Evolution of the Taste of a Bitter Camembert Cheese during Ripening: Characterization of a Matrix Effect

E. Engel,† S. Nicklaus, C. Septier, C. Salles,* and J. L. Le Quéré

Laboratoire de Recherches sur les Arômes, Institut National de la Recherche Agronomique, 17 rue Sully, B.P. 86510, 21065 Dijon Cedex, France

The objective of this study was to characterize the effect of ripening on the taste of a typically bitter Camembert cheese. The first step was to select a typically bitter cheese among several products obtained by different processes supposed to enhance this taste defect. Second, the evolution of cheese taste during ripening was characterized from a sensory point of view. Finally, the relative impact of fat, proteins, and water-soluble molecules on cheese taste was determined by using omission tests performed on a reconstituted cheese. These omission tests showed that cheese taste resulted mainly from the gustatory properties of water-soluble molecules but was modulated by a matrix effect due to fat, proteins, and cheese structure. The evolution of this matrix effect during ripening was discussed for each taste characteristic.

Keywords: *Camembert cheese; bitterness; ripening; taste; water-soluble extract; matrix effect; omission tests*

INTRODUCTION

Because of its fundamental role in consumer acceptance, the taste of cheese is one of its most important organoleptic attributes. Among cheese gustatory characteristics, bitterness has been particularly studied because of its economical consequences as a major cheese defect (*1*). It is generally accepted that bitterness in cheese results from the accumulation of an abnormally high concentration of hydrophobic peptides released during the proteolysis of casein (*2*).

However, many other substances such as amino acids, amines, amides, substituted amides, long-chain ketone, and some monoglycerides present in cheese might also add to its bitterness (*1*). Kubickova and Grosch (*3*) found that cadaverine, ornithine, and citruline, when present in a French Camembert cheese, may cause a bitter note. By determining the taste-active compounds in the water-soluble extract of a goat cheese, Engel et al. (*4*) showed that bitter taste resulted mainly from the bitterness of both calcium and magnesium chlorides, which in turn is partially masked by sodium chloride. This last study showed that peptides are not systematically responsible for cheese bitterness and that some compounds involved in other taste characteristics may disturb the perception of bitter compounds. However, the goat cheese studied was only slightly matured and, overall, had a probably weaker level of bitterness than a defective bitter cheese. Thus, to study the relative impact of minerals, peptides, or other compounds on bitter taste, it might be necessary to consider the total taste characteristics of a typically bitter cheese at different stages of ripening.

Because of its frequent occurrence, bitterness has often been investigated on surface mold cheeses such as Camembert cheese (*5*, *6*). However, as it is a known defect, it is difficult to select commercial products that are consistently bitter as they are generally removed by quality control procedures. Thus, such studies need to use pilot cheese-making facilities with appropriate treatments to obtain typically bitter cheese. To do this, some modifications to the cheese-making process such as an increased rennet quantity (*7*) or an accelerated draining (*8*) can be used. However, the use of selected *Penicillium camemberti* inoculum appears to be the most efficient process parameter that can be modified to obtain typically bitter Camembert cheese (*5*). Additionally, as the growth of *Geotrichum candidum* would seem to generally cause a decrease in bitterness (*9*), its absence would appear as a bittering factor. Molimard et al. (*6*), studying the correlation existing between nitrogen content and bitterness of an experimental cheese, discovered two strains of *P. camemberti* encoded Pc2 and Pc3 that yielded particularly bitter cheese when no *Geotrichum* was used.

Due to the metabolic activities of *Penicillium* on the cheese surface, concentration gradients are formed between the center of cheese and the surface. These are responsible for the migration of potentially taste-active components such as calcium or compounds acting on pH (*10*). These phenomena added to the progression of a proteolytic front may be responsible for the appearance of gustatory differences existing among the center, the under-rind, and the rind portions (*11*) and then for changes in cheese taste. Thus, knowledge of both gustatory characteristics and physicochemical compositions of these different portions seems to be necessary to explain the evolution of cheese taste during ripening. The first step of this approach, described in this paper, will focus mainly on the characterization of the sensory consequences of ripening on cheese taste.

To investigate cheese taste, the water-soluble fraction has been extensively studied because it is considered by numerous authors to be responsible for this sensory

* Corresponding author (telephone 33.380.69.30.79; fax 33.380.69.32.27; e-mail salles@arome.dijon.inra.fr).

† Present address: Unité Mixte de Recherches en Génic et Microbiologie des Procédés Alimentaires, Institut National de la Recherche Agronomique, 78850 Thirerval-Grignon, France.

10.1021/jf000967m CCC: $20.00
Published on Web 05/25/2001

property (*12*−*15*). However, bitter taste in cheese may be due to relatively hydrophobic peptides, which might be partially retained in the nonsoluble fraction. Thus, in the case of a typically bitter cheese it is necessary to evaluate the relative impact of water-soluble and nonsoluble fractions on the whole cheese taste prior to any investigation of these fractions. A methodology to evaluate the relative impact of fractions or components of a liquid mixture on its taste has been proposed in a previous study (*4*, *16*). It consists of omitting one or several subfractions of a mixture and evaluating the consequence of this omission by making the comparison between the taste profile of complete and incomplete mixtures. To study the relative impact of the water-soluble fraction on cheese taste, these omission tests need to be performed on a reconstituted cheese that is a mixture of fat, proteins, and water-soluble extract (WSE) recovered throughout water extraction.

However, in contrast with studies performed on mixtures made in physicochemical and sensory accordance with the WSE, it is impossible to obtain a reconstituted cheese with the same structural organization as a crude cheese. Even if the compositions of the two products are similar, the presence of an organized cheese matrix may probably change the perception of taste-active compounds. By analogy with interactions existing between some cheese matrix components such as proteins or fat and cheese aroma (*17*, *18*), it is probable that changes in matrix structure may change the perception of cheese taste. To confirm this hypothesis, McSweeney (*1*) stated that the saltiness of Cheddar cheese was perceived significantly more intensely in grated cheese compared to whole cheese. This result suggests that the gustatory properties due to taste-active compounds could be modulated by the cheese matrix structure. To fully explain cheese taste and its evolution during ripening, it is then necessary to consider this modulating effect and to follow its consequences throughout cheese maturation.

The first aim of this work was to select a typically bitter Camembert cheese and to characterize by sensory analysis the respective evolution of the taste of its rind, under-rind, and center portions to try to further link tastes and chemical changes occurring during ripening. Second, the influence of the cheese matrix and its components on each gustatory property was also characterized. The effect of the evolution of the cheese matrix during ripening was discussed for each taste attribute.

MATERIALS AND METHODS

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

Cheese Manufacture. Experimental French Camembert cheeses (45% fat in dry matter) were manufactured at the Lycée Agricole de Saint Lô Thère (Saint Lô, France). Whole fresh milk (210 L) was standardized to a fat concentration of 30 g/L after thermization of the milk (63 °C/30 s). After the milk had cooled to 13 °C, 0.6% mesophilic bacteria PAL 25 (Standa Industrie, Caen, France) and calcium chloride at 10.2 g for 100 L of milk were added to the milk to achieve its "prematuration". When the milk pH reached 6.4−6.45, it was pasteurized (75 °C/30 s). After the milk had cooled to 37 °C, 1% mesophilic bacteria PAL 25 and 0.2% *Streptococcus thermophilus* ST 82-87 (Standa Industrie) were added to the milk at the same time as calcium chloride (10.2 g for 100 L of milk).

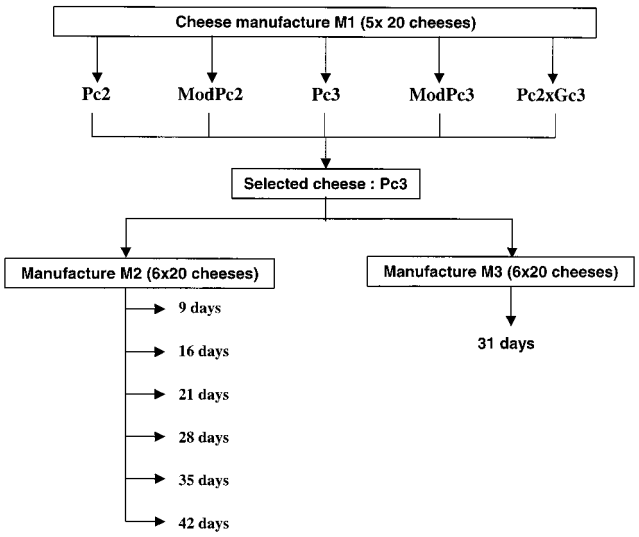

Figure 1. Organization of cheese-making.

Table 1. Manufacture Characteristics of the Five Experimental Cheeses Tested

cheese	fungal culture[a]	rennet quantity	draining conditions[b]
Pc2	*P. camemberti* Pc2	20 mL/100 L	normal
ModPc2	*P. camemberti* Pc2	25 mL/100 L	increased
Pc3	*P. camemberti* Pc3	20 mL/100 L	normal
ModPc3	*P. camemberti* Pc3	25 mL/100 L	increased
Pc2xGc3	*P. camemberti* Pc3 (99%) × *G. candidum* Gc3 (1%)	20 mL/100 L	normal

[a] Each fungal culture was inoculated at 2500 spores/mL of milk. In the case of the association (Pc2xGc3), the mixture is prepared with 99% Pc2 spores and 1% Gc3 spores. [b] Increased draining was obtained by an increase of the temperature during this step of the process.

The total amount of milk was divided into five batches of 40 L. Each of the five corresponding milk vats was then inoculated with 2500 spores/mL of milk of the appropriate fungal culture. When the pH reached 6.2−6.25, rennet "presure 520" (Laboratoire Central des Présures, Prat, France) was added at the concentration of 20 mL/L of milk for a "usual manufacture" process. After a first phase of coagulation of 28 min, the curd was cut up. The coagulum obtained after a resting time under serum (30 min) was transferred into hoops to obtain cylinder shapes, which were inverted three times: the first time just after the transfer, a second one when the pH reached 5.70, and a third one when the pH decreased to <5.1. Twenty-four hours after the beginning of manufacture, cheeses were salted in brine (density = 1180) for 35 min and placed at 16 °C and 80% humidity for 24 h. They were then ripened at 12 °C and 95% humidity and inverted two times. On the 12th day after manufacture, the cheeses were packed in a cellophane-based film and ripened at 8 °C. Thus, 20 (±1) Camembert cheeses of 250 g were obtained per vat.

Experimental Cheeses. Three series of experimental cheese-making were performed according to Figure 1. The first one, M1, was dedicated to the selection of the cheese-making process. M2 and M3 were two batches of the selected process. M2 allowed for the evolution of the cheese taste during ripening to be studied, whereas M3 was dedicated to evaluate the relative impact of WSE, fat, and proteins on cheese taste.

Cheese-Making M1. For the experimental Camembert selection, five series of 20 Camembert cheeses encoded Pc2, ModPc2, Pc3, ModPc3, and Pc2xGc3 were made. As Table 1 indicates, each series differed from the others only by the nature of the fungal culture used and/or by some eventual changes in the process. Three cheeses of each type were taken for microbiological and physicochemical analyses. All cheese series were tasted in triplicate: one session on the 29th and

two sessions on the 30th day. On the 30th day, they were frozen by submersion in a liquid nitrogen bath at −196 °C and held at −80 °C until further physicochemical analysis.

Cheese-Making M2. Six vats of the cheese Pc3 were manufactured according to the cheese-making process described above. 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 submersion in a liquid nitrogen bath for further physicochemical analysis and water extraction.

Cheesee-Making M3. Six vats of the cheese Pc3 were manufactured. At 31 days of ripening, 90 cheeses were frozen by submersion in a liquid nitrogen bath after elimination of the rind. These cheeses were dedicated to sensory evaluation, water extraction, and further physicochemical analysis.

Commercial Cheese Samples. Six samples of French Camembert cheese (45% fat in dry matter) encoded from c1 to c6 were purchased from commercial suppliers. All cheeses were tasted at ∼15 days before their optimum consumption date. Ten lots of 250 g of each cheese were obtained from retailers.

Twenty c6 cheeses were bought to be further compared to the five experimental cheeses obtained from M1. They were chosen to be tasted at 15 days before their optimum consumption date. Five of them were used for sensory evaluation, and the others were frozen for further water extraction and physicochemical analysis.

Fifty c1 cheeses were bought and frozen at −20 °C. They were used as control cheeses for the study of taste evolution during ripening. At each ripening time of Pc3 (9, 16, 21, 28, 35, and 42 days of M2 manufacture), 6 c1 cheeses were taken out of the −20 °C cold chamber, placed for 12 h at 2 °C for defrosting, and cut out in three parts to be tasted.

Cheese Partition. After 40 min at −20 °C, the cheeses were divided into three parts with a ham-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 cheese, the respective thicknesses of rind, under-rind, and center portions were 3, 7, and 15 mm in order to obtain a weight of the center portion equal to the weight of two under-rind portions.

Water Extraction: Extraction Procedure. The frozen cheese portions were grated, 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 upper layer, a liquid fraction including water-soluble molecules called C20000, and a pellet of proteins. The fat was collected in freezer bags, and the protein pellet was dissolved in water corresponding to 7.5% cheese weight. After filtration on gauze, the C20000 was recovered. All of these fractions were stored at −80 °C until further use.

Purification Procedure. Each C20000 obtained from cheese portions of M2 manufacture was pooled and 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 bar. For each fraction corresponding to one of the three cheese portions at one of the six ripening dates, two portions of 300 mL of C20000 were successively treated. The recovered permeate, called the water-soluble extract (WSE), was immediately frozen until further use. When they are used for sensory evaluation, these purified fractions are freeze-dried to compensate for the initial dilution with water due to water extraction.

The 37.5 kg of C20000 obtained from M3 manufactured cheeses were pooled and submitted to tangential microfiltration in a pilot apparatus equipped with two 0.05 μm membrane modules of 0.9 m^2 each (wide volume = 15 L; INRA, Laboratoire de Recherches de Technologie Laitière, Rennes, France). The retentate was rinsed with 43.5 L of osmosed water corresponding to four successive diafiltrations. This process allowed for the recovery of 19.7 kg of microfiltration retentate, named RUF0.05, and 63.2 kg of microfiltration permeate considered to be the WSE. All of the obtained fractions were frozen at −80 °C until further use. When they are dedicated to sensory evaluation, WSE and RUF0.05 were concentrated by freeze-drying to compensate their dilution with water during the extraction and purification steps.

Table 2. Nature and Concentration of Reference Solutions for Each Attribute

attribute	reference compound	concn in pure water
sour	lactic acid	1.38 g/L
bitter	L-leucine	10 g/L
salty	sodium chloride	3 g/L
umami	monosodium L-glutamate	0.6 g/L
sweet	D-lactose	23.75 g/L
astringent	potassium aluminum sulfate	0.33 g/L
sharp	capsaicin	0.15 mg/L

Elaboration of a Cheese Model. *Reconstituted Cheese.* Starting with the fractions recovered at each step of the extraction/purification process of M3 cheeses, it is possible to elaborate a reconstituted cheese if the corresponding fractions—fat, protein pellets, microfiltration permeate (WSE), and retentate—are at the same concentration as in the crude cheese. The dilution of both microfiltration permeate and retentate (WSE and RUF0.05) was compensated by freeze-drying. Thus, fat, proteins from centrifugation pellets, and freeze-dried microfiltration permeate and retentate were mixed using an Ultraturrax homogenizer (Polytron 3100, Kinematica, Littau, Switzerland) at 15000 rpm for15 min.

Omission of Fractions. In these cases, reconstituted cheeses were prepared by omitting one of the constitutive fractions. For each omission realized, the omitted fraction was replaced by ultrapure water. The mixing of the components was made in the same way as described above.

Physicochemical Analysis. Dry mater and pH values were measured on the whole cheese (*6*). For nitrogen determination an aliquot of grated cheese was suspended in a sodium citrate solution (*19*). Total nitrogen (TN), soluble nitrogen at pH 4.6 (SN), and soluble nitrogen in phosphotungstic acid (SNPTA) were determined according to the Kjeldahl method (*6*). The results are the average of three replications with a variation coefficient of <5%.

Sensory Evaluation. *General Conditions.* The evaluations were conducted under red light in an air-conditioned room (21 ± 1 °C). The room was fitted with 16 separated booths according to ISO 45486. To suppress olfactive sensations, panelists' nostrils were pinched. At each measuring session, products were presented in a monadic way, according to a Williams Latin square design (*20*) to balance report and position effects. For each sensation studied, an appropriate reference solution was chosen. The concentration of each reference was adjusted in accordance with its mean intensity in the studied cheeses. The quantification of each attribute intensity was evaluated in comparison with the perceived intensity of the corresponding reference solution adjusted to a given concentration (Table 2). Prior to every other task, panelists were asked to taste the reference solutions and to memorize their respective intensities corresponding, by mutual agreement, to 50% of their respective assessment scale. Afterward, they had to taste each product and to mark the intensity of each attribute on a 13-cm unstructured linear scale anchored from "no sensation" to "strong". A mark at the middle of the scale corresponded for each attribute to the intensity of the reference solution. During the tests, assessors could taste some reference solution to recall each gustatory sensation and its intensity. Data were recorded with the FIZZ computerized system version 1.20 (Biosystemes, Couternon, France).

Cheese Selection. Fourteen assessors were selected for their ability to recognize and quantify the taste of cheese. During six 1 h sessions, panelists were trained in the evaluation procedure and elaborated by consensus a list of adequate terms to describe the Camembert cheese gustatory properties (Table 2). The products, which had been stored at 4 °C, were placed at 15 °C for 2 h just before each session. The Camembert cheeses were cut in 16 equal pieces on cardboard plates and

were covered immediately with cellophane film to limit oxidation phenomena. At each tasting session, panelists were instructed to carefully eliminate the rind without losing too much under-rind, to taste the cheese portion, and to mark the intensity of each attribute. Between samples, they had to rinse their mouths with a piece of apple, some bread crumbs, and some mineral water in that order. At every measuring session, panelists had to taste two series of six cheeses. The six commercial cheeses c1−c6 were evaluated three times during three different sessions. The panelists were familiarized with the product at the first session, which was not taken into account in the data analysis. The five experimental cheeses, Pc2, ModPc2, Pc3, ModPc3, and Pc2xGc3, were tested together with c6. After the familiarization session, they were tasted five times: once at 29 ripening days and twice at both 30 and 33 ripening days.

Taste Evaluation of Cheese Portions at Different Ripening Stages. Fourteen other assessors were selected for their ability to perceive basic tastes with a special emphasis on bitterness. Thus, panelists were all sensitive to the bitter taste of 3 g/L of L-leucine and 2 g/L of calcium chloride. During seven sessions, they were familiarized with the quotation system and with the evaluation procedure of cheeses. After cheese partition, the obtained portions were cut into equal pieces on cardboard plates and were covered immediately with cellophane film to limit oxidation. At the beginning of each measuring session, the center of an ∼3 weeks old c3 Camembert cheese was tasted by the panelists. This sample, called "dummy", allowed on the one hand limited "first sample effect" (*21*) and on the other hand the ability to recall the evaluation procedure. Additionally, to reveal a possible drift in the panelists' way of scoring throughout the study, the same frozen control cheese c1 was tested at each measuring session. Fifty samples of control cheese c1 were frozen at −20 °C before the beginning of Pc3 ripening. At each ripening time when the Pc3 cheese was studied, an appropriate quantity of control cheese was defrosted and also divided into portions. Portion by portion they were evaluated together with Pc3 as an anonymous coded sample. During measuring sessions, a dummy sample, four centers, four under-rinds, and four rind portions were tasted by each assessor in that order. For each portion of the cheese, the four samples were in fact two replications of the Pc3 cheese and two replications of the control cheese. Two 250 g Camembert cheeses had to be used for each replication. The respective rind, under-rind, and center of one of the two cheeses were tasted by the same seven tasters. Each center was divided into eight pieces. The two under-rinds of the same cheese were both divided in 16, and a piece of each under-rind was given to each assessor. Each of the two rinds obtained from the same cheese was cut into quarters, giving a total of eight pieces. After the cutting, the cardboard plates were covered with cellophane film.

Evaluation of the Reconstituted Cheeses: Omission Tests. Sixteen assessors were selected for their ability to perceive basic taste with a special emphasis on bitterness. The sensitivity to bitterness of L-leucine, calcium chloride, and 6-*n*-propylthiouracil (PROP) was taken into account as the main selection criteria. The panelists were trained to the taste evaluation of mixtures of compounds in solution or in a complex matrix such as cheese. The reconstituted cheese and modified reconstituted cheeses in which one or several fractions were omitted were prepared just before the measuring sessions. A first measuring session allowed for the taste comparison between grated cheese and reconstituted cheese obtained from M3 manufacture. Taste profiles of both grated and reconstituted cheese were compared to the whole cheese evaluated in a previous session. During the second measuring session, reconstituted cheese, WSE, and reconstituted cheese in which WSE was omitted were evaluated for taste profile determination. At each of the two measuring sessions, 4 g of each product was tasted. Panelists were instructed as during the training period to take the sample with a small plastic spoon, to taste it, and to score the intensity of each attribute. Between each sample, assessors were instructed to rinse their mouths with apple, bread crumb, and mineral water in that order. At each session, the products were tasted twice.

Statistical Treatment. The data were processed with the SAS system release 6.12 (SAS Institute Inc., Cary, NC). Analysis of variance was performed at level $\alpha = 0.05$, according to the model attribute = product + subject + product × subject, with subject considered as a random effect. Means were compared with the Newman−Keuls multiple-comparison test.

RESULTS AND DISCUSSION

Selection of a Bitter Camembert Cheese. The aim of this step was to select a process which allowed for a Camembert cheese to be made that would appear as the best compromise among three main selection criteria: first, the selected cheese must be strongly and typically bitter; second, as the bitterness is often correlated to the presence of small peptides, it had to be rich in peptides and particularly in small ones; third, the selected Camembert cheese had to have a gustatory profile close to that of the bitter commercial Camembert cheese.

Before the experimental cheeses were manufactured, six 45% fat in dry matter Camembert cheeses were purchased from commercial suppliers. Among them, c6 was selected as a reference of bitter commercial cheese used for comparison with the experimental cheeses.

The five experimental Camembert cheeses, Pc2, ModPc2, Pc3, ModPc3, and Pc2xGc3, were manufactured and compared to c6 from a gustatory point of view. As shown in Figure 2, the significant differences among the cheeses concerned bitterness and saltiness. As the sweetness, astringency, sharpness, and umami notes were markedly weaker in intensity, those four attributes were not reported. Two cheeses, Pc3 and c6, had similar gustatory profiles and were perceived to be significantly more bitter than the others as their bitter intensity reached over 60/100. In contrast, the intensity was quoted as being weaker than the reference (50/100) for the other products. These results confirmed the fundamental role played by *Penicillium camemberti* in the development of cheese bitterness already claimed by several authors (*5*, *6*, *22*). A strongly bitter Camembert cheese may therefore be obtained by using Pc3 alone to make pasteurized Camembert cheese ripened for 30 days without modifying the level of rennet or increasing the draining.

To choose the experimental cheese to be further studied, it was necessary to compare their relative richness in peptides. Table 3 shows the results of pH, TN, SN, PTASN, and peptides determination. Pc3 and c6 appeared as being cheeses with the strongest SN, PTASN, and peptides values and consequently the richest products in peptides. Otherwise, pH values indicate that increasing draining and higher rennet level might delay the normal cheese maturation. This fact could explain why those manufacturing processes did not lead to their expected result concerning bitterness.

To conclude, Pc3 appeared to be a good compromise of making a typically bitter Camembert cheese with a relatively high level of peptides.

Cheese Taste Evolution during Ripening. Prior to any interpretation of the results concerning evolution of Pc3 cheese taste during ripening, it was necessary to examine a possible drift in the panelists' ways of scoring during that period by using a control cheese. Figure 3 shows the portions of the control cheese and the attributes for which significant differences appeared among the six sessions corresponding to the six studied

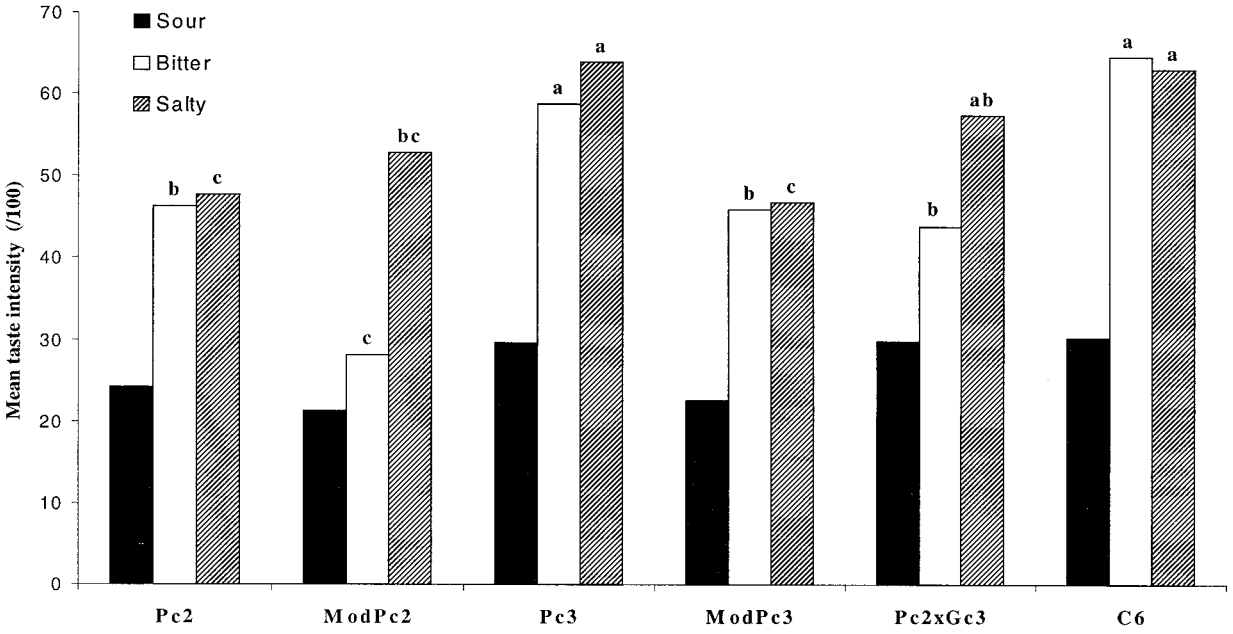

Figure 2. Taste profile of experimental cheeses evaluated together with one commercial cheese (c6). The bars indicate the mean gustatory intensity of the considered attributes for three replications. For each attribute, means with the same letter (a−c) are not significantly different at the level of 5% according to Newman−Keuls tests.

Table 3. Nitrogen Determination in Experimental Cheeses

exptl cheese	pH	TN (g/kg of cheese)	SN (%TN)	PTASN (%TN)	peptides (%TN)
Pc2	6.19	33.47	26.44	6.69	19.75
ModPc2	5.89	35.57	19.99	4.25	15.74
Pc3	5.94	31.80	30.31	8.14	22.17
ModPc3	5.71	35.96	25.22	5.78	19.44
Pc2xGc3	5.78	32.62	26.67	6.22	20.45
c6	6.47	34.49	36.13	9.63	26.50

[a] TN, total nitrogen; SN, soluble nitrogen; PTASN, phosphotungstic acid soluble nitrogen.

ripening stages of Pc3 cheese. For the center portion of the control cheese, saltiness was quoted around 60/100 at the first session (ninth day of Pc3 cheese ripening) and afterward decreased and stabilized at approximately 50/100. This phenomenon was not observed for the saltiness of under-rind or rind, which seemed to remain almost constant. Moreover, the decrease observed for the center of the control cheese was significant only when the 9th day (first session) was considered in comparison with the 21st day (third session). The other changes concerned bitterness of both under-rind and center of the control cheese, which decreased at, respectively, the 21st (third session) and 28th days (fourth session) of the study. This might be explained by a strong intensity of an analogue portion of Pc3 cheese. Anyway, those slight changes in control cheese evaluation did not express any systematic tendency and might be related to the magnitudes and directions of differences we observed in the taste profile of Pc3 cheese during its ripening.

As Pc3 cheeses have weak levels of sweetness, umami, and sharp notes, with means of less than 10/100, the results concerning these three attributes are not presented. Figure 4 shows the evolution of sourness, bitterness, saltiness, and astringency during ripening of the M2 manufacture serial for, respectively, Pc3 cheese rind, under-rind, and center.

Figure 4a shows a decrease of the perceived sour note between 9 and 35 days of ripening whatever the portions considered. The decrease in sourness appears as being first more pronounced in the rind, where it seems to be stabilized after 21 days of ripening. The two other portions seem to display similar decreases in sourness until reaching the same intensity evaluated at around 25/100. According to perception mechanisms at receptor level (*23*), the sour note in food products may be partially due to H_3O^+ concentration. Several authors, such as Le Graet et al. (*10*), showed the occurrence of a pH gradient in the cheese at the beginning of the ripening. This phenomenon would be due to lactic acid consumption by the *Penicillium* on the cheese surface, which may cause a fast pH increase. This could explain why a sourness decrease occurred first in the rind. However, as was previously showed by Biede and Hammond (*13*), pH and sourness may not be correlated. This last result is in accordance with those obtained by other authors (*1*, *4*) showing that the consideration of H_3O^+ concentration was not sufficient to fully explain the sour taste and that some other chemical species such as sodium chloride may act on this taste characteristic. The evolution of sourness in the other portions may be explained by the migration phenomena of taste-active compounds such as molecules that change the pH or those that are responsible for some enhancing effect such as sodium chloride. Thus, before a conclusion on the cause of the observed effect can be made, physicochemical analysis of the related taste-active compounds contained in the studied portions seemed to be necessary.

Changes in bitterness affecting the different portions of the cheese during ripening are given in Figure 4b. As the evolution of bitterness in cheese portions is far more important and opposite from punctual variations due to a slight panel drift observed in the control cheese (Figure 3), we conclude that it can be related to ripening effects on Pc3 cheese. Moreover, the relatively strong intensity of scored bitterness for Pc3 cheese under-rind and center at, respectively, 21 and 28 days of ripening may explain the weaker levels seen in the corresponding portions of the control cheese at these times. The

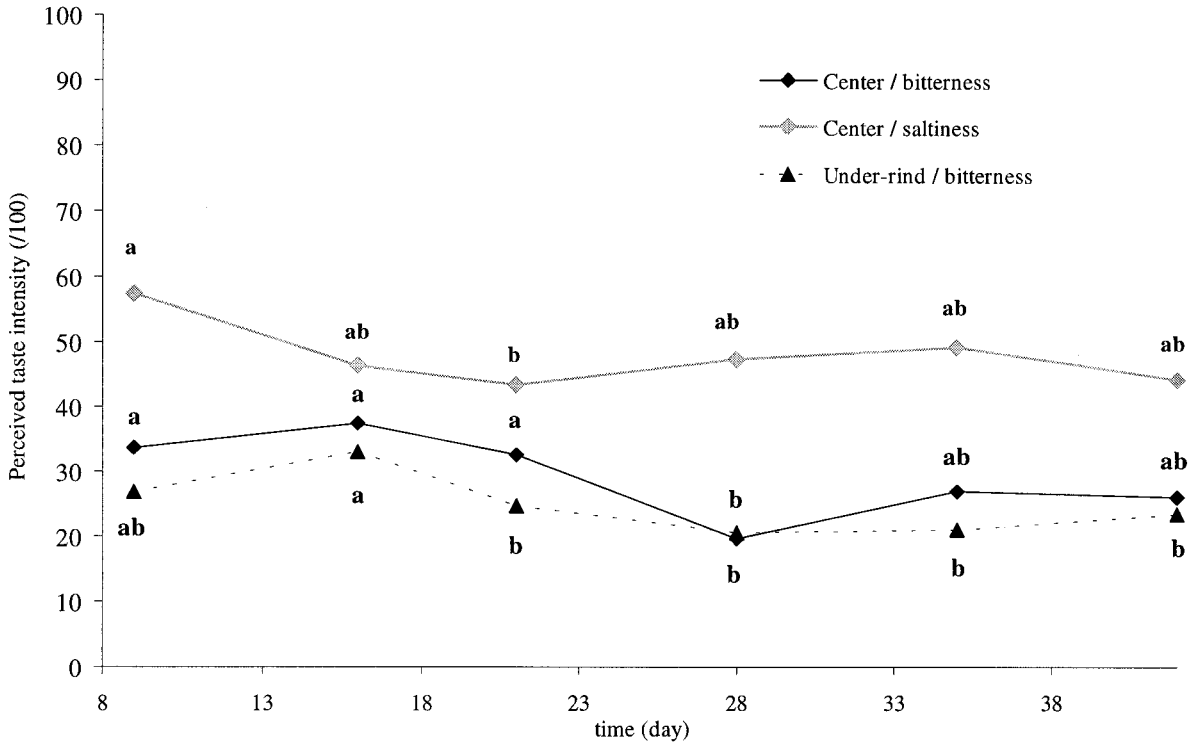

Figure 3. Evaluation of panel drift among the six sessions of the study of Pc3 cheese ripening: changes of the perceived taste intensity in the different portions of a control cheese. Control cheeses were evaluated at six sessions corresponding, respectively, to 9, 16, 21, 28, 35, and 42 days of Pc3 cheese ripening. The points represent the mean gustatory intensity of the considered attributes for two replications. For each attribute, means with the same letter (a−c) are not significantly different at the level of 5% according to Newman−Keuls tests.

evolution of Pc3 bitterness showed different tendencies among the portions considered (Figure 4b). In the rind portion, its intensity increased to 70 /100 between the 9th and 16th days of ripening and then decreased between the 16th and 21st days. Afterward, its value was almost constant. In the under-rind portion, the bitterness increased from 30/100 to over 80/100 between the 9th and 16th days and then appeared as being approximately constant. In the center, the increase was progressive between the 9th day, when its intensity was around 20/100, and the 28th day, when it reached more than 70/100. Many studies mention the probable causative linkage between proteolysis and cheese bitterness (*2*, *6*, *24*). The known progression of proteolytic activity from the surface of the cheese to its center during maturation gives us a good explanation of the sequential phenomenon observed. At the beginning of maturation, the proteolytic activity due to the development of *P. camemberti* on the surface is limited to the rind and releases bitter peptides exclusively in this portion. After 2 weeks, the rind portion is completely made up of mycelia, which appear to be less bitter than proteolyzed material. During the same time, the proteolytic front colonizes first the under-rind portion and then later the center. This would cause an increase in the intensity of bitterness in this portion. The stabilization of bitterness that occurs afterward might be explained according to Molimard et al. (*6*) by the absence of proteolytic activities generally due to *Geotrichum*. However, even if bitterness seems to be linked with proteolysis, there is no evidence showing bitter peptides and their metabolism as being responsible for the evolution of bitter taste. Other components such as mineral salts (*4*, *16*) or biogenic amines (*3*) and modification of the cheese matrix may also be implied in this evolution of bitter taste.

As for bitterness, the evolution of cheese astringency during ripening (Figure 4c) seems to be related to proteolysis. Instead of an increase, its intensity decreased in the under-rind and center portions; however, no significant intensity change was shown for the rind. These results agree with previous studies showing that proteins and/or big peptides might be responsible for cheese astringency (*16*, *25*). The progression of the proteolysis front could explain why astringency decreases first in the under-rind and more progressively in the center.

Concerning saltiness, the effect of ripening is shown in Figure 4d. First, even if the significant decrease of the control cheese saltiness was the result of a punctual change in the way of scoring (Figure 3), this panel drift may just allow for the decrease of Pc3 saltiness in the center portion to be explained. In contrast, it did not imply any other significant consequences on the evaluation of the experimental cheese. In the rind, the saltiness decreased until the 16th day of ripening; afterward, its intensity was almost constant. In both under-rind and center portions, the salty taste seemed to slightly increase even if this effect was not significant in the center. It is well accepted that minerals and, more especially, sodium, potassium, calcium, and magnesium chloride may be involved in the salty taste (*1*, *4*). Moreover, some of those compounds may be affected by a migration phenomenon occurring sometimes in the opposite direction (*10*) or by a concentration effect consecutive to water evaporation. Consequently, it would be hazardous to give any definitive interpretation without knowledge of the evolution of the composition of mineral salts.

This sensory study revealed some differences appearing in the cheese taste profile during ripening. To

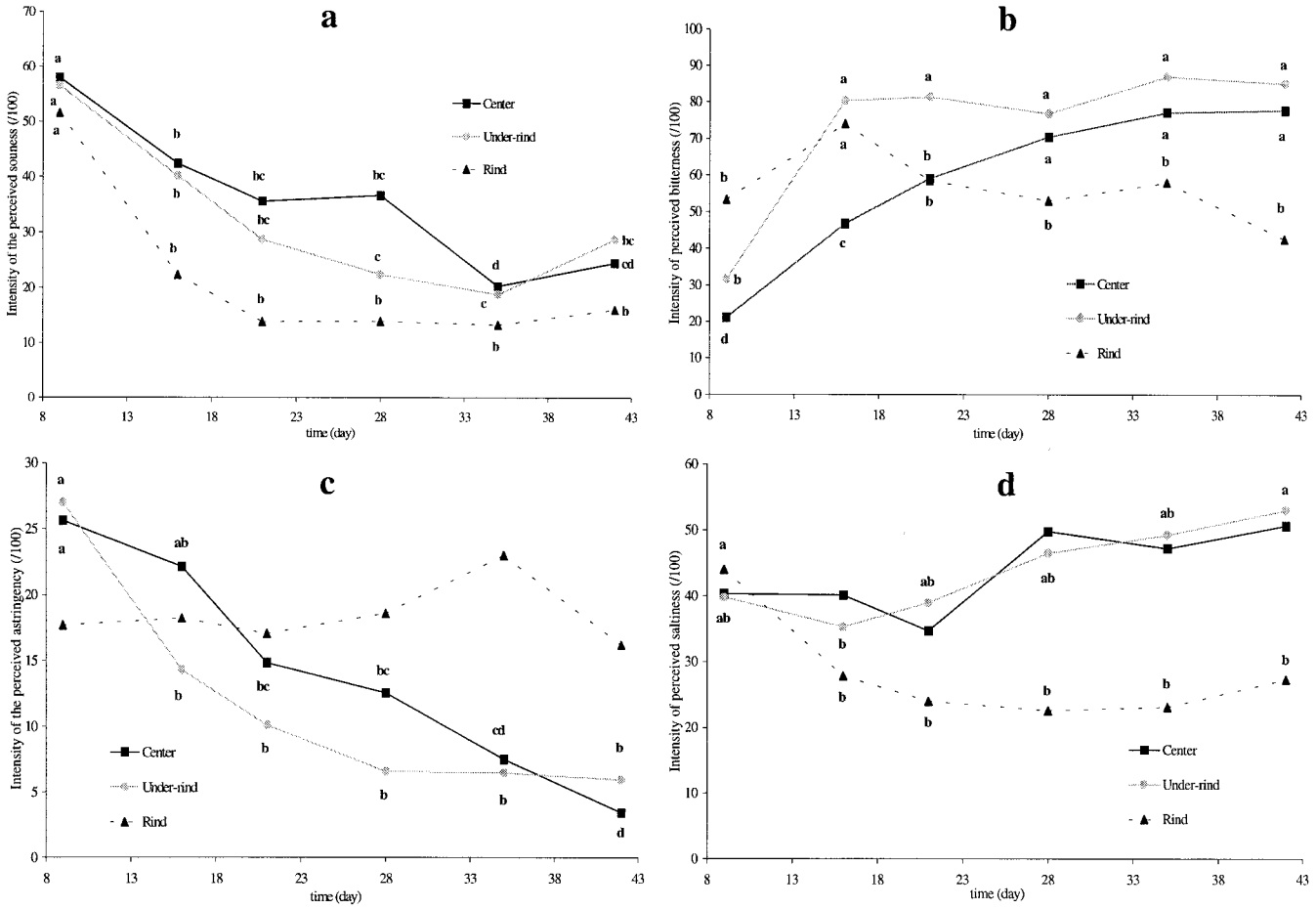

Figure 4. Evolution of the perceived taste intensity in the different portions of cheese during ripening for sourness (a), bitterness (b), astringency (c), and saltiness (d). The points represent the mean gustatory intensity of the considered attributes for two replications. For each attribute, means with the same letter (a−d) are not significantly different at the level of 5% according to Newman−Keuls tests.

explain this evolution, it was necessary to follow the concentration of compounds responsible for each of the main taste characteristics. According to previous work on goat cheese, the way to determine cheese taste-active compounds may be to use omission tests on a reconstituted WSE (*4*, *16*). The WSE is indeed considered by numerous authors as being responsible for cheese taste (*1*, *14*). However, the other less soluble fractions have to be taken into account in the case of bitter cheese because of the possible hydrophobic nature of peptides that are presumed to be the bitter stimuli. Thus, finally it was necessary to evaluate the exact role of the WSE in a context as close as possible to crude cheese.

Study of the Relative Impact of Fat, Proteins, and WSE on Pc3 Cheese Taste: Characterization of a Matrix Effect. To study the relative impact of fat, proteins, and WSE on cheese taste, each cheese fraction was totally recovered during the extraction process. By mixing all of these components and making adjustments when necessary at their appropriate concentration, we obtained a reconstituted Pc3 cheese.

Prior to any omission test, the reconstituted cheese must be compared to the crude one. Figure 5 shows the related gustatory profile of whole, grated, and reconstituted Pc3 cheese. Reconstituted Pc3 cheese appears to be saltier and less bitter than whole Pc3. Even if the comparison between whole and grated cheese shows only a tendency ($P < 0.1$) to increase the saltiness, the slight difference between grated and reconstituted cheese indicates that grating may be the main cause of saltiness difference between whole and reconstituted cheese. This phenomenon, already referred to by McSweeney (*1*), could be explained by an increase of mineral salts availability due to cheese grating. In contrast, the comparison between grated and whole Pc3 cheese indicates that even if grating might have a tendency ($P < 0.09$) to decrease bitterness, its impact was not sufficient to explain the highly significant difference between whole and reconstituted cheese. As freeze-drying, centrifugation, and homogenization in water are often used as nondestructive techniques in studies of bitter peptides, the specific loss of bitter material is unlikely. Thus, the loss of bitterness in reconstituted cheese may be linked with the difference between its matrix structure and that of whole cheese. In the reconstituted cheese, the matrix is almost totally degraded due to water extraction. Comparison between the taste profiles of whole and reconstituted cheeses reveals indeed that structural organization of the cheese matrix may increase the perceived bitterness due to cheese taste-active compounds. To conclude, the weaker bitterness and stronger saltiness in the reconstituted cheese may be explained, on the one hand, by a modification of taste-active compounds' availability due to cheese structure degradation and, on the other hand, by a change of texture interacting with taste.

The results of omission tests made on bitter cheese are given in Figure 6. First of all, when the WSE is omitted, the resulting reconstituted cheese (fat plus proteins plus RUF0.05) was tasteless, which indi-

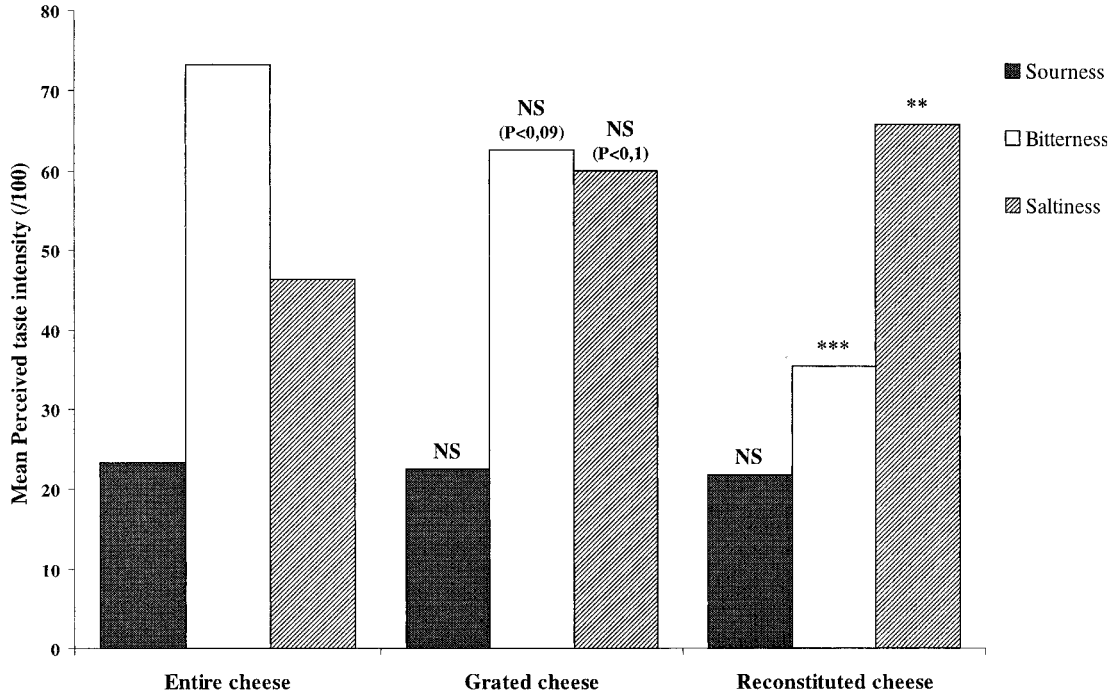

Figure 5. Gustatory profile of crude, grated, and reconstituted Pc3 cheese. For each attribute, *t* tests were performed between whole cheese and grated or reconstituted cheese. Significant ($P < 0.05$), very significant ($P < 0.01$), and highly significantly differences with the whole cheese are indicated with *, **, and ***, respectively. When the P value is >0.05 but <0.10, it is shown in parentheses. Each product was tasted twice by each panelist.

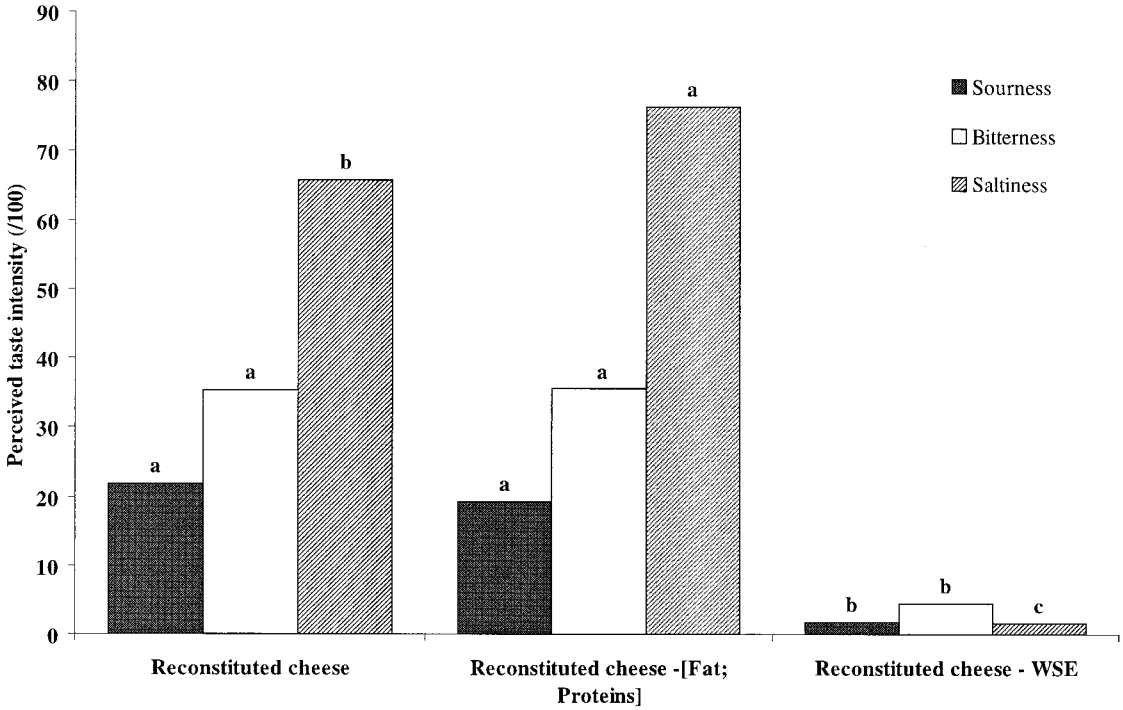

Figure 6. Comparison of gustatory profiles of reconstituted cheese, reconstituted cheese in which fat and proteins were omitted, and reconstituted cheese in which the WSE was omitted. For each attribute, means with the same letter (a−c) are not significantly different at the level of 5% according to Newman−Keuls tests. All omissions have been performed for two replicates.

cates that this WSE contains cheese taste-active compounds. In contrast, omission of fat and proteins together increased significantly the saltiness intensity of the reconstituted Pc3 cheese, implying that those fractions might act as masking agents for this taste characteristic.

Pc3 cheese taste may then be explained as resulting from the taste of the WSE containing taste-active compounds modulated by a matrix effect. This modulating effect may be characterized as composed first of masking due to fat and proteins, second of cheese matrix structure effects on the availability of taste-active compounds, and, finally, the effect of texture, which must interact differently with the taste perception of the cheese and its WSE. This effect can be quantified for a given taste A using an index defined as matrix

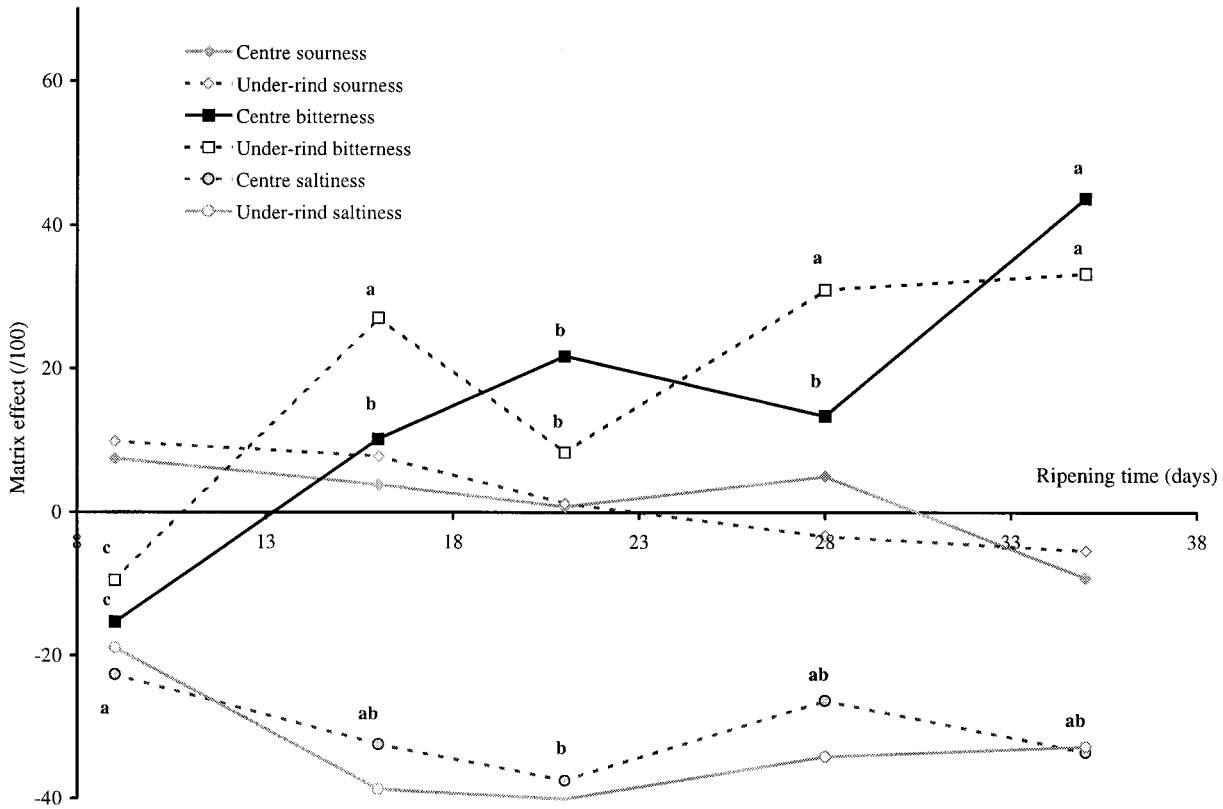

Figure 7. Evolution of the matrix effect on sourness, bitterness, and saltiness during ripening of a Pc3 Camembert cheese. Matrix effect on a taste *X* is defined as the difference between the mean perceived intensity of *X* in the cheese and the mean intensity of *X* in the corresponding WSE. For each attribute, means with the same letter (a−c) are not significantly different at the level of 5% according to Newman−Keuls tests. Calculation of each matrix effect value was performed starting with two replications of taste evaluation for both cheese and WSE.

effect (A) and equal to the difference between the perceived intensity of A in the cheese and the perceived intensity of A in the corresponding WSE.

Evolution of the Matrix Effect on Cheese Taste during Ripening. Every Pc3 portion already evaluated at the 9th, 16th, 21st, 28th, and 35th days of ripening as previously described (Figure 4) was submitted to water extraction and tasted. Using the under-rind and center portions, it was possible to calculate matrix effect values for sourness, bitterness, and saltiness for each ripening stage (Figure 7). The case of astringency is not shown because it was quoted at an almost constant low intensity of 5/100 in the WSE, confirming that the origin of this sensation might be due to non-water-soluble molecules, presumably protein or large peptides.

As attested by the weak values of the matrix effect, the sourness intensities quoted for the extracts were in good accordance with those obtained for the corresponding cheese portions. Whereas a slight decreasing tendency might be deduced from the curves, that taste characteristic did not seem to be affected by any modulating effect. Thus, the WSE gives a representative judgment of the cheese sourness.

Conversely, negative matrix effect values appeared for saltiness. They indicate a stronger value of WSE saltiness in comparison with that evaluated in the cheese, confirming results obtained previously with reconstituted cheese whatever the ripening stage considered. However, this masking effect of the matrix on saltiness seems to be weaker on the ninth day in comparison with the other ripening times. This lighter matrix effect might be partially explained by the brittle texture and the greater ability of Camembert cheese to release water when it is pressed at the beginning of its maturation (*26*). Then because of the easier release of water-soluble molecules, the taste of cheese would be more comparable to that of its WSE.

Figure 7 shows that the bitter taste of cheese portions and the corresponding WSE does not evolve in the same manner during maturation. In both center and under-rind portions, the matrix effect globally increased during ripening. The profiles of the curves obtained for the two portions are similar, and the evolution of the matrix effect seems to be faster for the under-rind than for the center. Considering a global tendency whatever the portion, the matrix effect clearly increased between the 9th and 35th days of ripening. It would have been possible to hypothesize a change of bitter material extractability during ripening because, for example, of the modification of cheese pH (*2*). However, the previous results on reconstituted cheeses obtained from a 31-day-old Pc3 Camembert cheese showed clearly that even if a part of bitter material was retained in the non-water-soluble fraction during water extraction, this part would have a negligible impact on cheese bitterness (Figure 6). Thus, this evolution may be better explained by a possible exhausting effect of the cheese matrix because of its modification by lipolysis and proteolysis during maturation. Additionally, a decrease of salty stimulus availability in the cheese in comparison with the water extract may increase the relative impact on the taste of less soluble compounds such as bitter molecules.

Conclusion. This study allowed for the selection of a typically bitter Camembert cheese and for the characterization of the relative impact of fat, proteins, and WSE on cheese taste. Although the WSE was shown to

contain the taste-active compounds, it was demonstrated that a modulating effect of the cheese matrix had to be taken into consideration to fully explain cheese gustatory properties. Starting from that result, a study has been performed to determine those taste-active compounds in studying the WSE of a 31-day Pc3 Camembert cheese.

Otherwise, the parallel study of different cheese portions revealed the existence of taste waves going through the cheese along maturation. Those phenomena might be due to migration of taste-active components. Thus, knowledge of the composition evolution of the different portions would enable the movements of potentially taste-active compounds to be followed and then to explain the changes appearing in cheese taste properties. The main results of these physicochemical analysis are discussed in a second paper.

ACKNOWLEDGMENT

We gratefully acknowledge Anita Garem and Nadine Leconte (Laboratoire de Recherches de Technologie Laitière, INRA, Rennes, France) for ultra- and nanofiltration attempts.

LITERATURE CITED

(1) McSweeney, P. L. H. The flavour of milk and dairy products: III. Cheese: taste. *Int. J. Dairy Technol.* **1997**, *50*, 123−128.
(2) Lemieux, L.; Simard, R. E. Bitter flavour in dairy products. II. A review of bitter peptides from caseins: their formation, isolation and identification, structure masking and inhibition. *Lait* **1992**, *72*, 335−382.
(3) Kubickova, J.; Grosch, W. Evaluation of flavour compounds of Camembert cheese. *Int. Dairy J.* **1998**, *8*, 11−16.
(4) Engel, E.; Nicklaus, S.; Septier, C.; Salles, C.; Le Quéré, J. L. Taste-active compounds in a goat cheese water-soluble extract. Part 2. Determination of the relative impact of water-soluble extract components on its taste using omission tests. *J. Agric. Food Chem.* **2000**, *48*, 4260−4267.
(5) Vassal, L.; Grippon, J. C. L'amertume des fromages à pâte molle de type Camembert: rôle de la présure et de *Penicillium caseicolum*, moyens de la contrôler. *Lait* **1984**, *64*, 397−417.
(6) Molimard, P.; Lesschaeve, I.; Bouvier, I.; Vassal, L.; Schlich, P.; Issanchou, S.; Spinnler, H. E. Amertume et fractions azotées de fromages à pâte molle de type camembert: rôle de l'association de *Penicillium camemberti* avec *Geotrichum candidum*. *Lait* **1994**, *74*, 361−374.
(7) Alais, C. *Science du Lait, Principes des Techniques Laitieres*, 4th ed.; SEPAIC: Paris, France, 1984.
(8) Choisy, C.; Desmazeaud, M.; Gripon, J. C.; Lamberet, G.; Lenoir, J.; Tourneur, C. Les phénomènes microbiologiques et enzymatiques et la biochimie de l'affinage. In *Le Fromage*; Eck, A., Ed.; Tec et Doc Lavoisier: Paris, France, 1983; pp 62−100.
(9) Mourgues, R.; Bergere, J. L.; Vassal, L. Possibilité d'améliorer les qualités organoleptiques des fromages de Camembert grâce à l'utilisation de *Geotrichum candidum*. *Tech. Lait.* **1983**, *978*, 11−15.
(10) Le Graet, 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* **1983**, *63*, 317−332.
(11) Lesage, L.; Sauvageot, F.; Voilley, A.; Lorient, D. Influence de la teneur en NaCl et de la durée d'affinage sur les caractéristiques sensorielles d'un fromage type Camembert enrichie en magnésium. *Lait* **1992**, *72*, 73−85.
(12) Biede, S. L.; Hammond, E. G. Swiss cheese flavor: I. chemical analysis. *J. Dairy Sci.* **1979**, *62*, 227−237.
(13) Biede, S. L.; Hammond, E. G. Swiss cheese flavor: II. organoleptic analysis. *J. Dairy Sci.* **1979**, *62*, 238−248.
(14) Aston, J. W.; Creamer, L. K. Contribution of the components of the water-soluble fraction to the flavour of Cheddar cheese. *N. Z. J. Dairy Sci. Technol.* **1986**, *21*, 229−248.
(15) Salles, C.; Septier, C.; Roudot-Algaron, F.; Guillot, A.; Etiévant, P. X. Sensory and chemical analysis of fractions obtained by gel permeation of water-soluble Comté cheese extracts. *J. Agric. Food Chem.* **1995**, *43*, 1659−1668.
(16) Engel, E.; Nicklaus, S.; Garem, A.; Septier, C.; Salles, C.; Le Quéré, J. L. Taste-active compounds in a goat cheese water-soluble extract. 1. Development and sensory validation of a model water-soluble extract. *J. Agric. Food Chem.* **2000**, *48*, 4252−4259.
(17) Delahunty, C. M.; Piggott, J. R. Current methods to evaluate contribution and interactions of components to flavour of solid foods using hard cheese as an example—review. *Int. J. Food Sci. Technol.* **1995**, *30*, 555−570.
(18) Piraprez, G.; Herent, M. F.; Collin, S. Flavour retention by lipids measured in a fresh cheese matrix. *Food Chem.* **1998**, *61*, 119−125.
(19) Gripon, J. C.; Desmazeaud, M. J.; Le bars, D.; Bergère, J. L. Etude du rôle des microorganisme et des enzymes au cours de la maturation des fromages. II. Influence de la présure commerciale. *Lait* **1975**, *55*, 502−516.
(20) MacFie, H. J.; Bratchell, N.; Greenhoff, K.; Vallis, L. V. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *J. Sensory Stud.* **1989**, *4*, 129−148.
(21) MacFie, H. J., Earthy, P.; Delza, R. Measurement of consumer preference. In *Consumer Preference and Sensory Analysis*; Poretta S., Ed.; Maarsen, The Netherlands, 1996; pp 9−20.
(22) Ribadeau-Dumas, B. Maîtrise de l'affinage des fromages de type Camembert. *Lait* **1984**, *64*, 448−468.
(23) Laing, D. G.; Jinks, A. Flavour perception mechanisms. *Trends Food Sci. Technol.* **1996**, *7*, 387−389.
(24) Habibi-Najafi, M. B.; Lee, B. H. Bitterness in cheese: a review. *CRC Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 397−411.
(25) Harwalkar, V. R.; Elliott, J. A. Isolation of bitter and astringent fractions from Cheddar cheese. *J. Dairy Sci.* **1971**, *54*, 8−11.
(26) Boutrou, R.; Gaucheron, F.; Piot, M.; Michel, F.; Maubois, J. L.; Léonil, J. Changes in the composition of juice expressed from Camembert cheese during ripening. *Lait* **1999**, *79*, 503−513.

Received for review August 1, 2000. Revised manuscript received March 8, 2001. Accepted March 15, 2001. This research was financed by INRA and the Regional Council of Burgundy.

JF000967M