# Evolution of the Composition of a Selected Bitter Camembert Cheese during Ripening: Release and Migration of Taste-Active Compounds

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The aim of this study was to add to the understanding of changes in taste that occur during the ripening of a bitter Camembert cheese by the evolution of its composition. Physicochemical analyses were performed on rind, under-rind, and center portions of a Camembert cheese selected for its intense bitterness. At each of the six steps of ripening studied organic acids, sugars, total nitrogen, soluble nitrogen, phosphotungstic acid soluble nitrogen, non-protein nitrogen, Na, K, Ca, Mg, Pi, Cl, and biogenic amines were quantified in each portion. Changes in cheese composition seemed to mainly result from the development of *Penicillium camemberti* on the cheese outer layer. Migration phenomena and the release of potentially taste-active compounds allowed for the evolution of saltiness, sourness, and bitterness throughout ripening to be better understood. Apart from taste-active compounds, the impact of the cheese matrix on its taste development is discussed.

**Keywords:** *Camembert cheese; bitterness; ripening; taste-active compounds; matrix effect*

## INTRODUCTION

A first part of this study (*1*) showed that during ripening of a typically bitter Camembert cheese, bitterness increased and reached a plateau, whereas sourness and astringency decreased. This evolution in taste appeared as being more rapid in the rind than in the center portions. In contrast with the other taste attributes, saltiness evolution was different in the rind, where it decreased compared to the other portions, where it increased. This sensory part of the study also showed that cheese taste was explained by the effect of taste-active compounds contained in the water-soluble extract (WSE), the gustatory impact of which was modulated by a matrix effect. Whereas the matrix contribution has already been characterized (*1*), this second part of the study will deal with the evolution in concentration of taste-active compounds.

As it was previously shown that cheese WSE contained cheese taste-active compounds (*1*), this fraction, which was obtained from the same bitter Camembert cheese ripened for 31 days, was used to determine which components are taste-active compounds. By use of a methodology already used to determine taste-active compounds in a goat cheese (*2*, *3*), the main WSE taste attributes of the mentioned Camembert cheese were elucidated (*4*). WSE sourness was explained by an enhancing effect of sodium chloride on the sour taste due to $H_3O^+$ concentration, whereas saltiness was due to the concentration of sodium chloride. WSE bitterness was mainly due to the bitterness of peptides of molecular weight ranging between 500 and 1000 Da (*1*). Thus, knowing the modulating effect of the matrix for each attribute and each ripening time, it was possible to explain a part of the evolution of cheese taste during ripening by following the concentrations of sodium chloride, nitrogen materials, and chemical species acting on pH.

However, omission tests were done on a cheese ripened for 31 days (*4*), and it may be possible that, due to a change of concentration during ripening, some compounds that are taste-active in the beginning of ripening become inactive after 31 days or vice versa. Thus, concentrations of some compounds that were found in other studies as potentially taste-active have also to be followed. A study performed on goat cheese (*3*) suggested that cheese bitterness may be due to chemical species other than peptides. Moreover, this study demonstrated that the bitter taste of the WSE was explained by the bitterness due to calcium chloride and magnesium chloride, which in turn is partially masked by sodium chloride. A possible role of magnesium chloride and/or calcium chloride on the bitterness of cheese had already been mentioned by other authors (*5*, *6*). As the calcium chloride concentration of mold-ripened cheese varies during ripening because of migration phenomena (*7*), it would be interesting to study the evolution of both calcium and magnesium concentrations in each cheese portion at each ripening stage of a cheese of known bitterness (*1*). In the same way, concentrations of selected biogenic amines were followed because Kubickova and Grosch (*8*) found that they were partially responsible for the bitter taste of a Camembert cheese.

The first goal of this study was to understand changes in composition occurring in the center, under-rind, and rind portions of a bitter Camembert cheese during its ripening. By following the concentration of potentially taste-active compounds, it might then be possible to explain the evolution of cheese taste attributes determined in a previous sensory study of the same portions (*1*).

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## MATERIALS AND METHODS

**Chemicals.** All synthetic components used in this study were of analytical grade and were purchased from commercial suppliers. Pure water was obtained from a MilliQ system (Millipore, Bedford, MA).

**Experimental Cheese Samples.** Six vats of the cheese Pc3 were manufactured according to the cheese-making process M2 described by Engel et al. (*1*). At each tasting session, on the 9th, 16th, 21st, 28th, 35th, and 42nd days, 4 cheeses were tasted and 13 cheeses were cut out in three parts (see below) and frozen by submerging in a liquid nitrogen bath for further physicochemical analysis and water extraction.

**Cheese Partition.** After 40 min at −20 °C, the cheeses were divided into three parts with a cutting machine. The portions obtained were named, respectively, rind, under-rind, and center. The rind part corresponded to the maximum thickness of mycelium. For a 35 mm thickness of cheese, the respective thicknesses of rind, under-rind, and center portions were 3, 7, and 15 mm, in order to have a weight of a center portion equal to the weight of two under-rind portions.

**Water Extraction.** *Extraction Procedure.* Each of the frozen cheese portions was grated. Grated cheese was dispersed in pure water (w/w 1:2) and homogenized for 4 min in a 1094 homogenizer (Tecator, Höganäs, Sweden). The suspension was centrifuged at 20000*g* for 30 min at 4 °C. Three phases were separated: a fat solid upper layer, a liquid fraction including water-soluble molecules called C20000, and a pellet containing mainly proteins. After filtration on gauze, the C20000 was recovered. All of these fractions were stored at −80 °C until required.

**Purification Procedure.** Each C20000 was submitted to a 100 kDa frontal ultrafiltration in a cell ($V = 400$ mL, $d = 76$ mm; Millipore, Bedford, MA) with regenerated cellulose membranes (Millipore). The filtration temperature and the transmembrane pressure were, respectively, maintained at approximately 8 °C and 4 Pa. For each of the three cheese portions and each of the six ripening times, two portions of 300 mL of C20000 were successively treated. The recovered permeate, considered as the diluted WSE, was immediately frozen until further use.

**Physicochemical Analysis.** All measurements were done in triplicate.

*pH.* It was measured using a pH meter HI 9017 (Hanna Instruments, Tannerie, France) equipped with a glass electrode.

*Dry Extract.* A drying oven (106 °C) was used to determine the dry matter content.

*Ashes.* One gram of grated cheese was dried for 12 h in an oven at 106 °C and placed for 6 h in a muffle furnace at 450 °C. After 6 h of cooling, 10 mL of 10% ammonium sulfate (Fluka, St Quentin Fallavier, France) was added to the ashes, which were dried for 12 h at 106 °C and placed again for 6 h in the muffle furnace at 450 °C. For further determinations of cation concentrations, the resulting ashes were dissolved in 100 mL of 0.1N HCl solution.

*Minerals.* The analysis of sodium, potassium, calcium, magnesium, and iron contents was performed on WSE with a Spectra 100 atomic absorption spectrophotometer (Varian, les Ulys, France). Determinations for entire cheese were made using ashes dissolved in 0.1 N HCl. Inorganic phosphorus and chloride ions were estimated on the WSE by diagnostic kits (Sigma, St Quentin Fallavier, France).

*Lactose, Galactose, and Organic Acids.* Lactose, galactose, and lactate WSE contents were quantified using external calibration by HPLC with an ion-exchange column (Aminex HPX-87H, 300 × 7.8 mm, Bio-Rad, Hercules, CA) warmed at 60 °C, according to a method adapted from that given by Bio-Rad. The eluent was 5 mM sulfuric acid. The flow rate was 0.8 mL/min. The detection was done by refractometry.

*Nucleotides.* They were quantified on WSE by HPLC with a Synchropack AX anion exchange column (250 × 4.6 mm, Synchrom Inc., Lafayette, IN) and UV detection at 254 nm (*9*).

*Biogenic Amines.* They were determined on WSE by RP-HPLC of the dabsyl derivatives (*10*).

*Nitrogen Determination.* An aliquot of grated cheese was suspended in a sodium citrate solution (*11*). Total nitrogen (TN), nitrogen soluble at pH 4.6 (SN), soluble nitrogen in phosphotungstic acid (PTASN), and soluble nitrogen in TCA (NPN) were determined according to the Kjeldahl method. Peptide content was estimated by the calculation of peptides = SN − PTASN (*12*).

**Statistical Treatment.** The data were processed with the SAS system release 6.12 (SAS Institute Inc., Cary, NC). ANOVA was performed at a level $\alpha = 0.05$. Linear regressions between physicochemical and sensory data were performed with PROC REG.

## RESULTS

**Respective Evolutions of Dry Extract, pH, Lactic Acid, and Sugars in the Different Portions during Ripening.** The rind was the only portion in which dry matter values seemed to change during ripening (Figure 1a). The rind dry matter increased between the 9th and 16th days of ripening and reached a plateau after this period. In the center and under-rind no significant change was observed during the whole maturation time studied. Figure 1b shows the evolution of pH in the WSE of the different cheese portions during ripening. In the rind, the pH increased from 4.8 to 7.0 between the 9th and 16th days of ripening and did not change afterward. This alkalization was more extensive in the under-rind portions, where the pH value reached 5.6 on the 16th day and 7.0 on the 21st day and continued to increase until near 8.0 after around 35 days of maturation. The evolution was the same in the center portions but with a delay of ∼10 days. In Figure 1c, it is noticeable that a lactic acid/lactate concentration decrease occurred more quickly in the rind than in the under-rind. In the same way as for pH the phenomenon seemed to be slower in the center. After 42 days of ripening, lactic acid/lactate concentrations appeared as being the same in each portion. As indicated by Figure 1d, similar results were found for lactose and galactose concentrations. The lactose concentration was already weaker in the rind (4 versus 5.5 g/kg of cheese in the other portions) at 9 days of ripening and subsequently fell in the following week. This was also observed in the under-rind. For the center, the disappearance of lactose occurred only after 28 days of ripening. The delay between portions was also observed for galactose consumption even if it appeared 1 week later than for lactose. No mononucleotide was detected in any cheese portion whatever the ripening time.

**Minerals.** Figure 2a shows that sodium had a tendency to migrate from the rind, where its concentration decreased from 5.7 to ∼4 g/kg of cheese. However, no clear tendency was observed for the sodium level of the under-rind and center portions despite a slight increase at the beginning of maturation. In contrast, whereas the chloride level seemed to be constant in rind throughout ripening, its level in the other portions increased gradually until a maximum (Figure 2b). It is noticeable that whereas the maximum concentration (∼12 g/kg of cheese) was reached at the 28th day of ripening in under-rind; the maximum value for the center portion was obtained around the 35th day. Figure 2b shows that for water-soluble phosphorus the evolution is almost the same within the portions until the 16th day of ripening. The concentration of phosphorus increased in the rind to recover its initial level (4 g/kg of cheese); however, it was stabilized in the other two portions.

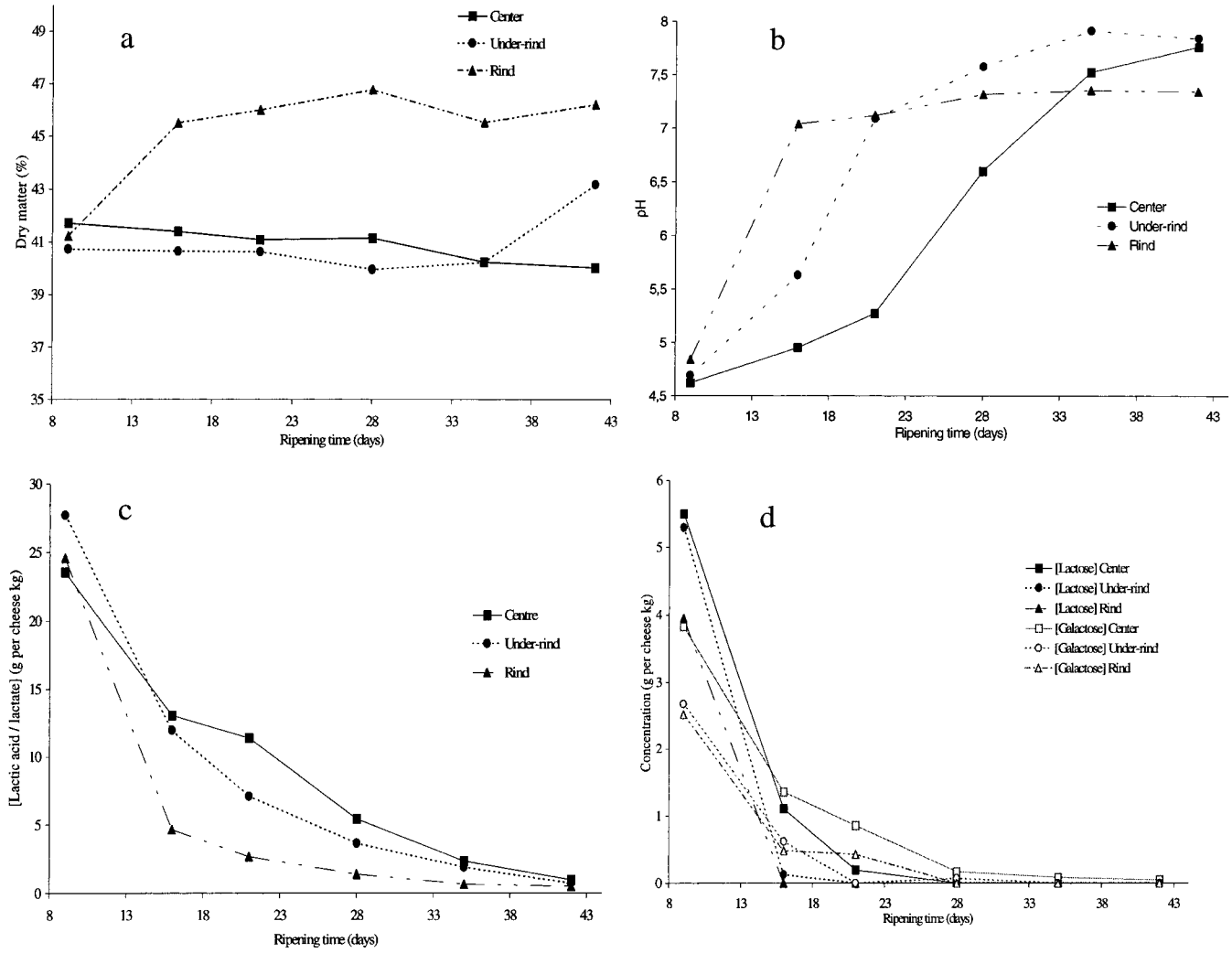


**Figure 1.** Evolution of (a) pH, (b) dry matter, (c) lactic acid, and (d) lactose and galactose, respectively, analyzed on rind, under-rind, and center portions of a Pc3 Camembert cheese during ripening. Results are expressed in pH units (a), percentage of cheese weight (b), and grams per kilogram of cheese (c and d). For each measurement done in triplicate, the standard deviation was <5%.

For both calcium (Figure 2c) and magnesium (Figure 2d) the same phenomenon was observed: whereas their level increased in the rind, it decreased in the two other cheese portions. Additionally, in the case of water-soluble calcium and magnesium their concentration decreased throughout ripening whatever the portion considered. However, apart from the higher concentration of calcium in the cheese (20-fold greater than the magnesium content in a 9-day-old cheese), a difference appeared between the two divalent cations: whereas calcium concentrations were almost the same between cheeses and their related WSEs, the concentration of water-extractable magnesium seemed to be lower than its concentration in the cheese.

**Nitrogen Determination.** Figure 3a indicates the respective evolution of total nitrogen within the cheese portions. In all cases, a slight TN increase occurred during ripening. It seemed to be more accentuated in the center, where it increased to 18.7% in comparison with under-rind (11%) or rind (5%).

However, parts b−d of Figure 3 indicate that the nitrogen composition of the rind differed from that of the two other portions, which both appeared to be affected by the same changes. On the 9th day the cheese rind had already a higher level of SN (30% TN). From the 16th day onward, the rind was characterized by having an almost constant and relatively high respective SN (∼80% TN), PTASN (∼50% TN), and NPN (∼70% TN) values. In contrast, increases of SN, NPN and peptides indicating migration of the proteolytic activity due to *Penicillium*, progressing through the cheese to center portions.

The evolution of the concentration in cadaverine, the main biogenic amine found in Pc3 Camembert cheese, is given in Figure 4. In rind and under-rind, cadaverine levels seemed to increase and reached a maximum value of at least 1.8 and 0.9 g/kg of cheese between the 16th and 28th days of ripening, respectively, and then decreased. In the center portion, the maximum concentration (at least 0.5 g/kg of cheese) occurred between the 28th and 35th days of ripening.

## DISCUSSION

**Evolution of Cheese Composition during Ripening.** Results of Figures 1−4 indicate that important composition changes occurred in cheese during its maturation.

For most of the cheese components those changes seemed to begin significantly between the 9th and 16th days of ripening, a period during which *P. camemberti* invaded the whole product surface as shown by the

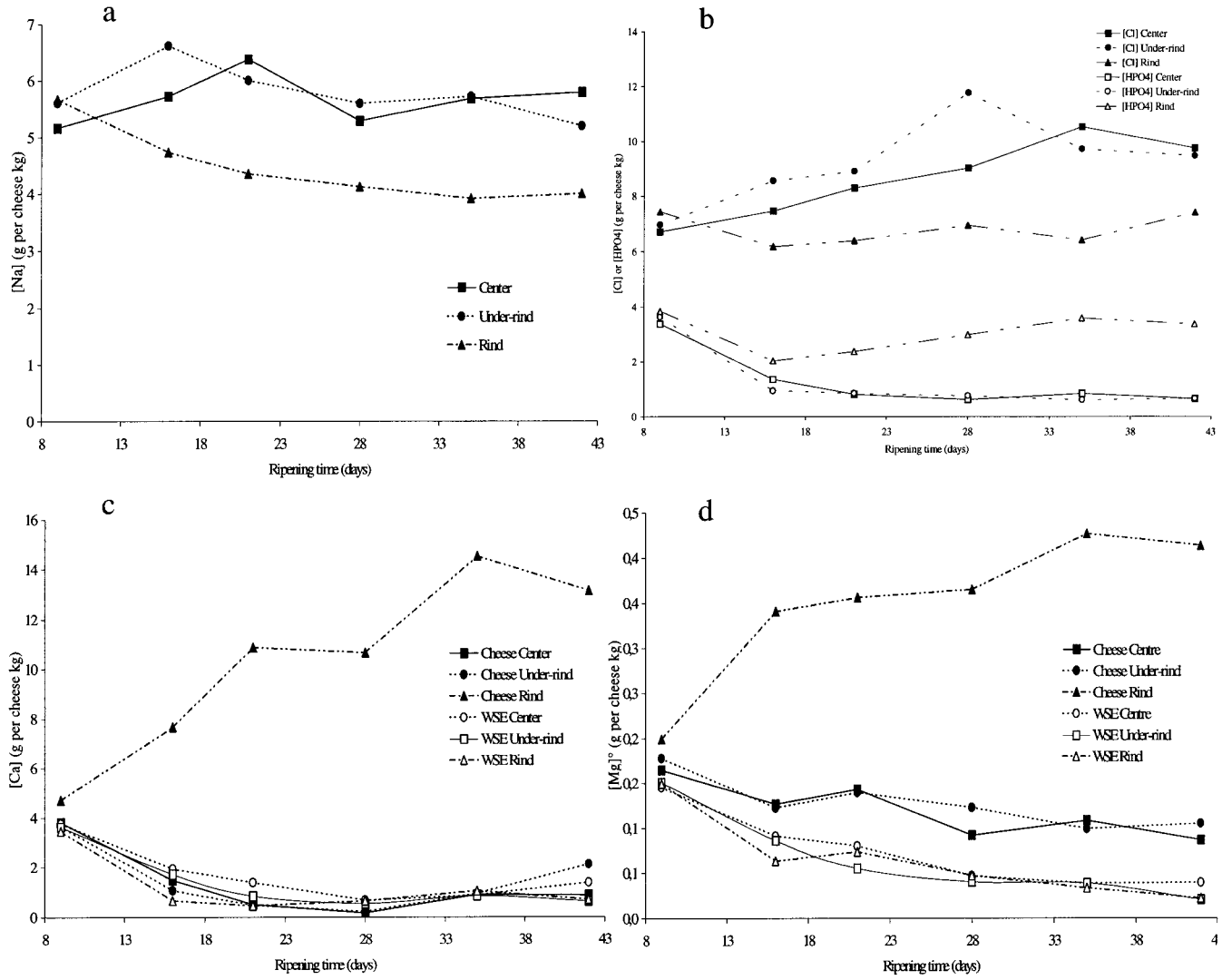


**Figure 2.** Migration of mineral salts during ripening as evidenced by evolution of (a) Na concentration, (b) Cl and $HPO_4$ concentrations (WSE and cheese), (c) Ca, and (d) Mg concentrations, respectively, analyzed on rind, under-rind, and center portions of a Pc3 Camembert cheese during maturation. Results are expressed in grams per kilogram of cheese. For each measurement done in triplicate, the standard deviation was <5%.

rapid increase in the respective dry matter (Figure 1a) and pH values (Figure 1b) of the rind. Moreover, Molimard et al. (*13*), using the same fungal inoculum (Pc3) cultivated in similar manufacture conditions, confirmed that whereas no growth of *P. camemberti* was observed for the first week of ripening, its population grew until the 14th day. The appearance of a pH gradient on the cheese surface would result from metabolic activities of *Penicillium* (*7*) and more especially from lactic acid consumption as confirmed by our results.

After the 16th day, analyses of the rind portions were done in fact more on a mycelium substrate than on a "dairy product". According to Molimard et al. (*13*), the number of living cells would be constant after 2 weeks of maturation. Additionally, it seemed that, apart from biogenic amines and calcium, the mycelium composition is then relatively constant, whatever the analyzed compounds considered. Thus, it was characterized by a dry extract of ~45%, a pH value near 7, and a relatively high soluble nitrogen content even in non-protein-soluble nitrogen. Although the composition of the rind was stabilized after 2 weeks, major changes occurred in both under-rind and center portions, and these might be related to *Penicillium* development.

Evolution of pH in a typically bitter Camembert cheese was similar to the tendency already observed by Le Graet et al. (*7*) for a not particularly bitter product. Successive increase of the pH value in under-rind and, subsequently, in the center might result from the advance of two phenomena acting in opposite directions: the migration of lactic acid and $H_3O^+$ from the center to compensate their decrease on the cheese surface and the release of basic compounds such as ammonia due to the advance of proteolysis (*14*).

Concerning sugars, lactose not already fermented to lactic acid was probably consumed on the surface by *Penicillium* (*15*). The decrease in other portions might be due to the residual activity of lactic acid bacteria activities and also to migration to the surface, where this sugar is immediately consumed. Additionally, the persistence of galactose until three ripening weeks has already been mentioned by Choisy et al. (*15*). Galactose would come from the hydrolysis of lactose. Its persistence level in the center might also be due to passive migration phenomena from the center to the rind, where it is consumed by *Penicillium*.

The decreasing value of sodium concentration in the rind has already been observed by other authors on nonbitter cheese and was explained by a migration

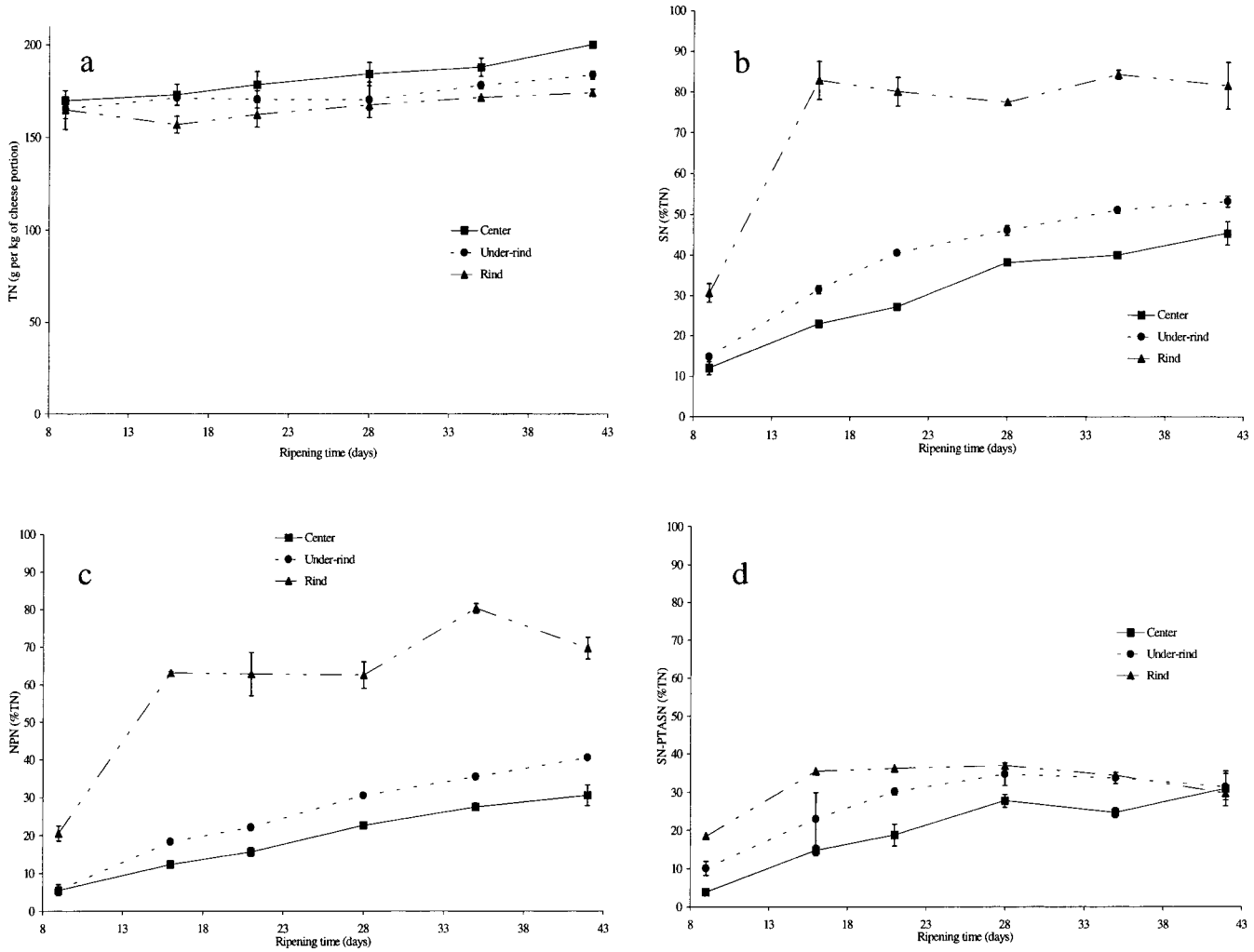


**Figure 3.** Evolution of (a) total nitrogen (TN), (b) soluble nitrogen (SN), (c) non-protein nitrogen (NPN), and (d) soluble nitrogen minus phosphotungstic acid soluble nitrogen (SN-PTASN) analyzed on rind, under-rind, and center portions of a Pc3 Camembert cheese during ripening. Results are expressed in grams per kilogram of cheese portion for (a) and in percentage of TN for (b−d). Standard deviations are given for each point indicating the mean value obtained for three replications.

phenomenon from the surface to the center whose origin would be from the brining of the surface *(16)*. However, as the sodium content of the rind at 9 days of ripening is comparable with that reached in the other portions (Figure 2a), the explanation of brining salt migration has to be rejected. On the contrary, the increase of the rind dry matter between the 9th and 16th days of ripening (Figure 1a), causing an increase of the sodium concentration in the rind aqueous phase, may be responsible for sodium migration in the internal portions. The same phenomenon may explain the increase of chloride concentration in both center and under-rind portions throughout ripening (Figure 2b).

As was previously shown by Le Graet et al. (*7*), calcium migrates together with inorganic phosphorus from inside to outside during cheese ripening. In the beginning of maturation, the pH values of the center and under-rind portions are sufficiently low to maintain almost all of the calcium present in its soluble form. This would explain why the level of total calcium quantified in those portions of cheese is almost the same as in their respective WSEs (Figure 2c). The mineral migration starts with *Penicillium* growth because of its effect on pH value. Indeed, the increase of pH value would decrease the solubility of calcium salts, leading them to precipitate with phosphates. Because of the decrease of its soluble form on the surface, calcium migrates from the center, where both total and soluble levels decrease as well as in under-rind portions (Figure 2c). Then, quickly, the concentration of total calcium in rind increases and stabilizes when the other portions have reached a sufficient pH value. The decreasing and then stabilized level of phosphate is in accordance with that hypothesis (Figure 2b). Despite its concentration being such lower than that of calcium, the same evolution was observed for magnesium (Figure 2d), suggesting that this ion may be subjected to the same phenomenon. However, it is noticeable that magnesium concentrations obtained for WSE after 2 weeks of ripening were at a lower level than that measured in the whole cheese. This would be due to the lower solubility of magnesium salts. A part of it could be already precipitated by the pH of the center and under-rind portions corresponding to the 16th day of cheese maturation.

According to Boutrou et al. (*14*), the slight increase in total nitrogen concentration (Figure 3a) would result from water loss due on the one hand to evaporation in the ripening room and, on the other hand, to the water-consuming hydrolysis of bound peptides. However, as the total dry matter levels of both center and under-rind portions were approximately constant (Figure 1a), this loss of water may have been compensated by migration phenomena from the center to the rind. Apart from residual whey protein, the TN would be a complex

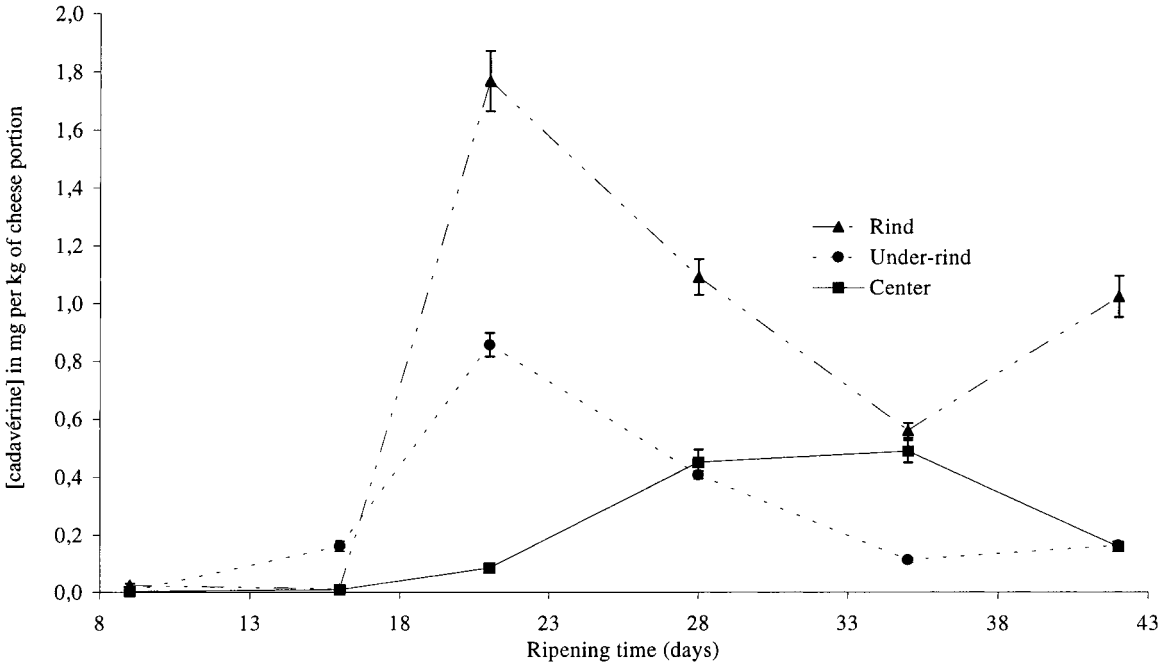


**Figure 4.** Evolution of the cadaverine concentration in different portions of a Pc3 Camembert cheese during ripening. Standard deviation are given for each point indicating the mean value obtained for three replications.

mixture of casein or its more or less soluble fragments, medium and small peptides, and ammonia compounds. As shown by the relative amounts of SN and NPN (Figure 3b−d), proteolysis advanced regularly from the rind to the cheese center. This indicates the predominant action of the proteinase and peptidase of *Penicillium* as already suggested by several authors (*12*, *14*, *17*). If we consider SN-PTASN, as an effective indicator of the relative quantity of peptides (*12*), Figure 3d indicates that they were globally released during the first 4 weeks of ripening. However, during the next 2 weeks, the balance between their release and their degradation to ammonia or amino acids seemed to be inverted. This last phenomenon would be due to the probable degradation of some previously released peptides.

Cadaverine has already been identified and quantified as the major biogenic amine in Camembert cheese (*8*, *18*). Its presence would be due to the decarboxylation of free lysine (*19*), one of the major amino acids quantified by Boutrou et al. (*14*) in 16-day-old Camembert cheese. Among microorganisms, bacteria are generally considered to be the main agents responsible for biogenic amine appearance. More particularly, some lactic acid bacteria are capable of decarboxylating one or more amino acids to produce cadaverine or other biogenic amines (*20*). Thus, if the presence of cadaverine seems to be associated with the advancement of proteolysis due to *Penicillium* enzymes (Figure 4), it is probably because they are responsible for the release of lysine, which is then decarboxylated by some bacteria also present in the studied cheese.

This study of the evolution of cheese composition at the rind, under-rind, and center during ripening allowed for a better understanding of the complex changes acting during maturation. Moreover, it enabled the evolution of cheese taste revealed by a parallel study (*1*) to be explained.

**Consequences on Cheese Taste.** *Cheese Sourness.* The study led in our laboratory (*4*) showed that the sourness of the same Camembert cheese ripened for 31 days was mainly due to $H_3O^+$ concentration, the sour taste of which was enhanced by sodium chloride. Thus, the sequential decrease of pH (Figure 1a) may partially explain the resulting diminution of sourness evaluated by a sensory panel (*1*): on the 9th day, cheese sourness was quoted at around 55/100 whatever the portion and decreased until a minimum level of 20/100 in 1 week for rind, in 3 weeks for under-rind, and in 4 weeks for the center. By studying the relationship existing between log(sourness) and pH or log(lactic acid), correlation coefficients were obtained of, respectively, $R = 0.81$ ($P < 0.0001$) and $R = 0.66$ ($P < 0.0001$). These correlations being significant, the evolution of sourness seemed to be in full accordance with the evolution of both pH and lactic acid/lactate concentration in the cheese. In contrast with our result, Biede and Hammond (*21*) found no correlation between "acid flavor" and either cheese pH or lactate concentration. However, they compared seven different Swiss cheeses with levels of salt varying from 21.98 to 45.04 (*22*): this difference may explain why they did not observe significant correlations. Thus, even if in our case the variations of salt concentration throughout ripening were weaker (Figure 2a,b), the evolution of sodium chloride concentration and its enhancing effect on sourness must be taken into account to fully explain cheese sourness.

*Cheese Saltiness.* In the same way as for sourness, the compounds responsible for the saltiness of the same Camembert cheese ripened for 31 days have been investigated (*4*). The salty note of the cheese was mainly due to sodium chloride. The sensory characterization of the saltiness of the same cheese portions as used for composition studies in this paper (Figure 2a,b) showed that if rind saltiness was decreasing between the 9th and 16th days before stabilization, its level in under-rind and center slightly increased throughout ripening. These sensory results seem in accordance with the migration of both sodium and chloride previously hypothesized. However, as it was shown in the sensory part of this study, any explanation of cheese saltiness needs to consider the global masking effect due to cheese

matrix on the WSE saltiness due to sodium chloride (*1*). This masking effect was characterized as composed first of masking due to fat and proteins, second because of cheese matrix structure effects on the availability of sodium chloride, and finally the effect of texture which must interact differently with the taste perception of the cheese and its WSE. Thus, changes of cheese saltiness during ripening are probably due to the migration of sodium and chloride but also to modification of the cheese matrix structure due to proteolysis and lipolysis.

*Cheese Bitterness.* According to the sensory evaluation made in parallel to physicochemical analysis (*1*), the bitterness of each cheese portion was in agreement with the advance of the proteolytic front: its intensity reached plateaus at 80/100 on the 16th day for under-rind and at 70/100 on the 28th day for the center. By studying the relationship existing between bitterness intensity and peptide (= SN-PTASN) or SN indices, correlation coefficients $R$ were obtained equal, respectively, to 0.95 and 0.87. Despite the correlation appears to be statistically highly significant ($P < 0.01\%$) for both indices, it must not be considered as an absolute attestation of a causative linkage between peptides and bitterness. The first reason is due to the use of the statistical correlations themselves. Therefore, apart from nitrogen content, a lot of factors might exist that vary within the sample, which could also explain the apparent differences in bitterness. Second, according to Molimard et al. (*12*), SN and SN-PTASN are two indices that could not permit bitter and nonbitter peptides to be distinguished.

In the same way as was mentioned before for sourness and saltiness, bitterness of a 31-day-old cheese was investigated using a methodology allowing for the study of the relative impact of cheese components on its taste (*4*). This previous study showed that peptides were the main factor responsible for the WSE bitter note. Bitter peptides ranged in molecular weight from <10000 Da to between 500 and 1000 Da. That result explains the correlation mentioned before and indicates that peptides are certainly involved in the evolution of cheese bitter taste during maturation.

However, the study of the evolution of matrix influence on Pc3 Camembert cheese taste during ripening (*1*) showed that the variation of cheese bitterness throughout ripening might also be due mainly to the modification of the cheese matrix. In fact, the bitter note of the WSE was found to be almost constant apart from a positive peak at 21 days for the under-rind and at 28 days for the center. This result reveals that the global pool of bitter peptides is almost constant in terms of bitter intensity. Moreover, the intensity of the bitter note perceived during maturation seems to get stronger and stronger in cheese in comparison with its WSE. This indicates an increasing exhaustive effect of the cheese matrix during ripening. This matrix effect was explained for bitterness, on the one hand, by the change in availability of bitter compounds contained in the WSE and, on the other hand, by the changing texture−taste interaction. Those changes seemed to be mainly due to structural changes of the cheese matrix and then to proteolysis and probably also lipolysis. Thus, the significant correlation coefficient between bitterness intensity and soluble nitrogen or peptides may result probably more from the effect of proteolysis on matrix structure than on the release of bitter peptides.

Additionally, it may be possible that, due to a change of concentration during ripening, some compounds that are taste-active in the beginning of ripening become inactive after 31 days or vice versa. Thus, the peak of bitterness intensity observed in the WSE (not shown) could be due to a contribution of cadaverine, the concentration of which increased at the same ripening time (Figure 4) even though the highest measured concentration was lower than its threshold value in pure water (0.13 mmol/kg) (*8*). Possible additive or synergistic effects can occur with other components of the complex solution (*23, 24*). However, as the profile of the whole cheese did not reveal any peak of bitterness intensity (*1*), its contribution did not seem to be enhanced by the cheese matrix. In the same way, calcium chloride may play a role in the bitter taste of young Camembert cheese, whereas it did not have any impact on the taste of a 31-day-old Pc3 cheese (*4*). On the 9th ripening day, it was indeed in a soluble state and sufficiently concentrated at ~4 g/kg to contribute to cheese bitterness (*2*). However, its impact would be expected to become negligible during the following weeks because of its migration and precipitation in the rind.

Thus, even if it can be caused in part by calcium chloride at the beginning, Camembert cheese bitterness would be due mainly to the taste of small peptides released throughout cheese ripening, the bitterness of which was modulated by a cheese matrix effect. The changes of this matrix effect during maturation could contribute strongly to the evolution of cheese taste by increasing the relative availability of bitter peptides and/or by modifying texture−taste interactions.

**Conclusion.** This study allowed for the evolution of the composition of a bitter Camembert cheese throughout ripening to be explained and confirmed the fundamental role of *P. camemberti* in this process. Moreover, the evolution of cheese taste may be explained not only by the evolution of the taste of its WSE containing potentially taste-active compounds such as $H_3O^+$, minerals, peptides, and biogenic amines but also by the changes affecting the modulating effect of the cheese matrix.

Thus, even if the fundamental role of proteolysis on the evolution of the cheese bitterness was confirmed, it might not be due only to its action on bitter peptides such as release or degradation. In fact, the evolution of bitterness may be explained also by the action of proteolysis and even probably lipolysis on the cheese matrix. This effect might be due partially to a change in the availability of bitter compounds due to degradation of the cheese matrix. It will be interesting to study those changes in availability of water-soluble compounds during mastication.

Additionally, as a previous study showed that small peptides from 500 to 1000 Da were the main bitter-active compounds of the same cheese at 31 days, the small peptides contained in the bitter Camembert cheese will be identified and quantified using HPLC-MS at each of the studied ripening stages. It will then be possible to study the consequences of proteolysis on potentially bitter hydrophobic peptides.

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