including a higher plant capacity, increased cheese yield, homogeneity in the quality of the resultant cheeses and decreased rennet usage in comparison to traditional practices. However, many issues still remain to be resolved and understood before the ripening of these types of cheeses more closely resembles that of traditional cheeses.

Many previous studies have reported that the ripening of cheeses made from ultrafiltered milk (UF-cheeses) is slower or delayed in comparison to cheeses manufactured by traditional methods [1, 5, 6, 8, 9, 14, 17, 23]. These studies have reported that in UF-cheeses, the level of proteolysis is low in comparison to traditional cheeses and consequently the ripening of these cheeses is delayed. Several hypotheses have been proposed in an effort to explain these phenomena: low residual rennet concentration [9], increased concentration of whey proteins that are thought to be largely resistant to rennet hydrolysis [6, 13], an inhibition of rennet or plasmin activity by whey proteins [1, 5, 13], the pumping and homogenisation of milk and a higher buffering capacity that retards lactic starter autolysis [25, 26].

A major difference between traditional cheese and UF-cheese is the different cheese matrix structures. Previous studies have reported that this modification in the matrix structure results in an alteration in the growth, lysis and distribution of the microflora which is thought to significantly contribute to the delayed ripening of UF-cheeses. For example, Saboya et al. [25, 26] reported that the *Lactococcus* starter used in the manufacture of UF-cheese did not lyse, but on addition of a crude broken bacterial suspension proteolysis increases, highlighting the important role that starter lysis plays in enhancing flavour development. It has also been shown in UF-cheese that the lysis of even highly autolytic strains of mesophilic *Lactococcus lactis* was delayed [11], with significant cell lysis beginning only after four weeks, while in traditional cheeses lysis of the same strain has been reported to occur from the start of ripening. This study also reported that strains of thermophilic *Lactobacilli* behave differently in UF-cheeses compared to their behaviour in traditional cheeses. In a more recent study [12], the pH at which rennet was added was shown to alter the microenvironment of the growing cells, affect the growth of the starter, and hence the induction of lysis. Nevertheless, in UF-cheeses a lower level of proteolysis has been reported, despite significant levels of lysis being detected (Lortal, personal communication). Therefore, other phenomena must be involved, which explain the delayed ripening observed in UF-cheeses and lead to alterations in the rates of diffusion and distribution of salts.

The aim of this study was to further investigate and understand the limited ripening of industrially manufactured UF-cheeses, through the characterisation of the migration of different minerals. Microscopic observations on the aspect of the mould layer at different stages of ripening were also performed and compared to those of traditional cheeses.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Raw milk was initially skimmed using a cream separator (Westfalia, Chateau-Thierry, France). The cream and skim milk were subsequently recombined to yield semi-skimmed milk (120 g·fa·kg^{-1} cheese). Following pasteurisation (LHLT), the milk was ultrafiltered to achieve a volume reduction factor of 6 using Membralox membranes having a pore size of 0.05 μm, a surface area 1.6 m^2 and a molecular weight cut-off of 100 kg·mol^{-1} (Pall Industrie, Saint Germain-en-Laye, France) at 50 °C. The resultant retentate was subsequently cooled to 35 °C before the addition of mesophilic starters MM 100 (10^{-4} units·100 kg^{-1} retentate, time of acidification ≈ 8–10 h at 10–12 °C, Danisco, Dangé-Saint-Romain, France), *Penicillium camemberti* LV2 (2 doses 1000 kg^{-1} retentate, Danisco) and rennet (Maxiren 600-DSM, Lille, France) (5 mL·100 kg^{-1} retentate). Finally, the retentate was poured into individual round moulds of 10-cm diameter and 4-cm height. The moulds were left for 10–12 h at 35 °C to acidify before the cheeses were cooled to 15 °C, demoulded the following day, salted by immersion into saturated brine and ripened for 9 days at 12 °C at a relative humidity of 95–98%. At the end of ripening, the cheeses were wrapped, placed into boxes and stored at 2–4 °C for up to 50 days.

2.2. Cheese samples

Cheeses from the same batch (same date of manufacture) were sampled throughout ripening on days: 2 (after brining), 5 (appearance of mycelia of *P. camemberti*), 9 (day before packaging), 12 (after packaging) and 40 and 50 (end of shelf life). Analyses were performed for each cheese on three zones: (1) the rind, (2) the cheese surface and (3) the core zones. The preparation of these three zones was standardised as follows (Fig. 1): cheeses were maintained at 4 °C before being cut. The rind zone was defined as the outer layer that was essentially composed of mycelia. This layer was scrapped off the surface of each cheese using the blade of a knife, which minimised the amount of cheese from the surface. The surface zone was defined as a layer of ~ 1 mm into the cheese from the upper surface and was cut using an electrically powered 'meat-slicer' rotary blade type in which the thickness of the 'slice' could be controlled and standardised. The core zone was taken from within the cheese mass in a similar fashion, as a 2-mm layer in the core of cheese. All cheese samples were grated, mixed and analysed immediately or stored at 4 °C until use. In this study, the rind fraction was mainly composed of mycelia and is not thought to be indicative of the cheese.

2.3. Physico-chemical analyses

The following analyses were performed at each time point for each cheese: pH, indices of proteolysis (non-casein nitrogen, NCN and non-protein nitrogen, NPN), minerals (potassium, phosphate and calcium) and lactate.

The pH was measured using a standard CG837 pH meter with InLAB 427 electrodes (Mettler Toledo, Viroflay, France) by direct insertion into the grated cheeses. Three measurements were taken and the average result calculated.

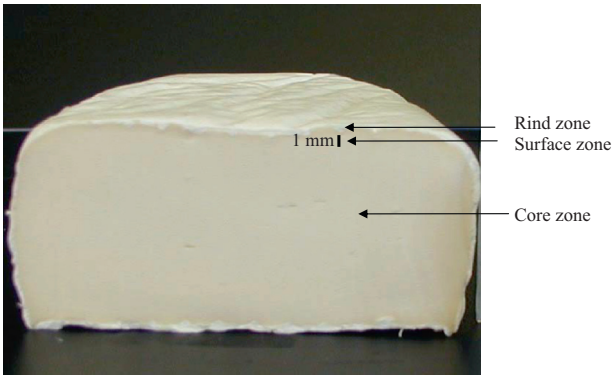

Figure 1. Definition of the three zones used in the analysis of the cheese: (1) rind, (2) surface and (3) core.

Total protein in cheese was determined by the Kjeldahl method using a conversion factor of 6.38 [15]. Nitrogen soluble at pH 4.4 (NCN) [10] and 150 g·kg^{-1} TCA-soluble N (NPN) [10] were measured and protein breakdown was assessed by the calculation of NCN/TN and NPN/TN.

Mineral contents were determined as follows: 3 g of grated cheese were homogenised with 100 g of 0.02 mol·L^{-1} nitric acid solution to obtain a pH of ~ 3 leading to total mineral solubilisation; samples were left for 1 h at room temperature before being filtered through 0.45 µm filters (Minisart, Sartorius, Göttingen, Germany). Calcium (Ca) and potassium (K) concentrations were determined using atomic absorption spectrometry (Varian AA 300 spectrometer, Les Ulis, France) according to Brulé et al. [4] and inorganic phosphate (P) and lactate were determined by ion chromatography (Dionex, Jouy-en-Josas, France) according to Gaucheron et al. [7].

2.4. Scanning electron microscopy of the rind

Samples (3 × 3 × 10 mm^3) of cheese were cut perpendicularly from the cheese surface, fixed in 12.5 g·kg^{-1} glutaraldehyde dissolved in water and stored at 4 °C. Samples were rinsed five times in Milli-Q water, dehydrated by washing in a series of gradually increasing concentrations of acetone (50%, 75%, 95%, 100% and twice in absolute acetone) and critical point dried using carbon dioxide. The dried samples were mounted with Araldite onto an observation holder of 32-mm diameter, cut with a razor blade before coating with gold palladium (40 nm thick) in a Polaron sputter (E5100) and examined using a scanning electron microscope (Hitachi 3000-N, Elexience, Verrières-le-Buisson, France) operating at 4 kV.

2.5. X-ray microanalyses in scanning electron microscopy

For X-ray microanalyses, the samples as described above were coated with a carbon coating (using an apparatus E5000-376) and examined using energy-dispersive spectroscopy (EDS) (INCA, Oxford Instruments, Saclay, France) on the same Hitachi microscope at 15 kV. This EDS technique was used to identify the photons emitting from carbon (C), oxygen (O), phosphorus (P), calcium (Ca) and sulphur (S).

For each of the two replicate cheese samples, five separate areas of 30 × 30 μm (10 pixels) were analysed from each of the three zones (rind, surface and core) to show the relative composition of the cheeses. Photons emitted from C, O, P, Ca, S and total X photons were counted and an average value calculated for each zone. To reduce the effect due to differential adsorption, the number of each type of photons (C, O, P, Ca and S) was divided by the total number of photons emitted from the sample and expressed as a percentage.

Also, photons emitted from Ca and P along a profile (a continuous series of 30 μm^2 areas) extending from the surface into the core of the cheese were measured. Along this profile, mineral contents were expressed as the percentage of (Ca + P) content of each area divided by the (Ca + P) content of the core.

Two samples per cheese at 22 and 50 days of ripening were prepared. Preliminary observations were performed at low voltage (1 kV) to facilitate the identification of relatively flat areas suitable for the analyses. Observations performed at low tension minimised the production of charge artefacts and resulted in more accurate images. For analyses, a tension of 15 kV was used.

3. RESULTS

For each of the analyses performed, the results were compared to the data previously reported in the literature for traditional Camembert cheeses [16, 18, 20, 22]. However, a direct comparison of the results of this study and those of previous studies can only be qualitative and not quantitative due to the definition of the zones analysed in each study. Moreover, the microbial flora of traditional Camembert-type cheeses is much more complex (containing several strains of lactic acid bacteria as well as yeast, mould, etc.) than the industrial cheeses investigated in this study.

3.1. Development of mycelia of *Penicillium* and evolution of pH

The mycelia of *P. camemberti* first became visible on the surface of the cheeses by day 5. By day 9, the white growth thickened and began to fill the grooves in the cheese rind. Between days 9 and 12, a very rapid growth was observed on the surface of the cheeses that were completely covered in a thick layer of mycelia of *P. camemberti* by day 12 (data not shown).

The pH of the rind, surface and core fractions throughout ripening is shown in Figure 2A. The pH increased rapidly in the rind between days 9 and 12 ($\Delta pH_{12\text{-}9}$ = 1.7), which corresponded closely to the rapid growth of *P. camemberti* during this period. The pH in the core fraction remained stable up to day 23 after which it increased from 5.15 to 6.50 by the end of ripening. An increase in the pH of all zones is typical for traditional Camembert cheese, especially at the rind and surface fractions [19, 23]. However, in comparison to traditional Camembert cheeses the pH of the UF-cheese was observed to be 0.5–1.0 pH units higher at the start of ripening, but 0.3 pH units lower in the surface zone by day 23 [18]. Despite the higher pH detected in the UF-cheese from day 0 to 9, a further ~ 2–3 days was required before the pH increased in the rind fraction. Moreover, in traditional Camembert cheese the pH of the surface fraction reached 6.5 after 20 days [18], while in the UF-cheeses in this study it took ~ 30 days.

3.2. Lactate

The concentration of lactate in each of the different fractions decreased from day 2 to the end of ripening, due to its use as a carbon source by *P. camemberti* (Fig. 2B). As mycelial growth occurs only in the rind fraction of cheese, a gradient of lactate was generated from the core

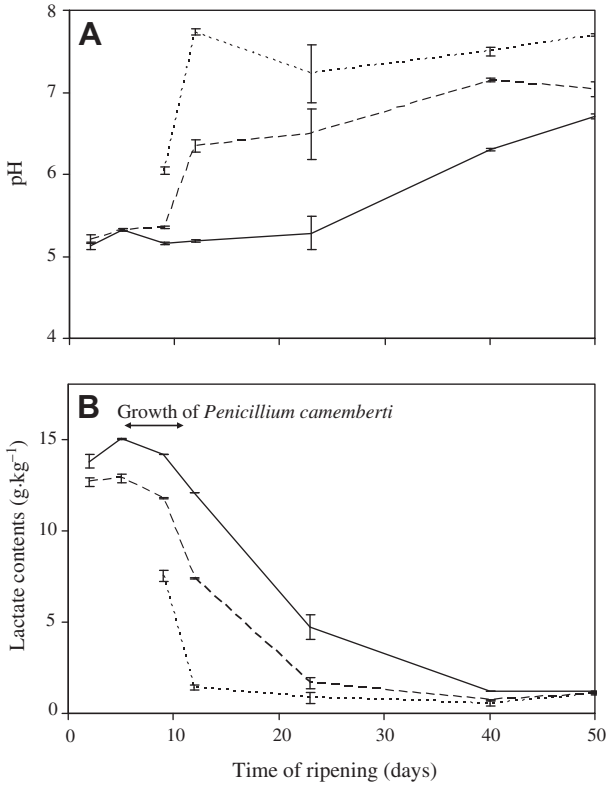

Figure 2. (A) pH and (B) lactate concentration of the rind (.....), surface (- - - - -) and core (——) fractions of UF-cheese throughout ripening. Mean values and SDs of pH values are presented. A larger number of samples (nine samples) were included in the data set at day 23, which resulted in a larger SD.

to the surface. In traditional Camembert-type cheese, the initial lactate concentration in both the rind and core fractions of the cheese is 10–17 $g \cdot kg^{-1}$ and decreases to 0–1 $g \cdot kg^{-1}$ at ~ day 30 [20]. In UF-cheese, a similar decrease in concentration took 40 days.

3.3. Proteolysis

The levels of NCN/TN and NPN/TN are used as indices of proteolysis and are shown in Figure 3. NPN/TN is an indication of the production of small peptides and free amino acids that are important for the development of texture and flavour. At day 2, NCN represented about 10% of the total nitrogen in both the surface and core fractions that increased to 24% and 14% by day 9, respectively. Indices of proteolysis (NCN or NPN) were much higher in the rind fraction throughout ripening (from 55% to 77%) due to the mycelial layer of *P. camemberti* and the extensive production of high and low molecular weight peptides, as well as free amino acids. Between days 9 and 12, a reduction in the level of proteolysis (from ~ 70% at day 9 to 50% at day 12) was

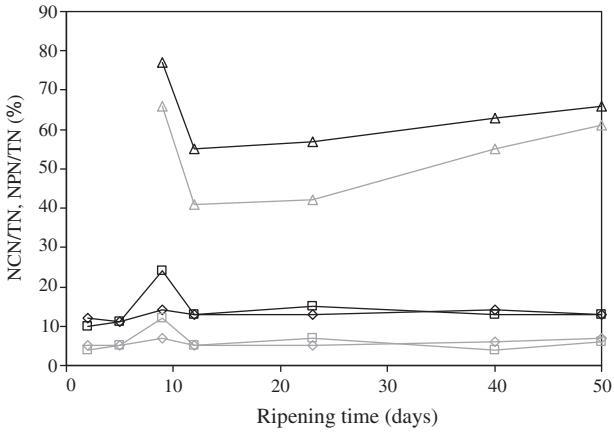

Figure 3. Levels of proteolysis expressed as the ratio of non-casein nitrogen to total nitrogen (NCN/TN in %, black symbols) and the ratio of non-protein nitrogen to total nitrogen (NPN/TN in %, grey symbols) in the rind (Δ, Δ), in the surface (□, □) and in the core (◊, ◊) of UF-cheese.

detected in the rind fraction and corresponded to the rapid growth of the surface flora. A similar reduction (from ~ 20% to 10%) was observed in the surface fraction.

3.4. Mineral concentration

The concentrations of calcium (Ca) and inorganic phosphate (P) in the surface and core fractions are shown in Figure 4. Both Ca and P concentrations increased in the surface fraction and decreased in the core fraction, such that, by day 9, at the moment of rapid growth of *P. camemberti,* the difference detected between the core and surface fractions was 125 mmol·kg^{-1} Ca and 150 mmol·kg^{-1} P. A similar increase in the concentrations of both Ca and P had previously been shown by Le Graët and Brulé [18]. These authors suggested that the solubility of both ions is reduced at the surface due to the increase in pH, and hence are deposited. Consequently, the levels of both ions decreased in the core fraction, due to their diffusion towards the surface of cheese.

The concentration of potassium (K) in the rind fraction rapidly increased between days 9 and 12, remained stable up to day 23 with a maximum at 7.7 g·kg^{-1} before decreasing to 5 g·kg^{-1} by day 40. In traditional Camembert cheese, the concentration of K in the rind has been reported to reach a maximum concentration of 3.2 g·kg^{-1} by day 9 before returning to its original value (1–1.5 g·kg^{-1}) by day 20 [18]. Hence, in UF-cheese, the concentration of K remained elevated in the rind throughout ripening and reached higher levels compared to those reported for traditional Camembert cheese. By the end of ripening, the concentration of K did not resume its initial concentration, while in traditional Camembert cheese this initial level was resumed after day 19.

3.5. Scanning electron microscopy

Figure 5A shows the scanning electron micrographs of cheeses at days 5, 9, 12 and 40 of ripening. Mycelia began to appear by day 5 and were seen as a thin layer of mycelial growth (80 ± 30 μm). By day 9, the entire cheese surface was fully covered with a layer of mycelia (500 ± 300 μm). A zone with more compacted mycelia was also observed as a layer next to the cheese surface, which may be composed

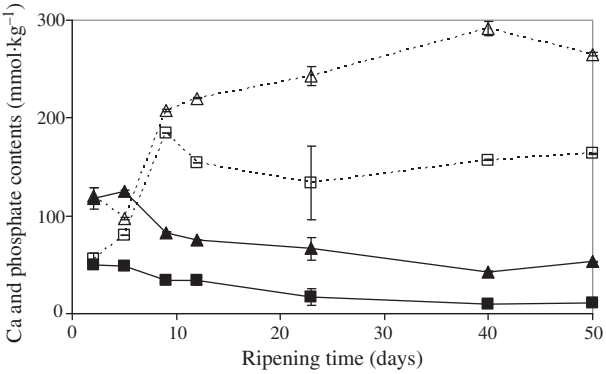

Figure 4. Concentrations of calcium (▲, Δ) and phosphorus (■, □) during the ripening of UF-cheese in the surface (open symbol and dotted lines) and the core fraction (close and continuous lines) of UF-cheese. Mean values and SDs are presented.

of degenerating mycelia. By day 12, a thick layer of mycelia (400 ± 100 μm) was observed and showed successive zones of compacted and more aerated mycelia. At the surface of the cheese, just under the layer of mycelia, a white zone (120 ± 40 μm) appeared in the upper surface of the cheese matrix, which probably contained a high concentration of calcium phosphate crystals. It is well known that as the pH increases, alkaline forms of calcium phosphate are formed with a higher ratio of Ca to phosphate, which has a much lower solubility [2, 19]. In some areas, a zone showing the clear separation of the mycelia from the cheese surface became visible (data not shown), and appeared to separate above the zone containing high concentrations of the white zone of calcium phosphate. At day 40, the surface flora was up to 900 ± 600 μm thick and appeared to have a layered structure containing several different zones. The zone of calcium phosphate is white and very visible with a thickness of 170 ± 70 μm.

In comparison, a commercial traditional low-fat (10% fat) soft mould surface-ripened cheese (Fig. 5B) bought from a local market at day 30 of ripening showed a much thicker rind zone and had no pronounced white layer in the cheese surface zone. Only, a crumbly zone at the cheese surface seemed to be present, containing traces of mycelia.

3.6. Analysis of the mineral layer by X-ray microanalysis

Results on the reproducibility of emitted photon measurements showed that C was homogeneously distributed in the three defined zones, whereas the concentrations of both Ca and P were greater in the cheese surface (Tab. I) that corresponded to the white mineral zone.

Figure 6 presents an example of the X-ray microanalysis on day 50. The concentration of each of the elements was measured from the surface into the core of UF-cheese by mapping a rectangular section composed of a continuous series of 30 μm^2 areas (coloured in black on Fig. 6A). The Araldite zone was sulphur rich (Fig. 6B), while a phosphate and calcium-rich zone was seen in the surface fraction of cheese (0.65 mm from the outer layer) at day 22 which became more intense by day 50 indicating increasing concentrations. The thickness of this zone was around

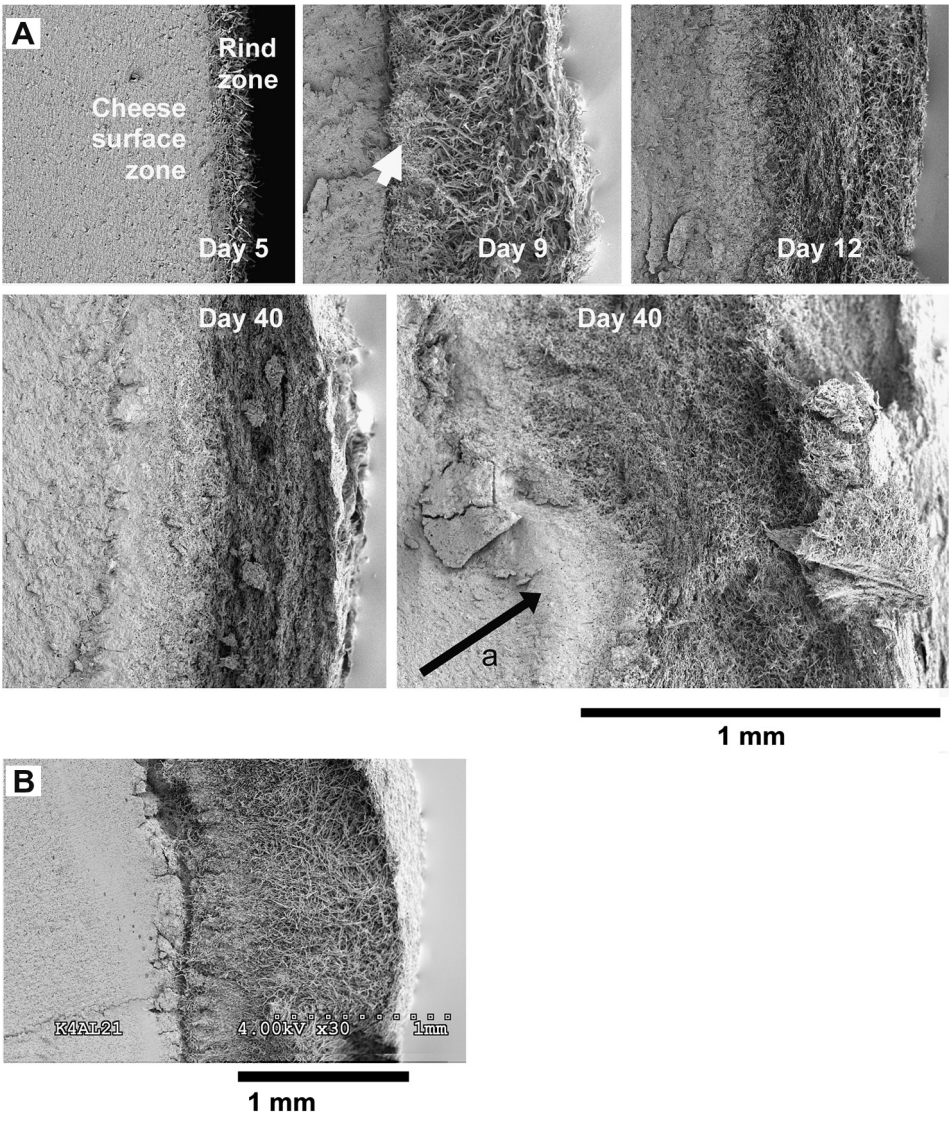

Figure 5. Scanning electron micrographs of cheese: (A) UF-cheese during ripening at days 5, 9, 12 and 40 showing the rind and the surface zones of cheese. White arrow shows the compacted zone of mycelia at day 9 and black arrow shows the white mineral zone at day 40. (B) Commercial mould-ripened cheese made using a traditional process.

118 ± 16 μm (± SD) at day 22, and had increased to 164 ± 14 μm by day 50. Calculation of the ratio of (Ca + Pi) detected at any point to that detected in the core reflected the increase in concentration of Ca and P as a function of distance

Table I. Number of counted emitted photons in the three defined zones of UF-cheese, expressed as a percentage of total emitted counted photons. The average of 10 points on two samples at each ripening time is given (mean value ± SD)[1].

Zones	Carbon	Oxygen	Phosphorus	Calcium	Total
Day 22					
Rind	42.9 ± 3.2^{a}	9.4 ± 1.8^{a}	3.0 ± 1.0^{a}	1.6 ± 0.3^{a}	56.9 ± 3.4^{a}
Surface	37.9 ± 4.6^{a}	8.6 ± 1.9^{a}	5.2 ± 0.9^{b}	4.8 ± 1.1^{b}	56.4 ± 1.3^{a}
Core	49.3 ± 3.6^{b}	6.4 ± 1.4^{a}	2.4 ± 0.4^{a}	1.9 ± 0.5^{a}	60.0 ± 4.5^{a}
Day 50					
Rind	41.1 ± 4.5^{a}	14.5 ± 2.5^{a}	3.0 ± 1.6^{a}	1.9 ± 1.9^{a}	61.4 ± 3.0^{a}
Surface	36.0 ± 4.0^{b}	10.8 ± 2.4^{b}	5.6 ± 0.9^{b}	5.7 ± 1.3^{b}	58.4 ± 4.5^{bc}
Core	50.6 ± 1.7^{c}	7.5 ± 0.6^{c}	2.4 ± 0.3^{a}	2.1 ± 0.4^{a}	63.7 ± 1.9^{ac}

[1] Values within a column not followed by the same letter differ significantly ($P < 0.05$).

(Fig. 6C). A maximum concentration of both these minerals was observed at a zone 0.65 mm from the rind at day 22 and had thickened by day 50. This analysis clearly indicates that this increase in intensity is a reflection on this mineral zone becoming richer in calcium and phosphorus as ripening progresses.

4. DISCUSSION

The industrial Camembert-type cheeses produced from UF retentate and analysed in this study had several surface defects: the mycelia of *P. camemberti* were observed to peel away from the cheese surface in places and the texture of the cheese was described as 'carton' like. The ripening of the cheeses was considered to be much slower than that of traditional Camembert cheese. Also, the core fraction of this study was taken as a 2-mm layer in the centre of the cheese and not the entire cheese mass as in some other studies.

The ripening of soft mould-ripened cheeses such as Camembert involves the mass transfer of minerals (sodium, calcium, etc.), protons, nitrogen and lactate that migrate between the core and the surface or in reverse from the surface to the core of the cheese due to the activity of the surface microflora. This migration leads to changes in the cheese micro-environment, which have further implications for the growth of micro-organisms, texture as well as the ripening and flavour development of these types of cheeses. In general, for traditional Camembert cheese, the faster this migration occurs, the quicker is the ripening. However, overstimulation of the growth of the surface microflora may lead to the exhaustion of carbon substrates, lactose and lactate, and hence enhance the breakdown of proteins and lipids by the proteolytic and lipolytic systems of the microflora for energy generation.

In the cheeses reported in this study, a severe increase of 1.7 pH units was observed in the rind fraction between days 9 and 12 and was concomitant with the visible growth of the mycelia on the surface. Also during this period, the concentration of lactate in the surface rapidly depleted, but remained elevated in the rind and core fractions. However, the levels of NCN/NT and NPN/NT showed a significant reduction between days 9 and 12, suggesting that these were used as an alternative source of nutrients and could potentially alter the metabolism of *P. camemberti* and may account for the layered structures

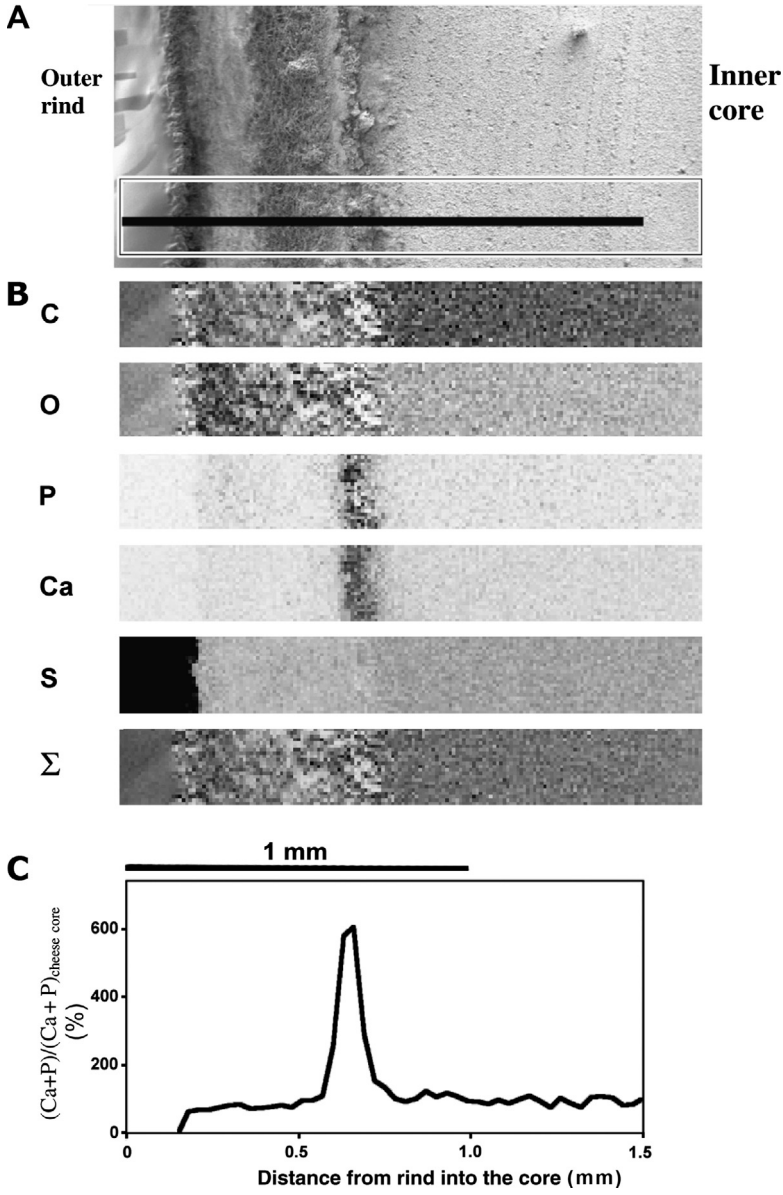

Figure 6. X-ray analysis of UF-cheese at day 50. (A) High-resolution image of secondary electrons showing the cartography zone of analysis. (B) Cartography of carbon (C), oxygen (O), phosphorus (P), calcium (Ca), sulphur (S) and of total X photons (Σ). (C) Changes in (Ca + P) along the black line drawn in (A) as a percentage of (Ca + P) in the core zone.

seen in the scanning electron micrographs. Leclercq-Perlat et al. [20] have reported that the concentration of viable *P. camemberti* can be correlated with the carbon surface concentrations, namely lactate.

Also at this time, a layer of calcium phosphate was deposited due to the increase in pH. This layer, not observed in traditional cheese, could be considered to act as a barrier to the diffusion of protons/lactate from the core to the surface, and hence alter the growth of the mycelia. The surface fraction also showed an increase of ~ 1 pH unit, while the pH of the core remained constant until day 23. This sudden increase in pH in the first few millimetres of the cheese body represented an enormous change in the cheese matrix environment and is considered to play a significant role in the precipitation of crystals of calcium phosphate in the surface zone that is clearly visible on the scanning electron micrographs on day 12. Precipitation of calcium phosphate depends on the solubility of calcium salts in relation to the pH of cheese as shown in Le Graët et al. [19].

In comparison to traditional Camembert cheeses, the UF-cheeses had a higher pH (5.2 compared to 4.7 in traditional Camembert) between days 0 and 9 and the increase in pH at the rind and surface zones was slower. This is thought to reflect the higher concentration of lactate, which remained in the UF-cheese (in the surface and core fractions), as well as the lower rates of proteolysis. Also, the rate at which the concentration of lactate decreased to 0–1 $g{\cdot}kg^{-1}$ in the core fraction of UF-cheese was observed to be slower (40 days) in comparison to traditional Camembert cheese (~ 30 days) and suggests that the consumption of lactate in the rind or its diffusion from the core to the surface was delayed. The pH also increased in the core region in the UF-cheese, but only after 23 days of ripening, while it begins to increase between 7 and 9 days in traditional Camembert cheese. This indicates a delay in the diffusion of protons during the initial stages of ripening, due either to a slow growth of *P. camemberti* or to a reduction in the rate of diffusion of protons due to the different structural network of UF-cheeses as compared to traditional Camembert cheese.

The indices of proteolysis detected in the core fraction did not increase throughout ripening (NCN and NPN were ~ 13% and 7%, respectively, at day 50). According to Schlesser et al. [27], in traditional Camembert cheese, NCN and NPN represented ≈ 9% of the total nitrogen at the beginning of ripening and increased to ≈ 60% and 50%, respectively, by the end of ripening. Lenoir [22] reported NPN values from 5% to 15% in the internal part of Camembert cheese and from 4% to 22% in the surface part of the cheese between days 5 and 32. The difference in these values highlights the low level of proteolysis in UF-cheeses compared to traditional cheese.

According to Le Graët and Brulé [18] and Le Graët et al. [19], the migration of K is due, firstly to a consumption of K by the growing mycelium of *P. camemberti* and secondly by the release of K in the rind fraction, due to a change in the permeability of the mould cell membrane or to the mould lysis. The high levels of potassium detected in the cheese rind fraction were considered as abnormal and reflected that some alteration in the metabolism of the growing mycelia has occurred compared to a traditional process. This result suggests that this ion may be used as an indicator of an altered growth or metabolism of the mycelia.

The occurrence of a mineral zone, in the surface part of the cheese, rich in calcium phosphate crystals, may prevent the mycelia from penetrating the cheese body and/or prevent the diffusion of solutes such as lactate towards the rind and possibly is the cause of the ‘carton’ texture of the rind and its break away from the cheese. Calcium phosphate crystals became clearly visible as a white zone at day 12 and were deposited in the surface fraction, concomitant with

the severe increase in pH and the growth of the surface flora. The mineral zone of crystals increased in thickness and quantity throughout ripening and appeared to be one of the reasons for the rind fraction to break away from the surface of the cheese. As this mineral layer was not present in traditional cheeses, this zone is thought to hinder the diffusion of solutes from the core to the surface of the cheese.

X-ray microanalysis using a scanning electron microscope clearly showed the presence of the calcium-phosphate-rich zone. Although this method is not well developed in substrates such as cheese, the digital X-ray mapping of a fracture surface of Camembert cheese [3] and the identification of calcium phosphate crystals in processed cheeses [24] have previously been reported. A zone just under the rind, 75–250-μm thick, with individual crystal aggregates of calcium phosphate has already been shown [3] and was confirmed in this study.

5. CONCLUSION

The analyses performed in this study showed the presence of a zone rich in calcium phosphate which was not present in traditional Camembert cheese. It is thought that this zone could act as a barrier to significantly reduce the rate of diffusion of various substrates and potentially alter the metabolism of the growing mycelia of *P. camemberti*, and hence contribute to the slow ripening of these cheeses and creation of the surface defects in comparison to traditional Camembert cheese.

REFERENCES

[1] Bech A.M., Characterising ripening in UF-cheese, Int. Dairy J. 3 (1993) 329–342.

[2] Bouler J.M., Caractérisation et propriétés des orthophosphates de calcium, in: Gaucheron F. (Ed.), Minéraux et produits laitiers, Tec et Doc, Paris, France, 2004, pp. 25–54.

[3] Brooker B.E., The crystallization of calcium phosphate at the surface of mould-ripened cheese, Food Microstr. 6 (1987) 25–33.

[4] Brulé G., Maubois J.L., Fauquant J., Study of mineral salts in products obtained by milk ultrafiltration, Lait 54 (1974) 600–615.

[5] Creamer L.K., Iyer M., Lelievre J., Effect of various levels of rennet addition on characteristics of Cheddar cheese made from ultrafiltered milk, N. Z. J. Dairy Sci. Technol. 22 (1987) 205–214.

[6] De Koning P.J., De Boer R., Both P., Nooy P.F.C., Comparison of proteolysis in a low-fat semi-hard type of cheese manufactured by standard and by ultrafiltration techniques, Neth. Milk Dairy J. 35 (1981) 35–46.

[7] Gaucheron F., Le Graët Y., Piot M., Boyaval E., Determination of anions of milk by ion chromatography, Lait 76 (1996) 433–443.

[8] Goudédranche H., Utilisation du lysozyme en tant qu'agent régulateur de l'affinage en fromagerie MMV, Lait 66 (1986) 189–206.

[9] Green M.L., Glover F.A., Scurlock E.M.W., Marshall R.J., Hatfield D.S., Effect of use of milk concentrated by ultrafiltration on the manufacture and ripening of cheddar cheese, J. Dairy Res. 48 (1981) 333–341.

[10] Gripon J.C., Desmazeaud M.J., Le Bars D., Bergère J.L., Étude du rôle des microorganismes et des enzymes au cours de la maturation des fromages. II – Influence de la présure commerciale, Lait 55 (1975) 502–516.

[11] Hannon J.A., Deutsch S.M., Madec M.N., Gassi J.Y., Chapot-Chartier M.P., Lortal S., Lysis of starters in UF cheeses: behaviour of mesophilic lactococci and thermophilic lactobacilli, Int. Dairy J. 16 (2006) 324–334.

[12] Hannon J.A., Lopez C., Madec M.N., Lortal S., Altering renneting pH changes microstructure, cell distribution and lysis of *Lactococcus lactis* AM2 in cheese made from ultrafiltered milk, J. Dairy Sci. 89 (2006) 812–823.

[13] Harper J., Iyer M., Knighton D., Lelievre J., Effects of whey proteins on the proteolysis of cheddar cheese slurries (a model for the maturation of cheeses made from ultrafiltered milk), J. Dairy Sci. 72 (1989) 333–341.

[14] Hickey M.W., van Leewen H., Hillier A.J., Jago G.R., Amino acid accumulation in

Cheddar cheese manufactured from normal and ultrafiltered milk, Aust. J. Dairy Technol. 38 (1983) 110–113.

[15] International Dairy Federation, Milk – determination of nitrogen content – Part 2: block-digestion method (macro method), IDF 20-2, ISO 8968-2, Int. Dairy Fed., Brussels, Belgium, 2001, pp. 1–10.

[16] Karahadian C., Linsay R.C., Integrated roles of lactate, ammonia, and calcium in texture development of mold surface-ripened cheese, J. Dairy Sci. 70 (1987) 909–918.

[17] Lawrence R.C., The use of ultrafiltration technology in cheesemaking, Bull. Int. Dairy Fed. 240 (1989) 1–15.

[18] Le Graët Y., Brulé G., Migration des macro et oligo-éléments dans un fromage à pâte molle de type Camembert, Lait 68 (1988) 219–234.

[19] Le Graët Y., Lepienne A., Brulé G., Ducruet P., Migration du calcium et des phosphates inorganiques dans les fromages à pâte molle de type camembert au cours de l'affinage, Lait 63 (1983) 317–332.

[20] Leclercq-Perlat M.N., Buono F., Lambert D., Latrille E., Spinnler H.E., Corrieu G., Controlled production of Camembert-type cheeses. Part I: Microbiological and physicochemical evolutions, J. Dairy Res. 71 (2004) 346–354.

[21] Lenoir J., L'activité protéasique dans les fromages à pâte molle de type camembert, Rev. Lait. Fr. 275 (1970) 231–243.

[22] Lenoir J., Les flores de surface et leur rôle au cours de l'affinage des fromages, Bull. Int. Dairy Fed. 171 (1984) 3–19.

[23] Mistry V.V., Maubois J.L., Application of membrane separation technology to cheese production, in: Fox P.F. (Ed.), Cheese Chemistry, Physics and Microbiology, Chapman & Hall, London, UK, 1993, pp. 493–522.

[24] Pommert J.F., Klaebe A., Perie J., Lebugle A., Puech J., Observation and analysis of crystalline phases in processed cheese, J. Food Sci. 53 (1988) 1367–1447.

[25] Saboya L.V., Goudédranche H., Maubois J.L., Lerayer A.L.S., Lortal S., Impact of broken cells of lactococci or propionibacteria on the ripening of Saint-Paulin UF-cheeses: extent of proteolysis and GC-MS profiles, Lait 81 (2001) 699–713.

[26] Saboya L.V., Lortal S., Lerayer A.L.S., Maubois J.L., Absence of lactococcus lysis in ultrafiltered cheeses, in: 23rd IDF World Dairy Congress, Congrilait, 24–27 September 2002, Paris, 2002.

[27] Schlesser J.E., Schmidt S.J., Speckman R., Characterization of chemical and physical changes in Camembert cheese during ripening, J. Dairy Sci. 75 (1992) 1753–1760.