Microbiological and Biochemical Aspects of Camembert-Type Cheeses Depend on Atmospheric Composition in the Ripening Chamber

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ABSTRACT

Camembert-type cheeses were prepared from pasteurized milk seeded with *Kluyveromyces lactis*, *Geotrichum candidum*, *Penicillium camemberti*, and *Brevibacterium aurantiacum*. Microorganism growth and biochemical dynamics were studied in relation to ripening chamber CO₂ atmospheric composition using 31 descriptors based on kinetic data. The chamber ripening was carried out under 5 different controlled atmospheres: continuously renewed atmosphere, periodically renewed atmosphere, no renewed atmosphere, and 2 for which CO₂ was either 2% or 6%. All microorganism dynamics depended on CO₂ level. *Kluyveromyces lactis* was not sensitive to CO₂ during its growth phases, but its death did depend on it. An increase of CO₂ led to a significant improvement in *G. candidum*. *Penicillium camemberti* mycelium development was enhanced by 2% CO₂. The equilibrium between *P. camemberti* and *G. candidum* populations was disrupted in favor of the yeast when CO₂ was higher than 4%. Growth of *B. aurantiacum* depended more on O₂ than on CO₂. Two ripening progressions were observed in relation to the presence of CO₂ at the beginning of ripening: in the presence of CO₂, the ripening was fast-slow, and in the absence of CO₂, it was slow-fast. The under-rind was too runny if CO₂ was equal to or higher than 6%. The nitrogen substrate progressions were slightly related to ripening chamber CO₂ and O₂ levels. During chamber ripening, the best atmospheric condition to produce an optimum between microorganism growth, biochemical dynamics, and cheese appearance was a constant CO₂ level close to 2%.

Key words: chamber atmospheric composition, CO₂ and O₂, microbial and biochemical aspects, mold soft cheese ripening

INTRODUCTION

Cheese quality depends on simultaneous control of the raw material and curd production as well as pro-

cesses and conditions of ripening. A large number of microbiological and biochemical parameters influence the ripening of soft mold-ripened cheeses like Camembert. Specific microbiological or enzymatic and biochemical aspects have already been studied (Gripon, 1993). A kinetic approach to microbial and biochemical phenomena during Camembert cheese ripening has been recently published (Leclercq-Perlat et al., 2004). These authors have established relationship between the different microbiological and biochemical changes during cheese ripening in continual renewal atmospheric conditions.

Environmental factors (temperature, hygrometry, and gaseous atmospheric composition in ripening chamber) play a determining role in microbial development and enzymatic process (van den Tempel and Nielsen, 2000; Bonaïti et al., 2004). For French mold soft cheeses, ripening temperature is kept at 12°C. Although increasing the ripening temperature (3 to 4°C) is one of the easiest and most economically feasible strategies to accelerate ripening (Nunez et al., 1991), soft cheeses (in which the surface microflora has important enzymatic activities) cannot undergo substantial temperature increases without significantly modifying cheese quality (poor texture, unpleasant taste; Ramet, 2000). For mold cheeses, ripening chamber hygrometry is close to 95%. Consequently, a part of the cheese water evaporates to the atmosphere leading to a decrease in cheese mass and water content (Hardy et al., 2000). If relative humidity (RH) is higher than this value, *P. camemberti* development is poor and mycelia are brown. Moreover, if RH is lower than 95%, the water loss is excessive; therefore, the resulting cheese is too dry.

Although more data are available on packaging films and their effects on soft cheeses, little information is available on the effect of the gaseous environment in ripening chambers on the growth of microorganisms and its consequences on cheese qualities. Carbon dioxide extends the shelf life of cheeses by inhibiting mold growth (Doyon et al., 1997; Pintado and Malcata, 2000a,b; Colchin et al., 2001). However, the atmospheric composition of the ripening chamber has been shown to change the overall ripening process due to

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its effects on microorganism physiology (Kiermeier and Wolfseder, 1972a,b; Champagne et al., 2003). A low O₂ concentration reduces aerobic microorganism metabolisms (Champagne et al., 2003), whereas addition of CO₂ to the atmospheric composition may increase bactericidal or fungicidal effects (Taniwaki et al., 2001). Moreover, according to Champagne et al. (2003), CO₂ and O₂ concentrations used in experiments focused on postwrapping changes (10 to 30% CO₂ and 10 to 40% O₂) are very different from those used in industrial ripening chambers. Little work has involved the effects of CO₂ and O₂ on the growth of flora throughout the ripening process and the consequences on cheese quality (Kiermeier and Wolfseder, 1972a,b; Weissenfluh and Puhon, 1987; Roger et al., 1998). Kiermeier and Wolfseder (1972a,b) have shown that oxygen consumption is correlated to CO₂ production during the ripening of soft mold-ripening cheeses. Weissenfluh and Puhon (1987) and Roger et al. (1998) have shown that respiration rate of *P. camemberti* decreased when O₂ partial pressure was reduced. However, the growth dynamics of strains used to ripen Camembert cheeses and their contribution to overall ripening need more investigation, in relation to time and gaseous atmospheric composition of chamber.

The aim of this study was to monitor microflora development and biochemical changes of Camembert-type cheeses throughout all the ripening process (from d 0 to 40), in relation to gaseous atmospheric composition of the ripening chamber (from d 0 to 16), especially CO₂ concentration. The approach involved manufacturing a Camembert-type cheese on a pilot scale under aseptic environmental conditions, seeding with a ripening culture (*K. lactis*, *G. candidum*, *P. camemberti*, or *B. aurantiacum*). After draining and molding, the cheeses were ripened under 5 different gaseous atmospheric compositions and under controlled conditions of temperature and RH. The effects of CO₂ and O₂ levels during chamber ripening were studied in relation to microbial growth, biochemical dynamics, and appearance of cheeses. The set of cheeses used here was the same as used in the companion study (Picque et al., 2006).

MATERIALS AND METHODS

Biological Material, Preparation of Cultures, and Cheese Production

The ripening cultures were *Kluyveromyces lactis* (GMPA collection, 44₈), *Geotrichum candidum* (Degussa, D), *Penicillium camemberti* (Degussa, R), and *Brevibacterium aurantiacum* (ATCC9175; Gavriš et al., 2004).

The preparations of *K. lactis* and *B. aurantiacum* were carried out as previously described in Leclercq-Perlat et al. (2004). *Geotrichum candidum* (strain D) and *P. camemberti* (strain R) were lyophilized. Their preparation was described in Leclercq-Perlat et al., 2004. One week before use, a single tube of each starter was thawed and the viable cell concentration of each was determined. For *K. lactis* and *G. candidum*, this was done by surface plate counting on yeast extract glucose chloramphenicol (YEGC, Biokar, Beauvais, France) after 2 to 3 d of incubation at 25°C. For *B. aurantiacum* it was determined by using a lactate medium after 5 to 6 d of incubation at 25°C, and for *P. camemberti* by using YEGC containing 6 g of sodium chloride/100 g (6 to 8 d; 20°C).

Flora Danica lyophilizate (CHN11, Chr. Hansen, Arpajon, France) was the chosen mesophilic lactic acid bacteria culture. It was prepared according to Leclercq-Perlat et al. (2004). Before each cheese making run, 1 aliquot of the starter was cultured at 22°C in 1.5 L of sterile skim milk for 16 h.

The cheeses were prepared on a pilot scale under aseptic conditions in a sterilized 2-m³ cheese-making chamber in which coagulation, cutting, draining, and shaping of the curds were carried out (Leclercq-Perlat et al., 2004). For each cheese-making trial, 100 L of milk were used to make 45 cheeses. The raw milk was obtained from the experimental farm of the Institut National Agronomique Paris-Grignon (Thiverval-Grignon, France). It was standardized at 2.9 g of fat/100 g by mixing skim and full-cream milk, and contained more than 3.2 g of protein/100 g. The cheese making and ripening of the Camembert-type cheeses was described in detail previously (Leclercq-Perlat et al., 2004). Ripening took place under the same conditions (13°C for all chamber ripening, 24 h at 85% RH, then under defined RH from d 2 to 14, and thereafter at 85% RH) except for RH (95 ± 2% instead of 93 ± 1%) and the wrapping time (on d 16 instead of d 14). However, the system used to maintain RH in the chamber in previous experiments was changed to account for salt evaporation. Indeed, in this study, RH was controlled without salt baths (potassium nitrate, KNO₃ in saturation) because during the preliminary CO₂ runs, a significant quantity of water evaporated into the atmosphere and was not absorbed by the salt baths. Thus, a system based on the principle of cold point, involving a cooling and heating system plunged into a water tank, was used.

From d 0 to 16, cheeses were ripened in chamber under 5 controlled atmospheres as described in detail I the companion article (Picque et al., 2006): 1) continuously renewed atmosphere (**CRA**) under which CO₂ and O₂ levels remained at 0 and 21%, respectively; 2) period-

ically renewed atmosphere (**PRA**) under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); 3) no renewed atmosphere (**NRA**) under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 4) CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18% (**2CO₂**); and 5) CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13% (**6CO₂**).

Each controlled atmosphere trial was carried out in duplicate, except NRA. Only one run was carried under NRA because the rind color was slightly brown, and cheeses exhibited another major flaw: the surface was greasy and irregular; the underrind was completely runny.

For all runs, the effects of CO₂ and O₂ on the microbiological and biological aspects of cheeses were recorded. Under PRA and NRA, CO₂ concentration increased in relation to time and it was calculated by determining the average for each phase of the timeline described below.

For each run, all the cheeses were turned on d 5. Between d 0 and 16, 1 cheese was removed daily for analysis. On d 16, all remaining cheeses were wrapped, and left to finish ripening at 4°C under unknown RH until d 40. During this period (d 16 to 40), 1 cheese was removed weekly for analysis.

Microbial Counts and Biochemical Analysis

These analyses were carried out as previously described by Leclercq-Perlat et al. (2004).

Viable cell counts of *K. lactis* and of *G. candidum* in both the rind and the core were measured by counting in YEGC dishes (25°C; 2 to 3 d). Spores of *P. camemberti* in the rind were determined by counting on YEGC medium containing 60 g of NaCl/L to limit *G. candidum* growth (20°C, 6 to 8 d). Viable cells of *B. aurianticum* in the rind were determined on amphotericin-containing lactate agar (25°C, 7 d).

Dry matter and pH as well as lactose and lactate concentrations in both the rind and the core were measured. Nitrogen fractions [total N, acid-soluble nitrogen (**ASN**), NPN, and free ammonium concentrations] were measured for the rind only. The thickness of the cheese creamy underrind was measured using the first 15 mm at 6 points on each face. Then, the arithmetic average of these 12 measurements was calculated. The appearance of the cheese (microorganism cover, surface appearance, and texture of the underrind) was estimated as proposed by Bonaïti et al. (2004).

Statistical Analyses

The evolutions of microbiological concentrations, biochemical kinetics, and the cheese appearance during the entire ripening period (d 0 to 40) were described by 2 or 3 descriptors. Except for *K. lactis*, the descriptors were determined by a Weibull model (using Statistica for Windows, Statsoft, Maisons-Alfort, France). The descriptors for *K. lactis* were calculated by linear regression. All the statistical tests were carried out using the 95% confidence level ($P < 0.05$). The reproducibility of the microbiological (viable cell counts) and the biochemical measurements (carbon substrate concentrations, pH, DM, nitrogen fractions, and underrind thickness) were studied according to Leclercq-Perlat et al. (2004). This analysis showed that 1) the hypotheses of equality of the means were satisfactory ($1 - \alpha = 0.95$), and 2) the risks of a false interpretation (α) were less than 0.05. For all statistical analyses, explained variance (Weibull modeling; R) or correlation coefficient (linear regression; R) was between 0.90 and 0.99, at a 95% confidence level, except for NPN maximal rate of PRA and 6CO₂. For this descriptor, explained variance (R) was between 0.81 and 0.83 at a 95% confidence level. If R was higher than 0.9, the descriptor was highly significant, and if it was between 0.81 and 0.90, the descriptor was simply significant.

For each single measurement, and each sampling day, a 1-way ANOVA was used to determine the influence of CO₂; ANOVA was carried out with Newman-Keuls test at a P -value of 0.01.

RESULTS

Microbiological and Biochemical Behavior Under CRA

Under CRA conditions, during which total air volume was renewed every 2 h, no accumulation of CO₂ was observed and O₂ level remained constant at 21% (Picque et al., 2006). These CRA runs were the reference CO₂ treatment. The effects of CO₂ and O₂ on microbiological and biochemical parameters and appearance of cheeses were compared with those obtained under the other 4 atmospheric conditions.

Microbiological Changes in Ripening Cheeses

For the average of 2 runs, the log₁₀ of ripening microorganism counts of the rind vs. time were similar to those described in a previous study (Leclercq-Perlat et al., 2004). In the core, the overall changes in *K. lactis* and *G. candidum* concentrations were similar to those in the rind. However, their concentrations were around 250 times lower than in the rind (data not shown). As

expected, *P. camemberti* and *B. aurantiacum* did not grow in the core.

Three phases were apparent in the timeline of *K. lactis* growth and several kinetic descriptors were calculated (Table 1). Firstly, between d 0 and 5, the specific growth rate ($\mu_{\max}$) during the exponential phase was $0.46 \pm 0.04 \text{ d}^{-1}$ and the associated generation time was close to 1.5 d. Secondly, from d 5 to 19, the viable count ($X_{\max}$) remained constant, close to $4 \times 10^7 \text{ cfu/g}$. Thirdly, after d 19 and until d 40, the viable *K. lactis* count diminished slowly and exponentially with a specific death rate ($D_{\max}$) equal to $0.100 \pm 0.007 \text{ d}^{-1}$.

Three phases were apparent in the timeline of *G. candidum* growth (Figure 1A, Table 1). Firstly, growth of *G. candidum* was measurable only after d 3 (lag phase). The second phase, between d 4 and 8, had a maximum specific growth rate ($\mu_{\max}$) on d 6 equal to $0.7 \pm 0.2 \text{ d}^{-1}$ and associated generation time was about 1.0 d. In the third phase (stationary phase), between d 9 and 40, the maximal viable count remained constant at $\sim 1 \times 10^7 \text{ cfu/g}$ of DM.

Viable *P. camemberti* spore counts in the rind were close to 10^4 cfu/g of DM during the first 6 d of ripening (Figure 1B, Table 1). Then, from d 6 to 19, growth was slow and close to exponential with a specific growth rate ($\mu_{\max}$) of $0.23 \pm 0.05 \text{ d}^{-1}$ occurring on $9 \pm 3 \text{ d}$ (Table 1). After d 20 and until d 40, these spore concentrations remained constant, close to $7.5 \times 10^5 \text{ cfu/g}$ of DM. Because the sampling technique destroyed the mycelia, only visual observation of the surface was possible; *P. camemberti* mycelia were seen from d 4. Between d 5 and 12, the mycelia grew and uniformly covered the entire cheese surface by d 12.

Three phases were apparent in the timeline of *B. aurantiacum* growth as previously shown (Leclercq-Perlat et al., 2004); *B. aurantiacum* counts in the rind remained nearly constant (at $6.3 \times 10^4 \text{ cfu/g}$ of DM) during the first 10 d of ripening. Between d 12 and 19, this species grew exponentially with a specific growth rate ($\mu_{\max}$) equal to $0.46 \pm 0.07 \text{ d}^{-1}$ and the time taken to reach $\mu_{\max}$ reached $15 \pm 1 \text{ d}$ (Table 1). From d 20 to 40, *B. aurantiacum* counts remained constant, close to $3.4 \times 10^8 \text{ cfu/g}$.

Biochemical Changes in Ripening Cheeses

pH dynamics under CRA conditions were similar to those observed in previous studies (Leclercq-Perlat et al., 2004) and showed 3 phases. Firstly, between d 0 and 3, pH values remained constant at 4.60 ± 0.06 . Secondly, between d 5 and 7, pH values increased strongly with a deacidification rate ($V_{\max}$) close to 1.3 pH unit/d, reaching 7.5 on d 7. During this phase, rind pH presented an important standard deviation due to

important differences between the 2 sides of each cheese. Thirdly, from d 7 to 40, rind pH did not significantly change, remaining close to 7.6 ± 0.2 . The pH of the core changed slowly during ripening (Table 2). From d 0 to 8, the pH remained constant at 4.65 ± 0.05 , and it increased slightly until the end of ripening, reaching 7.0 ± 0.2 on d 40.

Lactose and lactate concentrations (mmol/kg of DM) in the rind and in the core under CRA conditions were similar to those obtained by Leclercq-Perlat et al. (2004). On d 0, the lactose in the rind or the core was around 80 and 100 mmol/kg of DM, respectively. Lactose concentrations decreased quickly with a maximal consumption rate (C_{LO}) in the rind close to 18 mmol/kg of DM per d and with a lactose rate (D_{LO}) decreasing to 12 mmol/kg of DM per d in the core (Table 3). Lactose concentration was negligible on d 6 in the rind and on d 10 in the core.

Between d 0 and 4, lactate concentrations increased in the rind and in the core due to the postacidification phenomenon. On d 4, lactate concentration was maximal, and close to 450 and 500 mmol/kg of DM for the rind and core, respectively. On d 4 and until d 9 (rind) or d 16 (core), lactate concentration decreased rapidly with a decreasing rate close (C_{LT}) to 106 mmol/g of DM per d for the rind and (D_{LT}) close to 32 mmol/kg of DM per d for the core (Table 3). Lactate concentration became negligible on d 16 (wrapping) for the rind and on d 40 for the core.

The descriptors of changes in ASN and NPN indexes as well as NH_3 concentrations of the rind during all the ripening (d 0 to 40) are shown in Table 4. The ASN index remained constant at $18 \pm 4 \text{ g}$ of ASN/100 g of total N (%) during the first 3 d of ripening. Then, it increased from d 3 to d 6 at a maximal rate of 26%/d, reaching 100% on d 8. Afterwards, and until d 40, ASN remained constant at 100%.

The NPN index remained constant at almost 4 g of NPN/100 g of total N (%) during the first 4 d of ripening. Between d 4 and 7, it increased at a maximal rate of 16.0%/d, reaching about 44% on d 7. Afterwards, and until d 40, it increased slowly, reaching about 50% on d 40 (Figure 2A; Table 4).

Ammonia concentrations remained at about 1 g of NH_3 /kg of DM during the first 5 d of ripening. After d 5, and until d 16, NH_3 concentrations increased at a maximal rate of 1.13 g of NH_3 /kg of DM per d to reach about 13.5 g of NH_3 /kg of DM. After d 16 and until the end of ripening, it remained nearly constant at 14 g of NH_3 /kg of DM (Figure 2B, Table 4).

Changes in Cheese Appearance

The rind color of the cheeses provided information about the ripening and microorganism dynamics. From

Table 1. Kinetic descriptors of microorganism dynamics in relation to atmospheric conditions during chamber ripening of a Camembert-type cheese¹

Parameters ²	Atmospheric conditions ³				
	CRA	2CO ₂	PRA	6CO ₂	NRA
<i>Kluyveromyces lactis</i>					
Timing of phase 1	d 0 to 4	d 0 to 4	d 0 to 5	d 0 to 4	d 0 to 5
$\mu_{\max}$, d ⁻¹	0.46 ± 0.04	0.6 ± 0.1	0.6 ± 0.1	0.50 ± 0.07	0.47 ± 0.02
[CO ₂], %	0.0	2.0	0.2	6.0	4.3
R ₁	0.95	0.94	0.98	0.91	0.97
Timing of phase 2	d 5 to 19	d 6 to 19	d 7 to 16	d 6 to 13	d 16 to 40**
X _{max} , cfu/g of DM	4.0 × 10 ⁷	7.9 × 10 ⁷	6.3 × 10 ⁷	5.1 × 10 ⁷	1.0 × 10 ⁷
[CO ₂], %	0.0	2.0	3.7	6.0	21.7
Timing of phase 3	d 19 to 40	d 19 to 40	d 14 to 40	d 16 to 40	d 8 to 16
D _{max} , d ⁻¹	0.100 ± 0.007	0.072 ± 0.005	0.062 ± 0.009	0.099 ± 0.006	0.122 ± 0.007
R ₁	0.98	0.95	0.93	0.99	0.98
<i>Geotrichum candidum</i>					
Timing of phase 1	d 4 to 8	d 3 to 6	d 3 to 8	d 4 to 8	d 4 to 9
$\mu_{\max}$, d ⁻¹	0.7 ± 0.2	1.5 ± 0.3	1.3 ± 0.2	1.4 ± 0.3	1.5 ± 0.3
Time to reach $\mu_{\max}$, d	5 ± 1	4.2 ± 0.4	4.9 ± 0.8	5.5 ± 0.7	4.9 ± 0.7
[CO ₂], %	0.0	2.0	2.0	6.0	20.7
R ₂	0.97	0.99	0.99	0.98	0.98
Timing of phase 2	d 9 to 33	d 6 to 26	d 8 to 33	d 8 to 40	d 9 to 40
X _{max} , cfu/g of DM	1.0 × 10 ⁷	6.3 × 10 ⁷	1.3 × 10 ⁸	14.5 × 10 ⁸	3.2 × 10 ⁸
<i>Penicillium camemberti</i>					
Timing of phase 1	d 6 to 19	d 4 to 7	d 5 to 9	d 5 to 14	d 6 to 12
$\mu_{\max}$, d ⁻¹	0.23 ± 0.05	0.6 ± 0.1	0.48 ± 0.08	0.43 ± 0.09	0.37 ± 0.05
Time to reach $\mu_{\max}$, d	9 ± 3	5 ± 2	8 ± 2	8 ± 3	9 ± 2
[CO ₂], %	0.0	2.0	3.4	6.0	15.9
R ₂	0.97	0.92	0.96	0.90	0.98
Timing of phase 2	d 19 to 40	d 7 to 40	d 14 to 40	d 16 to 33	d 14 to 40
X _{max} , cfu/g of DM	7.5 × 10 ⁵	7.4 × 10 ⁵	7.7 × 10 ⁵	1.0 × 10 ⁶	2.5 × 10 ⁶
<i>Brevibacterium aurantiacum</i>					
Timing of phase 1	d 6 to 19	d 4 to 7	d 5 to 9	d 5 to 14	d 6 to 12
$\mu_{\max}$, d ⁻¹	0.23 ± 0.05	0.6 ± 0.1	0.48 ± 0.08	0.43 ± 0.09	0.37 ± 0.05
Time to reach $\mu_{\max}$, d	9 ± 3	5 ± 2	8 ± 2	8 ± 3	9 ± 2
[CO ₂], %	0.0	2.0	3.4	6.0	15.9
R ₂	0.97	0.92	0.96	0.90	0.98
Timing of phase 2	d 19 to 40	d 7 to 40	d 14 to 40	d 16 to 33	d 14 to 40
X _{max} , cfu/g of DM	7.5 × 10 ⁵	7.4 × 10 ⁵	7.7 × 10 ⁵	1.0 × 10 ⁶	2.5 × 10 ⁶

¹Values are the means of duplicate runs carried out under the same conditions; only a single run was carried out under NRA conditions.

²Parameters: $\mu_{\max}$ = maximum specific growth rate determined by modelling; [CO₂] = CO₂ concentration; R₁ = linear regression coefficient; X_{max} = mean cell concentration obtained under stationary growth phase; D_{max} = maximal death rate; R₂ = explained variance of Weibull modelling; phase 1 = exponential growth; phase 2 = stationary phase.

³Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

** = mean value obtained during the stationary phase.

d 0 to 1, color was uniformly white and bright. From d 1 to 5, cheeses became slightly cream colored and extremely dull due to growth of *K. lactis*. On d 4, growth of *P. camemberti* aerial mycelia was observed. These mycelia began to grow in spots on the aired face of cheeses. From d 10 to 16 (wrapping), rind color was white with short and uniform mycelium growth typical of *P. camemberti*. After d 10, the rind was close to 3 mm thick and the surface color remained white. However, on d 40, the outer circumference of each side of the cheeses was light cream colored, but no relationship with *B. aurantiacum* growth could be established.

The thickening of the underrind in relation to ripening time provided information about the ripening level because the underrind was linked to proteolysis and lipolysis dynamics (Table 5, Figure 3). Between d 0 and 5, the underrind thickness values remained negligible. Between d 5 and 14, thickness increased slightly, reaching about 2.0 mm on d 16. After d 16 and until the end of ripening (d 40), thickness greatly increased with a maximal rate ($V_{\max}$) equal to 0.63 ± 0.09 mm/d (Table 5), which occurred on d 27. On d 40, the underrind thickness was maximal and corresponded to about half the thickness of the cheese (15 mm).

Figure 1. Changes in A) *Geotrichum candidum* cell concentration and B) *Penicillium camemberti* spore concentration in the rind in relation to ripening time (from d 0 to 40). All progressions were made using Weibull modeling. D = surface drying period (relative humidity = 85%); W = wrapping on d 16. Except under NRA, each progression vs. time and its standard deviation (error bars) represent the mean of 2 runs carried out under conditions of CRA (●), 2CO₂ (△), PRA (■), 6CO₂ (▲), and NRA (○) in the ripening chamber (from d 0 to 16, 12°C, relative humidity = 95%). — = modeling results. Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

Influence of Gaseous Atmospheric Composition on Ripening Dynamics

Microbiological Changes in Ripening Cheeses.

Table 1 lists the 3 descriptors ($\mu_{\max}$, $X_{\max}$, $D_{\max}$) for growth of *K. lactis* as well as the 2 descriptors ($\mu_{\max}$, $X_{\max}$) for growth of *G. candidum*, *P. camemberti* spores, and *B. aurantiacum*, in relation to ripening chamber CO₂ concentration. Under PRA and NRA conditions, CO₂ concentration increased in relation to time, and it was calculated by determining the average for each phase of the time line described below.

Whatever the CO₂ concentration, *K. lactis* growth began on d 0 and finished between d 4 (CRA, NRA, and 6CO₂) and d 6 (2CO₂, PRA, Table 1). The mean specific growth rate ($\mu_{\max}$) was statistically $0.5 \pm 0.1 \text{ d}^{-1}$. The concentration of CO₂ in the chamber atmosphere did not have any statistically significant effects on $\mu_{\max}$ and $X_{\max}$. Except under NRA conditions, the death phase began around the end of chamber ripening (d 14 to 19) and it finished on d 40. It was characterized by mean specific death rates between 0.062 and 0.10 d^{-1} . Under NRA, the *K. lactis* death period was completely shifted in relation to ripening time. It took place between d 8 and 16, when CO₂ completely replaced O₂, and out of all the atmospheric conditions, the death rate of *K. lactis* was the highest ($0.122 \pm 0.007 \text{ per d}$) in this case (NRA). The absence of O₂ probably explained this highest rate and earliest mortality. The overall dynamics of *K. lactis* depended on CO₂ concentration, and in particular, the timing of the death phase.

The ANOVA (data not shown) showed no effect of CO₂ concentration on dynamics of *K. lactis*.

Regardless of CO₂ concentration, growth of *G. candidum* vs. time exhibited the same overall changes (Figure 1A). Concentration of CO₂ present in the ripening chamber showed very significant effects on *G. candidum* growth descriptors ($\mu_{\max}$, $X_{\max}$; Table 1). Maximal growth rates of *G. candidum* were about twice as high as those obtained under CRA conditions (0% of CO₂), but the associated time (time for $\mu_{\max}$) was about the same value due to standard deviation. The lowest $X_{\max}$ value was obtained under CRA. Under 6CO₂ conditions the maximal concentration of *G. candidum* rose to $145 \times 10^7 \text{ cfu/g}$. However, when CO₂ concentration was higher than 6% under NRA, *G. candidum* $X_{\max}$ ($32 \times 10^7 \text{ cfu/g}$) was reduced.

Analysis of variance (Table 6) confirmed that these dynamics were the lowest under CRA. The presence of CO₂ during chamber ripening promoted *G. candidum* growth, and after wrapping (d 16) the effect of CO₂ on cell concentrations ($X_{\max}$) was obvious: $X_{\max}$ presented a maximum in relation to CO₂ concentration (Figure 1A).

Table 2. Values of kinetic descriptors (obtained from Weibull modeling) of rind pH dynamics in relation to atmospheric conditions during chamber ripening (from d 0 to 16) of a Camembert-type cheese¹

Parameters ²	Atmospheric conditions ³				
	CRA	2CO ₂	PRA	6CO ₂	NRA
Timing of phase 1	d 5 to 7	d 5 to 7	d 5 to 7	d 5 to 7	d 5 to 7
V _{max} , pH unit/d	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.2	1.26 ± 0.06	1.18 ± 0.09
[CO ₂], %	0.0	2.0	3.3	6.0	5.4
Timing of phase 2	d 7 to 40	d 7 to 40	d 9 to 40	d 8 to 40	d 9 to 40
pH _{max}	7.6 ± 0.2	7.9 ± 0.1	7.8 ± 0.1	7.8 ± 0.3	7.6 ± 0.1
[CO ₂], %	0.0	2.0	3.7	6.0	19.8
R	0.983	0.982	0.984	0.980	0.983

¹Values are the means of duplicate runs carried out under the same conditions; only a single run was carried out under NRA conditions.

²Parameters: V_{max} = maximal rate; [CO₂] = CO₂ concentration; pH_{max} = maximal pH value reached; phase 1 = increasing phase; phase 2 = constant phase.

³Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

Changes in CO₂ concentration induced changes in maximal sporulation rate ($\mu_{\max}$; Figure 1B, Table 1). Indeed, out of all the atmospheric conditions, the highest maximal sporulation rate value was under 2CO₂. The highest CO₂ concentration (NRA) had a significant effect on the maximal X_{max} value, which reached about 2.5×10^6 spores/g (around 3 times higher than for the other conditions).

Analysis of variance (Table 6) confirmed that for *P. camemberti* sporulation, 1) CRA and 6CO₂ showed the same progressions; 2) 2CO₂ runs had the highest progressions between d 3 and 16; and 3) PRA (CO₂ between 2 and 8%) was intermediate between 2CO₂, 6CO₂, and NRA.

Regardless of CO₂ concentration, *B. aurantiacum* counts presented several progressions vs. time (data

Table 3. Values of kinetic descriptors of carbon substrate in relation to atmospheric conditions during chamber ripening (from d 0 to 16) of a Camembert-type cheese¹

Parameters ²	Atmospheric conditions ³				
	CRA	2CO ₂	PRA	6CO ₂	NRA
Timing of phase 1	d 0 to 6	d 0 to 6	d 1 to 7	d 0 to 7	d 3 to 8
C _{LO} , mmol/kg of DM per d	18 ± 2	16 ± 1	18 ± 1	14 ± 2	22 ± 3
[CO ₂], %	0.0	2.0	1.5	6.0	4.6
Timing of phase 2	d 0 to 9	d 1 to 9	d 0 to 8	d 0 to 9	d 0 to 14
D _{LO} , mmol/kg of DM per d	12 ± 2	11 ± 2	15 ± 2	12 ± 3	10 ± 1
[CO ₂], %	0.0	2.0	1.2	6.0	10.5
Timing of phase 3	d 4 to 9	d 5 to 9	d 5 to 8	d 5 to 9	d 5 to 9
C _{LT} , mmol/kg of DM per d	106 ± 7	81 ± 6	104 ± 11	74 ± 7	61 ± 7
[CO ₂], %	0.0	2.0	3.3	6.0	9.2
Timing of phase 4	d 4 to 16	d 3 to 12	d 5 to 14	d 5 to 16	d 5 to 14
D _{LT} , mmol/kg of DM per d	32 ± 2	42 ± 2	38 ± 6	27 ± 2	20 ± 3
[CO ₂], %	0.0	2.0	3.8	6.0	16.1

¹Values are the means of duplicate runs carried out under the same conditions; only a single run was carried out under NRA conditions.

²Parameters: C_{LO} = lactose maximal consumption rate in rind; D_{LO} = lactose maximal decreasing rate in the core; C_{LT} = lactate consumption rate in the rind; D_{LT} = lactate decreasing rate in the core; [CO₂] = CO₂ concentration; phase 1 = linear decreasing phase.

³Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

Table 4. Values of kinetic descriptors of nitrogen concentration dynamics in relation to atmospheric conditions during chamber ripening (from d 0 to 16) of a Camembert-type cheese¹

Parameter	Atmospheric conditions ²				
	CRA	2CO ₂	PRA	6CO ₂	NRA
Acid-soluble N (ASN)					
Initial ASN	18 ± 4	11 ± 4	15 ± 2	14 ± 3	16 ± 3
Timing	d 3 to 6	d 4 to 6	d 4 to 6	d 4 to 6	d 4 to 7
V _{max} (± SD), g/100 g of total N per d	26 ± 2	43 ± 3	38 ± 3	33 ± 3	27 ± 3
[CO ₂], %	0.0	2.0	3.3	6.0	7.1
d for ASN = 100%	7	6	8	7	8
R	0.98	0.96	0.98	0.97	0.95
NPN					
Initial NPN	6 ± 3	6 ± 2	4 ± 1	6 ± 2	4 ± 1
Timing	d 4 to 7	d 4 to 8	d 4 to 13	d 4 to 15	d 4 to 15
V _{max} (± SD), g/100 g of total N per d	16 ± 2	11 ± 1	3.1 ± 0.4	2.6 ± 0.3	2.3 ± 0.3
[CO ₂], %	0.0	2.0	3.4	6.0	6.1
[NPN] _{max}	50 ± 6	46 ± 4	37 ± 3	43 ± 4	49 ± 1
d for [NPN] _{max}	6	7	13	19	18
R	0.96	0.97	0.82	0.82	0.91
Free ammonium (NH ₄ ⁺)					
Initial NH ₄ ⁺	1.4 ± 0.6	1.9 ± 0.2	2.3 ± 0.3	2.3 ± 0.5	2.3 ± 0.2
Timing	d 5 to 16	d 5 to 16	d 5 to 16	d 8 to 16	d 12 to 24
V _{max} (± SD), g of NH ₃ /kg of DM per d	1.13 ± 0.08	0.54 ± 0.04	0.91 ± 0.04	0.54 ± 0.04	0.65 ± 0.05
Δ[NH ₄ ⁺] _{max} , g of NH ₃ /kg of DM	12.0	6.3	10.4	9.6	8.2
R	0.92	0.99	0.97	0.90	0.97

¹Values are the means of duplicate runs carried out under the same conditions; only a single run was carried out under NRA conditions.

²Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

not shown). Under all conditions except NRA, bacterial concentration showed a lag phase from d 0 to 9. After this lag phase, and until d 16 (6CO₂), d 20 (CRA, PRA) or d 33 (2CO₂), *B. aurantiacum* grew with a maximal growth rate ($\mu_{\max}$) depending on the CO₂ concentration during chamber ripening (Table 1). In the absence of CO₂ (CRA), $\mu_{\max}$ was the highest (0.46 ± 0.07 d⁻¹)—35% higher than that obtained when the CO₂ concentration was under 2 or 6%. After this growth phase, *B. aurantiacum* concentration remained constant, but its value depended on the CO₂ level. When CO₂ levels were less than 6%, $X_{\max}$ was between 3×10^8 and 10×10^8 cfu/g. When CO₂ levels were 6.0% (6CO₂), $X_{\max}$ was divided by about 300 in relation to 2CO₂ and CRA. Under NRA (the highest CO₂ level), *B. aurantiacum* did not grow, and its concentration remained constant, about 4×10^4 cfu/g during chamber ripening. After d 16 and until 40, no bacteria were found in the rind. The ANOVA confirmed that the highest CO₂ concentrations present in the ripening chamber significantly inhibited *B. aurantiacum* growth (data not shown).

Biochemical Changes in Ripening Cheeses in Relation to Atmospheric Conditions

Table 2 shows the descriptors of cheese rind pH (V_{max} and pH_{max}) in relation to CO₂ concentration. Regardless

of the CO₂ concentration used during chamber ripening, no significant differences of pH descriptors were observed. The ANOVA confirmed that no significant differences existed between these dynamics (data not shown).

The atmospheric conditions of ripening (CO₂ level) did not have a significant effect on lactose consumption rate in the rind (C_{LO}, mean value 18 ± 3 mmol/kg of DM per d) and lactose disappearance in the core (D_{LO}, mean value = 12 ± 2 mmol/kg of DM per d) at 95% confidence level (Table 3). The ANOVA confirmed that there were no significant differences (data not shown).

Initial presence (d 0) of CO₂ seemed to have slightly negative effect on lactate consumption by the microorganisms present on the surface (C_{LT}), but it did not have any effect on lactate disappearance rate (D_{LT}), consecutive to lactate consumption in the core by *G. candidum* and *K. lactis* (Leclercq-Perlat et al., 2004), as well as to its diffusion from the core to the rind. Indeed, C_{LT} of CRA and PRA was higher than for the other conditions (Table 3). Under NRA (results not shown) lactate consumption in the rind and in the core was stopped on d 10 and 23, reaching about 97 and 10 mmol/kg of DM, respectively. In the chamber, between d 10 and 16, CO₂ concentration was the highest: equal to 19.2% on d 10 and remaining constant at 20% from

Figure 2. Changes in A) NPN indices and B) free NH_4^+ concentration in the rind in relation to ripening time (from d 0 to 40). Atmospheric conditions: CRA (●) = continuously renewed atmosphere under which CO_2 and O_2 levels remained at 0 and 21%, respectively; PRA (■) = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO_2 concentration was decreased to 2% by air injection (O_2 decreased to 17 to 18%); NRA (○) = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO_2 up to 20%, disappearance of O_2); 2CO₂ (△) = CO_2 concentration maintained at 2% from d 0 to 16, with O_2 close to 17 to 18%; and 6CO₂ (▲) = CO_2 concentration kept constant at 6% from d 0 to 16, with O_2 ~12 to 13%.

d 11 to 16. At these CO_2 levels, all microorganism activities slowed down. Lactate diffusion from the core to the rind continued until d 23 and stopped when the 2 lactate concentrations were equal. This could explain why these lactate rates (C_{LT} and D_{LT}) obtained under NRA were lower than under the other atmospheric conditions. The ANOVA confirmed that the lactate dynamics in the rind were similar under 6CO₂, CRA, 2CO₂, and PRA. The dynamics in the rind and in the core were the lowest under NRA conditions (data not shown).

Regardless of the CO_2 concentration used during chamber ripening, the kinetic increases occurred during the same ripening time (from d 3 or 4 to d 6 or 7). No significant difference of initial ASN concentration was observed (Table 4). The maximal rate (V_{max}) was the highest for 2CO₂ and PRA conditions (Table 4). The maximal rate was lowest when CO_2 level was negligible (CRA) or the highest when CO_2 level was maximal (NRA). Moreover, when V_{max} was the highest, the time for which ASN level was 100% was the lowest (2CO₂); indeed, it seemed that this proteolytic index became quickly maximal. However, ANOVA showed that there were no significant differences (data not shown).

When CO_2 concentration was lower or equal to 2% (CRA, 2CO₂), the NPN rates (V_{max}) (Figure 2A, Table 4) were between 4 and 5 times higher than those obtained for the other runs (CO_2 level $\geq 3\%$), and the time for which NPN level was maximal was the lowest (d 6 to 7). No significant differences of the maximal NPN were found regardless of CO_2 concentration in the chamber. Under 6CO₂ and NRA (the highest CO_2 level) conditions, the maximal concentration of NPN was obtained after wrapping, on d 19 and 18, respectively.

Analysis of variance (Table 7) confirmed that under PRA, the NPN dynamic was the lowest. The NPN dynamics of CRA (starting on d 9) were the same as for 2CO₂ (starting on d 7) but with a delay of 2 d. Under 6CO₂ and NRA conditions, there was a weak acceleration of NPN production, from d 19 and d 16 until d 40, respectively.

For NH_3 evolutions, 3 observations can be made (Figure 2B; Table 4). Firstly, no production of NH_3 was observed before d 7. Secondly, except under NRA, NH_3 production occurred during chamber ripening and finished with wrapping (d 16). Under NRA, NH_3 increase occurred from d 12 until 8 d after wrapping. Thirdly, an initial presence of CO_2 or an important concentration of CO_2 induced lower production of NH_3 .

Whatever the atmospheric conditions, the ANOVA study detected no significant effect from d 0 to 6 (Table 7B). Under NRA, regardless of day, the NH_3 concentration was the lowest. From d 6 until 40, CRA and PRA runs presented the fastest dynamics and the highest values of NH_3 concentrations. Under 2CO₂ or 6CO₂, ammonium concentration dynamics formed an intermediate group.

Influence of Atmospheric Composition on Cheese Appearance

Figure 3 shows the progression of underriind thickness in relation to ripening time (from d 0 to 40) and to atmospheric conditions used during chamber ripening. Table 5 presents the duration of lag period ($T_{\text{UR}} = 0 \text{ mm}$)

Table 5. Values of kinetic descriptors of underrind thickness (T_{UR} , mm) in relation to atmospheric conditions during chamber ripening (from d 0 to 16) of a Camembert-type cheese¹

Parameters ²	Atmospheric conditions ³				
	CRA	2CO ₂	PRA	6CO ₂	NRA
d for $T_{UR} = 0$	d 0 to 5	d 0 to 7	d 0 to 6	d 0 to 8	d 0 to 8
Timing	d 14 to 40	d 6 to 26	d 14 to 40	d 6 to 26	d 14 to 40
V_{max}	0.63 ± 0.09	0.63 ± 0.09	0.60 ± 0.08	0.7 ± 0.1	0.65 ± 0.08
Time to reach V_{max} , d	27 ± 1	14 ± 1	30 ± 2	16 ± 2	32 ± 2
Type of ripening	Slow-fast	Fast-slow	Slow-fast	Fast-slow	Slow-fast
R	0.992	0.997	0.996	0.995	0.998

¹Values are the means of 2 runs carried out under the same conditions; only a single run was carried out under NRA conditions.

²Parameters: V_{max} = maximal rate of increase.

³Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

and fast increase phase as well as its kinetic descriptors (V_{max} , time for V_{max}) and the type of ripening.

Two different ripening progressions were observed (Figure 3). In the presence of CO₂ on d 0 (2CO₂ and 6CO₂), the underrind thickness increased greatly be-

Figure 3. Changes in creamy underrind thickness (mm) throughout the entire ripening process (from d 0 to 40). All the underrind thickness progressions were made using Weibull modeling. D = surface drying period; W = wrapping on d 16. Atmospheric conditions: CRA (●) = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA (■) = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA (○) = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ (△) = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ (▲) = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

tween d 6 and 26. After this phase, the underrind thickness remained constant, reaching about half the thickness of the cheeses on d 34. The ripening of these 2 tests was termed fast-slow. For the runs free of CO₂ on d 0 (CRA, PRA, and NRA), the underrind thickness increased at a low rate from d 6 to 16. After that, and until d 40, it increased quickly, reaching half the thickness of the cheeses on d 40; this ripening was termed slow-fast. The CO₂ concentration of atmospheric composition in the chamber did not have a significant effect on V_{max} (Table 5) but it did have a significant effect on time associated with V_{max} , as can be seen by the shape of the curves (Figure 3).

When CO₂ concentration was higher than 6% during the ripening period (PRA, 6CO₂, NRA) the underrind was too runny. The higher the CO₂ concentration and the longer the time in ripening, the poorer the underrind quality.

Regardless of CO₂ concentration, *P. camemberti* mycelium began to grow on the same day (d 5). However, when growth started, appearance of cheese surface, color, and density of mycelium as well as microorganism growth depended on CO₂ level. When CO₂ concentration was 0 or 2%, the appearance of the cheese surface corresponded to the 3 targeted visual criteria (white or light cream color, density of mycelium, and the uniformity of these 2 criteria). Increases in CO₂ concentration above 2% resulted in worse cheese surface appearance.

On d 12, under NRA conditions, the cheese color was gray with some brown spots not significantly distributed; the cheese edges were also brown. The aerial mycelia of *P. camemberti* were no longer observed in the middle of the surface. Concerning color and appearance, the cheese surface was similar to *G. candidum* biofilms

Table 6. Analysis of variance¹ of *Geotrichum candidum* and *Penicillium camemberti* concentrations throughout the entire ripening process (from d 0 to 40) in cheeses ripened using different CO₂ concentrations during the chamber ripening phase (from d 0 to 16)²

Ripening time (d)	Atmospheric conditions ³				
	CRA	2CO ₂	PRA	6CO ₂	NRA
<i>G. candidum</i>					
0	*	*	*	*	*
1	*	*	*	*	*
2	*	***	**	*	*
3	*	****	***	**	***
5	*	***	**	**	**
6	*	***	***	**	***
7	*	**	**	**	**
8	*	**	**	***	***
9	*	**	**	***	***
12	*	**	**	***	***
14	*	**	**	***	**
16	*	**	**	****	***
19	*	**	**	***	***
26	*	*	**	***	**
33	*	*	**	***	**
40	*	*	*	***	**
<i>P. camemberti</i>					
0	*	*	*	*	*
1	*	*	*	*	*
2	*	*	*	*	*
3	*	**	*	*	*
5	*	**	*	*	*
6	*	**	*	*	*
7	**	****	***	**	*
8	**	***	**	*	*
9	*	***	**	*	*
12	*	**	**	*	**
14	*	**	*	*	**
16	*	*	*	*	*
19	*	*	*	*	*
26	*	*	*	*	*
33	*	*	*	*	*
40	*	*	*	*	*

¹Newman-Keuls test with *P*-value of 0.01.²Data represent log₁₀ of the concentrations of viable *G. candidum* and *P. camemberti*. On each day, data with the same score are not significantly different. * = statistical group with the lowest values; ** and *** = statistical groups with intermediate values; **** = statistical group with the highest values.³Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.

found on cheese ripened by this yeast. Moreover, the cheese completely lost its shape: the rind collapsed all around the core such that the cheese acquired a bowler-hat shape; wrapping did not change the shape. Under 6CO₂ and PRA conditions, the cheese color was white and gray and the central core collapsed completely. Moreover, the CO₂ level present in the chamber de-

Table 7. Analysis of variance¹ of NPN index and change in free ammonium (NH₃) concentration throughout the entire ripening process (from d 0 to 40) in cheeses ripened using different CO₂ concentrations during the chamber ripening phase (from d 0 to 16)²

Ripening time (d)	Atmospheric conditions ³				
	CRA	2CO ₂	PRA	6CO ₂	NRA
NPN index					
0	*	*	*	*	*
1	*	*	*	*	*
2	*	*	*	*	*
3	*	*	*	*	*
5	*	*	*	*	*
6	*	*	*	*	*
7	*	**	*	*	*
8	*	**	*	*	*
9	**	**	*	*	*
12	**	**	*	*	*
14	**	**	*	*	*
16	**	**	*	*	**
19	***	***	*	**	***
26	**	**	*	**	**
33	**	**	*	**	**
40	**	**	*	*	**
Δ[NH ₃] ⁴					
0	*	*	*	*	*
1	*	*	*	*	*
2	*	*	*	*	*
3	*	*	*	*	*
5	*	*	*	*	*
6	*	*	*	*	*
7	***	**	**	*	*
8	****	**	***	**	*
9	*****	**	****	***	*
12	****	**	***	**	*
14	****	**	***	**	*
16	****	**	***	**	*
19	***	*	***	**	*
26	***	*	**	**	*
33	**	*	**	**	*
40	**	*	**	**	*

¹Newman-Keuls test with *P*-value of 0.01.²Data represent log₁₀ of the concentrations of viable *G. candidum* and *P. camemberti*. On each day, data with the same score are not significantly different. * = statistical group with the lowest values; **, ***, and **** = statistical groups with intermediate values; ***** = statistical group with the highest values.³Atmospheric conditions: CRA = continuously renewed atmosphere under which CO₂ and O₂ levels remained at 0 and 21%, respectively; PRA = periodically renewed atmosphere under which the ripening chamber was sealed but each day, if necessary, the CO₂ concentration was decreased to 2% by air injection (O₂ decreased to 17 to 18%); NRA = no renewed atmosphere under which the chamber was sealed during chamber ripening (accumulation of CO₂ up to 20%, disappearance of O₂); 2CO₂ = CO₂ concentration maintained at 2% from d 0 to 16, with O₂ close to 17 to 18%; and 6CO₂ = CO₂ concentration kept constant at 6% from d 0 to 16, with O₂ ~12 to 13%.⁴On each ripening day, Δ[NH₃] was equal to [NH₃] obtained on that d minus its initial (d 0) value.

graded the shape. The rind center was more or less collapsed, and the experts characterized this underrind as runny.

DISCUSSION

It is thought that the role of *K. lactis* and *B. aurantiacum* in CO₂ production is negligible. In our case, what-

ever the atmospheric conditions, CO₂ production was insignificant during *K. lactis* growth. It was likewise for *B. aurantiacum*, and no significant CO₂ production has been reported in the literature. For the runs without CO₂ control (NRA, PRA), CO₂ production was concomitant to *P. camemberti* mycelium growth (d 5 to 6). Adour et al. (2002) have shown that *P. camemberti* and *G. candidum* produced higher amounts of CO₂. In our case, whatever the CO₂ levels in the presence of an important O₂ concentration (>12 to 13%), *K. lactis* grew in a similar manner and its specific growth rate ($\mu_{\max}$) as well as its maximal concentration ($X_{\max}$) did not significantly differ regardless of atmospheric conditions. This could be due to CO₂ or O₂ levels in the chamber during the growth phase. Except under NRA, *K. lactis* death phase occurred after wrapping. This could be explained by an osmotic shock due to the reoxygenation of cheese during the wrapping as shown by Skjerdal et al. (1995) for some ripening bacteria. Under NRA, the death period of *K. lactis* took place during chamber ripening (d 10 to 16) at the highest rate (<10%). This could be explained by both an important CO₂ quantity in the chamber after d 8 ($\geq 20\%$) and low levels of O₂ ($\leq 1\%$) in the chamber, which induced lysis of *K. lactis* cells. In fact, *K. lactis* growth was not sensitive to CO₂ and O₂ concentrations. However, its death (period and rate) was related to atmospheric conditions (CO₂ and O₂).

In absence of CO₂, *G. candidum* dynamics were the lowest and, in presence of 6% of CO₂, they appeared maximal (Table 6). These results were in accordance with those of Wells and Spalding (1975). Indeed, those authors have shown that *G. candidum* is able to grow under microaerophilic conditions and under elevated CO₂ levels, and that it is stimulated by low O₂ and high CO₂ atmospheres. Moreover, Couriol et al. (2001) have shown that CO₂ production was associated with *G. candidum* growth whereas Adour et al. (2002) have considered that *G. candidum* produced lower CO₂ levels than *P. camemberti* mycelia in liquid media and pure cultivation.

Sporulation of *P. camemberti* was also increased by significant CO₂ concentration in the chamber (>6%). The specific rate ($\mu_{\max}$) was maximal for a CO₂ level close to 2% (2CO₂), whereas the maximal spore concentration ($X_{\max}$) was slightly higher under 6CO₂ or NRA (Figure 1B). Using ANOVA, *P. camemberti* spore dynamics appeared maximal under 2CO₂ and PRA (Table 6). These results are in accordance with those of Roger et al. (1998). Roger et al. (1998) have shown that a CO₂ concentration of about 4% allowed for the best development of *P. camemberti* mycelia on Brie cheeses, but only if the O₂ level was >16%. Similarly, Magan and Lacey (1984) have shown that CO₂ concentrations

close to 5% with low O₂ concentrations stimulate growth of some *Penicillium* species on wheat extract medium if water activity and temperature are correctly chosen. Adour et al. (2002) have also shown that under their study conditions (liquid media, pure culture), CO₂ promoted *G. candidum* growth (Table 1) but disadvantaged *P. camemberti* mycelium development. The poor development of *P. camemberti* visually observed for CO₂ higher than 3% could also be explained as proposed by van den Tempel and Nielsen (2000). These authors have shown that 1) *G. candidum* was adapted to growth at low levels of oxygen and high levels of CO₂; and 2) growth and sporulation of *Penicillium roqueforti* was negatively affected in the presence of *G. candidum* at high CO₂ levels, irrespective of O₂ levels. Although van den Tempel and Nielsen (2000) used *P. roqueforti*, the effects of CO₂ and O₂ could be similar or more important due to the higher sensitivity of *P. camemberti* to O₂ (Doyon et al., 1997; Roger et al., 1998). The development of *P. camemberti* (mycelia as well as sporulation) in the presence of *G. candidum* was related to the CO₂ level in chamber ripening. In fact, CO₂ changed the equilibrium existing between these 2 strains. Indeed, the higher the CO₂ concentration, the more significant the *G. candidum* concentration was in the stationary phase ($X_{\max}$) and the worse *P. camemberti* mycelium development (visual appearance) was.

In the absence of CO₂ (CRA), exponential *B. aurantiacum* growth occurred on d 9 and continued after wrapping. The pH of the rind was favorable for *B. aurantiacum* but, surprisingly, its growth occurred 3 d after the rind pH reached its maximum (d 6), whereas in our previous study (Leclercq-Perlat et al., 2004), we have shown that growth of this species started as soon as pH of the rind reached neutral pH. Its maximal growth rate was obtained in absence of CO₂ (Table 1). However, maximal concentration ($X_{\max}$) was the same if the O₂ level in the chamber was higher than 16% (CRA, 2CO₂, PRA). In the other runs (6CO₂, NRA), its growth ($X_{\max}$) was negligible. Lower levels of O₂ (<12 to 13%) with high CO₂ concentration had significant inhibitor effects on *B. aurantiacum* evolution; *B. aurantiacum* is considered a strict aerobic strain, and no growth occurs in absence of O₂ (Ratnay and Fox, 1999).

No significant effect on carbon substrate dynamics of CO₂ levels during chamber ripening was observed on the cheese rind or in the cheese core, except under NRA. Indeed, O₂ concentration was high (≥ 11 to 18%) and did not limit glycolysis. However, under NRA, the lactate consumption was stopped on d 11 when CO₂ was 20% and O₂ was negligible. The absence of O₂ explains the limitation of these oxidative metabolisms as observed here.

The CO₂ level present in the chamber seemed to have no significant effects on ASN dynamics, but a slight effect of CO₂ on ASN maximal rates was observed. However, it had a significant effect on NPN maximal rates (Table 7). Indeed, the V_{max} values of NPN kinetics were the highest when CO₂ concentration was the lowest (0 to 2% for CRA and 2CO₂), and the time NPN concentration took to become maximal was the shortest (6 to 7 d). However, the final NPN concentration (measured to NPN_{max}) was not significantly different. According to Sousa and et al. (2001), *P. camemberti* is the main agent of amino acid release in Camembert cheeses. This suggests that high CO₂ (>2%) could have an inhibitor action on peptidasic activities during ripening.

Under CRA, the quantities of ammonium obtained in the rind were much higher than those found by Leclercq-Perlat et al. (2004). However, they were in accordance with those obtained by von Mrowetz (1979), who studied ammonium contents during Camembert cheese ripening without reporting cheese-making and ripening conditions. The following sequence of events could explain these significant differences. When a saturated salt (KNO₃) system was used to regulate RH, a part of KNO₃ was vaporized into the atmosphere, and by consequence, was deposited in the rind. This salt absorption induced a significant decrease of water activity in the rind (Gobbetti et al., 1999). These authors have also shown that a decrease of water activity involved a negative effect on the peptidase activity of some lactic acid bacterium strains. This negative effect on proteolysis could be verified for the other strains. Under CRA and PRA, ammonium production was higher and their dynamics were the fastest compared with those of the other atmospheric conditions. Similar to NPN dynamics, this could be due to an inhibition of aminopeptidase and deaminase enzymes by CO₂. Indeed, according to Hemme et al. (1982), deaminases (aminoxidases or oxidoreductases) convert amino acids (or their amines) into NH₃ and cetic acids or aldehydes, which requires O₂. Many ripening microorganisms, in particular, surface bacteria, are considered to be the most active in proteolysis. Rattray and Fox (1999) have reviewed the proteolytic system of *Brevibacterium linens* and shown the presence of several aminopeptidases. In our study, when CO₂ contents higher than 2% were associated with lower O₂ concentration, *B. aurantiacum* growth was decreased; therefore, inducing lower oxido-oxidase activities. The same explanation could be made for *P. camemberti* mycelium growth.

The thickness of the creamy underrind provides information about overall proteolysis and lipolysis of the cheeses. The presence or absence of CO₂ on d 0 induced different ripening patterns. Under 2CO₂ and 6CO₂ (CO₂

level was not 0% on d 0), the overall ripening measured by the underrind thickness was termed fast-slow, whereas under other conditions (CO₂ = 0% on d 0), it was termed slow-fast. However, for a CO₂ level above 4%, the cheese underrind was extremely runny on d 40. *G. candidum* and *P. camemberti* are well known for their significant lipolytic and proteic systems (Choisy et al., 1997; Boutroun and Guéguen, 2005). The effects of CO₂ on their growth (notably 6% on *G. candidum* and 2% on *P. camemberti* mycelia) and on their equilibrium might explain the underrind dynamics. *B. aurantiacum* is also well known for its important exo- and endocellular proteolytic activities (Lecocq and Guéguen, 1994; Rattray and Fox, 1999), and *K. lactis* has few endocellular proteolytic activities (Klein et al., 2002). These 2 strains have no significant lipolytic activities in comparison to *G. candidum* and *P. camemberti*. However, the important death rate of *K. lactis* in presence of the highest CO₂ level could also favor the cheese underrind liquefaction by release of endocellular enzymes in the cheese (Klein et al., 2002). The role *B. aurantiacum* played was difficult to determine because it grows in the presence of significant CO₂ levels when the O₂ level was above 12% in relation to CO₂ and O₂ levels.

The CO₂ atmospheric conditions had an effect on overall cheese ripening. Indeed, high concentrations of CO₂ (>2%) degraded cheese color and *P. camemberti* mycelium growth as well as cheese shape and underrind quality (texture).

CONCLUSIONS

Whatever the ripening atmospheric conditions in the chamber, the presence of CO₂ resulted in numerous changes in the microbiological and biochemical aspects of the cheeses. It seemed to set in motion some enzymatic dynamics related to *G. candidum* and *P. camemberti* cells or mycelia that appeared linked to CO₂ levels. The higher the CO₂ concentration, the higher the *G. candidum* cell concentrations and the poorer the appearance of *P. camemberti* mycelium and cheese appearance (underrind thickness and color). Moreover, the ripening was accelerated in the presence of CO₂ on d 0. The best ripening conditions to obtain an optimum between microorganism growth and biochemical dynamics as well as cheese appearance was around 2% of CO₂ in the presence of an O₂ concentration of 17 to 18%. The above results may be helpful if chamber ripening is to be shortened while maintaining the quality of mold soft cheeses.

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