Temperature and relative humidity influence the microbial and physicochemical characteristics of Camembert-type cheese ripening

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ABSTRACT: To evaluate the effects of temperature and relative humidity (RH) on microbial and biochemical ripening kinetics, Camembert cheeses were prepared from pasteurized milk seeded with *Kluyveromyces marxianus*, *Geotrichum candidum*, *Penicillium camemberti*, and *Brevibacterium aurantiacum*. Microorganism growths and biochemical changes were studied under different ripening temperatures (8, 12, and 16 °C) and RH (88, 92, and 98 %).

Temperature had significant effects on the growth of both *K. marxianus* and *G. candidum* while RH did not affect it. Whatever the temperature, under 98 % RH, the specific growth rate of *P. camemberti* spores was significantly higher (between 2 (8 °C) and 106 times (16°C) higher). However at 16 °C, the appearance of the rind was no longer suitable because mycelia were damaged. *B. aurantiacum* growth depended on both temperature and RH. At 8°C under 88 % of RH, its growth was restricted ($1.3x10^7$ CFU/g) while at 16°C under 98 % RH, it was favored, reaching $7.9x10^9$ CFU/g, but the rind had a dark brown color after d20.

Temperature had a significant effect on carbon substrate consumption rates in the core as well as in the rind. In the rind, when temperature was 16 °C rather than 8 °C, lactate consumption rate was 0.7 times higher under 88 % RH. Whatever the RH, temperature had significant effects on the increase in rind pH (from 4.6 to 7.7 ± 0.2). At 8°C the increase in rind pH was observed between d6 and d9 while at 16°C between d2 and d3. Temperature and RH had an effect on the increase in underrind thickness: at 16°C, half of the cheese thickness appeared ripened on d14 (wrapping day). However, under 98% RH, the underrind was runny.

The best ripening conditions to achieve an optimum between microorganism growth and biochemical kinetics were 13°C and 94 % RH.

Keywords: cheese ripening, temperature, relative humidity, microbial growth, biochemical evolutions.

Interpretative summary. To evaluate the effects of temperature and relative humidity (RH) on the ripening kinetics of Camembert, microbial growth and biochemical changes were studied under different temperatures and relative humidities.

Some factors only depended on temperature, such as yeast growth and the pH in the cheese rind. Often, the factors depended on both temperature and RH, as *P. camemberti* sporulation, *B. aurantiacum* growth, carbon substrate consumption rates, and the increase in the cheese underrind thickness. The best ripening conditions to achieve an optimum between microorganism growths and biochemical kinetics were 13°C and 94 % RH.

Abbreviation keys: θ, ripening temperature (°C). RH, relative humidity (%). t, ripening time (d). μ_x, maximal growth rate of *X* (d^{-1}). $t_{\mu x}$, time for which μ_x was obtained (d). D_{KM}, maximal death rate of *K. marxianus* (d^{-1}). t_{DKM}, time for which D_{KM} was obtained (d). C_{LO}, lactose maximal consumption rate of the rind ($g.kg^{-1}$ per.d). $D_{LO,}$ lactose maximal decreasing rate of the core ($g.kg^{-1}.d^{-1}$). C_{LA}, lactate maximal consumption rate of the rind ($g.kg^{-1}.d^{-1}$). $D_{LA,}$ lactate maximal decreasing rate of the core ($g.kg^{-1}.d^{-1}$). t_{CLO}, t_{DLO}, t_{CLA}, and t_{DLA}, time for which C_{LO}, D_{LO}, C_{LA}, and D_{LA} were obtained (d). V_{pH}, maximal rate of deacidification (pH unit.d^{-1}). t_{VpH}, time for which V_{pH} was obtained (d). T_{UR}, underrind thickness (mm). V_{TUR}, maximal increasing rate of underrind thickness ($mm.d^{-1}$). t_{TUR}, time for which V_{TUR} was obtained (d). L_{H2O}, relative weight loss (%).

Indexes: X = KM for *Kluyveromyces marxianus*, GC for *Geotrichum candidum*, BA for, *Brevibacterium aurantiacum*, and PC for *Penicillium camemberti*; 14 for d14 (wrapping day) and 40 for d40 (shelf live).

Many environmental parameters, mainly temperature, relative humidity (RH), and gaseous atmosphere composition of ripening chambers, affect the ripening of surface mould-ripened soft cheeses (Choisy et al., 2000; Ramet, 2000). In modern cheese plants, for a better control of the quality, the ripening microorganisms essentially come from starters. The ripening starter is generally composed of yeasts (mainly *Kluyveromyces species* and *Geotrichum candidum*), *Penicillium camemberti*, and surface bacteria. The evolution in microbial and biochemical characteristics in the case of Camembert-type cheeses throughout the entire ripening process, carried out under constant controlled temperature (12 ± 1 °C) and RH (93 ± 1 %), has already been investigated by Leclercq-Perlat et al. (2004, 2006).

For other types of cheeses, the effects of ripening conditions on cheese characteristics have been studied. For goat cheese, Malcata et al. (1995) have shown the effect of ripening temperature and relative humidity on proteolysis and lipolysis. For a smear soft cheese, Bonaïti et al. (2004) have pointed out the influence of temperature on the ripening dynamics but these authors were yet able to reveal the effect of RH on the microbial and biochemical parameters of ripening, except in the case of water mass loss. For hard cheese (Reggianito Argentino), Sihufe et al. (2007, 2010 a,b) have shown the influence of ripening temperature on proteolysis and lipolysis and they were able to estimate the optimal ripening time. For an uncooked pressed cheese, Callon et al. (2011) have shown the importance of temperature and RH to control the growth of *Lactobacilli* and their inhibition activities on *L. monocytogenes*. To our knowledge, little work has focused on the influence of temperature and relative humidity on the cheese ripening qualities of Camembert-type cheeses.

According to Hemme and Richard (1986), the growth of *G. candidum*, *P. camemberti*, and *B. aurantiacum* on the surface of Camembert-type cheeses is an essential prerequisite for the development of the aroma, flavor, color, and texture. In consequence, environmental factors (such as ripening temperature and the hygrometry of ripening chamber) that act on microorganism growth play a determining role in microbial development and enzymatic reactions (van den Tempel et al., 2000). Increasing temperature 3 to 4°C is the easiest and most economically feasible strategy to accelerate ripening (Nunez et al., 1991). However, in

the case of mould soft cheeses, there are important enzymatic activities trigged by the surface flora. When temperature is increase so is the level of these activities, largely modifying cheese qualities such as texture and taste (Reps, 1993; Ramet, 2000). From the point of view of relative humidity (RH), throughout ripening it changes in both the total water content and water activity on the cheese surface (Gripon, 1993; Reps, 1993; Hardy et al., 2000; Simal et al., 2001). A decrease in RH from 98 to 95 % has been shown to increase cheese water losses 2.5 times (Mirade et al., 2004). Airflow rate has also been shown to have an effect on water evaporation (Weissenfluh and Puhan, 1987). Weight losses have been known to increase 6 times when air velocity changes from 0 to 0.5 $m.s^{-1}$ (Mirade et al., 2004). Airflow rate also changes atmospheric composition, which acts on ripening phenomena. Chamber hygrometry varies between 90 to 95 % for mould soft cheeses (Lesage-Meessen et al., 1998). Consequently, a part of the water in the cheese evaporates into the atmosphere, leading to a decrease in cheese mass and water content (Hardy et al., 2000). This water loss is related to different cheese parameters (initial water quantity, specific surface, bound water quantity, and surface density) (Reps, 1993; Hardy et al., 2000). If RH is higher than 95 %, *P. camemberti mycelium* development is poor (Lenoir et al., 1985), Moreover, if the RH value is lower than 90 %, water loss is excessive and the cheese is therefore too dry. Whatever the soft cheese undergoing ripening, the total water weight loss throughout the ripening must not vary any more than 10 to 15 % of the initial weight. The hygrometry also plays a role in restricting undesirable microorganism growth, but it must do so without inhibiting the growth of ripening microorganisms. Bonaïti et al. (2004) have defined the best ripening conditions in terms of temperature and RH (13°C and 95 ± 1 %, respectively) of a smear soft cheese seeded with a very simple starter (*Debaryomyces hansenii* and *B. aurantiacum*). However, little data have been published about the influences of temperature and relative humidity in cheese ripening, explaining, in part, why the regulation of these conditions in ripening chambers remains empirical.

The aim of this study was to evaluate the effects of temperature and relative humidity (RH), used in the ripening chamber, on microbial developments, biochemical changes, and the

corresponding kinetics of Camembert-type cheeses throughout the ripening process (from d0 to d40).

MATERIAL AND METHODS

Biological material.

Kluyveromyces marxianus (GMPA, 44_8), *Geotrichum candidum* (strain D, Cargill, La Ferté-sous-Jouarre, France), *Penicillium camemberti* (strain R, Cargill), and *Brevibacterium aurantiacum* (ATCC 9175; ATCC standard, Manassas, Virginia, USA) were the given ripening culture. Flora Danica lyophilisat (CHN11 for 1000 L of milk, Chr Hansen, Saint-Germain-les-Arpajon, France) was the given mesophilic lactic acid bacteria culture (LAB). Before each cheese-making trial, 2.5 g of this LAB was mixed in 1.5 L sterile (115°C, 10 min) skim milk and then cultured at 30°C for 16 h without stirring. The preparations of ripening strains and their numerations were carried out as previously described by Leclercq-Perlat et al. (2004).

Cheese-making.

The cheeses were prepared on a pilot scale under aseptic conditions, in a sterilized 3 m^3 chamber in which coagulation, cutting, draining, and shaping of the curds were carried out. The chamber and all the non-autoclavable pieces of equipment were cleaned and sterilized as previously described by Leclercq-Perlat et al. (2004). The temperature of the cheese-making chamber was maintained at 28 ± 1°C.

For each cheese-making trial, 200 L of milk were used to make 100 Camembert-type cheeses. The raw milk and the skim milk were obtained from the AgroParisTech experimental farm (Thiverval-Grignon, France). It was standardized at 29 g/L of fat matter by mixing skim and full-cream milk and at 35 g/L of total protein matter by adding milk protein powder (852, Ingredia, Arras, France). After mixing, the milk was pasteurized (2min30sec at 77 ± 1°C) and cooled to the incubation temperature (34 ± 1°C). The pH of the milk varied between 6.5 and 6.6. After pouring the first liters of milk into the coagulation tank, that milk was inoculated with the LAB starter (1.5 % V/V) and the ripening starters. After filling up the tank with the remaining pasteurized of milk, the concentrations of *K. marxianus*, *G. candidum*, *P. camemberti*, and *B. aurantiacum* were $4x10^3$ CFU.mL^{-1}, 80 CFU.mL^{-1},

$8x10^3$ spores.mL^{-1}, and $2x10^4$ CFU.mL^{-1}, respectively. Due to the LAB activity, the milk pH reached 6.3 after 80 to 110 min. Then, the rennet (520 mg/L of chymosine, Chr Hansen) was added (20 mL/100L). Coagulation took 20 min and the hardening that followed took 40 min. Then the curd was cut into 2 cm cubes. After another 40 min., roughly 80 liters of whey were drained to obtain an average cheese dry matter between 40 and 42 %. The curd was then shaped in polyurethane moulds (diameter 107 mm, height 77 mm), producing cheeses weighing 300 ± 20 g. The moulds were turned after 30 min., 5h and 19h after the end of molding. Three hours after the end of molding the temperature of the cheese-making chamber was reduced to 21 ± 1 °C. The cheeses were turned out 20 hours after molding and 24 hours after the end of molding, the cheeses were pickled for 25 min. in sterile (2x30 min. at 120°C) brine (330 g NaCl/L at pH 5.5) at 14 ± 1°C. Then, after 1 hour of drying, to eliminate the excess brine, they were transferred to the ripening chambers, sterilized beforehand with peracetic acid. This was initial ripening time (d0).

For each cheese-making trial, between d0 and d1, the cheeses were maintained at 12°C and 85 % RH. Then, between d1 and d13, they were kept at a given controlled temperature (8 ± 1°C, 12 ±1°C or 16 ± 1°C) and a given controlled RH (88 ± 1%, 92 ± 1 % or 98 ± 2%), with the same periodically renewed atmosphere. To that end, on d0, the ripening chamber which contained air (CO_2 = 0 %) was sealed. Knowing that when respiration takes place the CO_2 concentration increases, when, in our case, the CO_2 concentration reached 0.5 %, it was automatically decreased down to 0.1 % by injecting humid sterile air as described by Picque et al. (2006). On d5, the cheeses were turned. On d13, no matter the given temperature and RH, the temperature and RH were regulated at 12°C and 85 % to dry the cheese surface. On d14, they were wrapped in a reference film (AMCOR Flexibles, Barbézieux, France) and stored to continue ripening at 4°C until d40. Between d1 and d14, a cheese was removed and analyzed on a daily basis and between d14 and d40, this was done on a weekly basis.

Analyses performed on the cheeses.

The rind (2 mm of height on all cheese surfaces) and core of each cheese were separated and analyzed as previously described (Leclercq-Perlat et al., 2004). Viable cell counts of ripening strains were measured only in the cheese rind because only *K. marxianus* and *G. candidum* grow in the core and reached concentrations 500 to 1000 times lower than the ones of the rind (Leclercq-Perlat et al., 2004, 2006). The microbial analyses in the rind were carried out as previously described by Leclercq-Perlat et al. (2004). pH of the rind as well as lactose and lactate concentrations in both the rind and the core were measured as previously described (Leclercq-Perlat et al., 2004, 2006). For each sampling time, the underrind thickness of the cheese was measured using the first 14 mm at 6 points per face. Then, the arithmetic average of these 12 measurements was calculated. For each run, 20 cheeses were weighted on d1 and on d14 with an accuracy of 0.01 g to determine the average water mass loss.

Experimental design.

The effects of temperature and RH on the cheese ripening qualities were examined using a two-factor, three-level complete factorial experimental design (3^2). The 9 combinations of temperature and RH are shown in Tables 1, and 3 (left part). The levels of each factor were 8, 12 and 16 °C for temperature and 88, 92 and 98 % for RH. Levels were chosen in accordance with the ones of interest during Camembert cheese ripening. Due to the length of the experiments, the trial corresponding to the central point of the experimental design was quintupled (runs 6 to 10). The runs under 8°C and 98 % RH (runs 3 and 4) and under16°C and 88 % RH (runs 12 and 13) were duplicated.

Statistical analyses.

For the runs carried out under the same conditions (runs 3 and 4; runs 6 to 10, and runs 12 and 13), cell counts, lactose and lactate concentrations, pH of the rind, and water mass loss were compared by a two factor (run and time) analysis of variance (ANOVA) with Statistica software 6.1 (Statsoft, Maisons-Alfort, France). The hypotheses examined were the equality of the runs and the absence of false interpretation. To control the equality of runs, this test was significant for the risk α ($\alpha = 1 - p(F_{obs}<F_{crit})) \leq 0.01$). To evaluate the risk of false inter-

pretation, the test power (1–β) was determined according to Mann and Whitney's method (Cohen, 1992).

For each run, the changes in each ripening parameter, in relation to time, were described by two descriptors calculated by Weibull model (Schepers et al., 2000) with Statistica and defined in the first part of results.

The general linear model (Statistica Software) was performed to calculate quadratic models that allowed determining the influence of the two factors (θ; RH) on the kinetic descriptors. The non-significant terms were omitted one by one, using the procedure Stepwise Backwards (Statistica), consequently only the terms significant at 99 % of confidence level (*p-value* < 0.01) were considered. The three-dimensional response surfaces of some descriptors versus θ and RH factors were plotted to illustrate the main (linear or quadratic) and/or interactive effects.

RESULTS AND DISCUSSION

Whatever the run, on d0, the average decimal logarithm of viable cells concentration calculated from all the trials were 5.2 ± 0.2, 2.1 ± 0.1, and 4.9 ± 0.3 CFU/g for *K. marxianus, G. candidum,* and *B. aurantiacum*, respectively, and 3.8 ± 0.1 spores/g for *P. camemberti*. The chemical composition of the rind was: dry matter content = 41.3 ± 0.6 %, pH = 4.80 ± 0.05, lactose concentration = 13.7 ± 0.4 g/kg, lactate concentration = 14.0 ± 0.6 g/kg while the one of the core was: dry matter content = 39.0 ± 0.8 %; pH = 4.80 ± 0.05, lactose concentration = 14.3 ± 0.5 g/kg, and lactate concentration = 15 ± 1 g/kg.

Description of phenomena observed at 12°C and 92 % of RH (central point)

Statistical trial reproducibility.

For the runs carried out under 12°C and 92 % of RH, the reproducibility of the microbiological (viable cell counts) and the physicochemical measurements (carbon substrate concentrations, pH, and cheese underrind thickness) in relation to ripening time were studied. From the two factor (run, time) ANOVA results, it was apparent that (1) the hypotheses of equality of the evolutions were satisfactory with a risk α = 0.01 and (2) the risks of a false interpretation estimated by the test power (1-β) were less than 0.94. Throughout the experimental period, the runs carried out in the same conditions were

statistically identical. This result is shown in Figure 1, where the four microbial evolutions, in relation to ripening time, are given.

Microbial evolutions.

Figure 1 gives the decimal logarithm evolutions of *K. marxianus*, *G. candidum*, and *B. aurantiacum* viable cell concentrations and spore concentration of *P. camemberti*, in relation to time, for the five central point runs (runs 6 to 10) and their Weibull modeling. Whatever the microorganism, the evolution of their concentration showed the same behaviors that thosepreviously described by Leclercq-Perlat et al. (2004, 2006) using similar ripening conditions.

For *K. marxianus*, the maximum growth rate μ_{KM} was 0.50 ± 0.07 d^{-1} (Table 1) and was obtained on 1.50 ± 0.05 d ($t_{\mu KM}$) (results not shown). Its maximal cell concentration was around $3.2x10^7$ CFU per g. From d14 to d40, *K. marxianus* cell concentration showed a death phase with a maximal death rate (D_{KM}) of 0.050 ± 0.007 d^{-1} occurring at 29.8 ± 0.2 d (Table 1). For *G. candidum*, the growth maximal growth rate μ_{GC} was 1.0 ± 0.1 d^{-1} and occurred at 3.7 ± 0.1 d ($t_{\mu GC}$) (Table 1). After d 8 *G. candidum* viable cell concentration remained constant around $2.6x10^7$ CFU/g. For *B. aurantiacum,* from d9 to d25, maximal growth rate (μ_{BA}) was 0.58 ± 0.09 d^{-1} occurring at 15.0 ± 0.2 d ($t_{\mu BA}$) (Table 1). From d26 to d40, a second growth phase took place and reached a value close to $7.9x10^8$ CFU/g on d40. For *P. camemberti* the maximal sporulation rate (μ_{PC}) was 0.5 ± 0.1 d^{-1} and it occurred at 9.7 ± 0.6 d ($t_{\mu PC}$) (Table 1)

Except for *K. marxianus*, the values of the kinetic descriptors (Table 1) were in accordance to the ones previously obtained (Leclercq-Perlat et al., 2006). For *K. marxianus*, growth and death rates were higher than the ones found by Leclercq-Perlat et al. (2006) due to modeling method: in 2006 these authors have calculated mean rates.

Biochemical changes

For the central point runs, the evolution of pH, carbon substrate concentrations, and cheese underrind thickness showed the same phases of the ripening time as the ones previously described by Leclercq-Perlat et al. (2004, 2006) using similar ripening conditions. Their

kinetic descriptors were determined using Weibull modeling (Bonaïti et al., 2004; Leclercq-Perlat et al., 2006).

pH of surface. Figure 2 shows the evolutions of pH and their Weibull curves, in relation to ripening time for the nine conditions of temperature and RH. For the five central point runs (runs 6 to 10), the average of maximal increasing rate (V_{pH}) was 1.3 ± 0.1 pH unit/d and it occurred at 6.60 ± 0.1 d (Table 2). After wrapping, rind pH remained constant at 7.7 ± 0.2.

Carbon substrates. Figure 3 shows the evolutions of mean lactose (A) and lactate (B) concentrations (g/kg) in the rind throughout ripening whatever the nine conditions of temperature and RH used. For the runs carried out at 12°C under 92 % of RH, mean lactose concentrations in the rind and in the core decreased and became negligible on d8-d9 and 19 d, respectively. The kinetic descriptors were the maximal consumption rate C_{LO} equal to 3.05 ± 0.07 g/kg/ d occurring at t_{CLO} = 2.6 ± 0.1 d and maximal decreasing rate D_{LO} equal to 1.55 ± 0.06 g/kg/d occurring at t_{DLO} = 3.9 ± 0.1 d, respectively (Table 2).

Whatever the cheese part, from d0 to d4-d6, mean lactate evolution presented a first increasing phase corresponding to post-acidification phenomenon (Lenoir et al., 1985; Leclercq-Perlat et al., 2004, 2006). And then until d20 the mean lactate concentration kinetic descriptors in the rind were (C_{LA}) 3.11 ± 0.05 g/kg/d obtained at (t_{CLA}) 6.8 ± 0.1 d while the ones in the core (D_{LA}) 1.82 ± 0.07 g/kg/d observed at (t_{DLA}) 7.6 ± 0.1 d (Table 2). From d20 to d40, lactate concentrations in the two cheese parts continued to decrease reaching 0.5 and 0.9 g lactate per kg in the rind and in the core on d40, respectively (Figure 3).

Under similar ripening conditions, Leclercq-Perlat et al (2006) have found the same range of kinetic descriptor values for pH of the rind as well as lactose and lactate concentrations.

Cheese water mass loss. From d0 to d14, the mean water weight loss decreased linearly as previously shown by Bonaïti et al. (2004) and for the standard ripening conditions the mean water mass loss rate (L_{H2O}) was 1.6 ± 0.3 g per d (Table 3).

Underrind thickness. The development of the cheese underrind, in relation to ripening time, gives information about ripening level. Figure 4 shows the mean underrind thickness evolutions of Camembert cheeses (mm), throughout the ripening carried out under the nine

ripening conditions. Under standard ripening conditions (runs 6 to 10), from d0 to d7, the underrind was not measurable, allowing defined the day on which the underrind became observable ($t_{UR,i}$ = 7 d). The mean underrind thickness was measurable a day after. Then and until the end of ripening, the mean cheese underrind thickness increased with maximal increasing rate (V_{TUR}) equal to 0.60± 0.05 mm per d occurring on d29.5 (t_{VTUR}) (Table 3).

In conclusion, it has been shown that 1) the repeatability and the reproducibility of the central point runs of ripening, and 2) for each microbial and biochemical parameter, it was possible to define two kinetic descriptors. This can allow us studying the effects of temperature and RH in the microbial and biochemical characteristics of Camembert ripening.

Influences of ripening temperature and RH on the ripening kinetics

All statistical tests were carried out using 99 % confidence levels ($p < 0.01$). Table 1 gathers the kinetic descriptors of *K. marxianus* (μ_{KM} for growth and D_{KM} and t_{DKM} for death), the ones of *G. candidum* (μ_{GC} and $t_{\mu GC}$) and *B. aurantiacum* (μ_{BA} and $t_{\mu BA}$) growths, and of *P. camemberti* sporulation (μ_{PC} and $t_{\mu PC}$). Tables 4a and 4b give the quadratic models associated with ripening descriptors in relation to temperature and RH. Some representative three-dimensional response surfaces are plotted in Figure 5.

Influences of ripening temperature and RH on K. marxianus (KM) evolutions.

μ_{KM} and D_{KM} increased from 0.021 ± 0.06 d^{-1} and 0.044 ± 0.08 d^{-1} (runs 3 and 4 : 8°C - 98% RH) to 0.098 ± 0.06 d^{-1} and 0.072 ± 0.06 d^{-1} (run 14: 16°C – 92% RH), respectively. The highest values of μ_{KM} and D_{KM}, corresponding to a generation time of 0.7 d (≈ 17 h) and a death time of 9.9 d, respectively, were obtained at 16°C under 98 % RH. Temperature had statistically significant influences on the three *K. marxianus* kinetic descriptors and the equations describing its effects on them only included temperature with quadratic term (Table 4a). The three-dimensional response surfaces of maximal growth rate (μ_{KM} in d^{-1}) and the time associated to D_{KM} (t_{DKM} in d) are given in Figures 5A and 5B, respectively. These surfaces, showing an increase only along the temperature axis, confirmed both the temperature preponderance over maximal growth rate (μ_{KM}) as well as the time associated to maximal death rate (t_{DKM}) of *K. marxianus* and the absence of RH influence on them.

Whatever the temperature and the RH used throughout the ripening, the time for which μ_{KM} was obtained was equal to 1.50 ± 0.05 d and the maximal *K. marxianus* viable cell concentration was ranged between $2.3x10^7$ and $3.7x10^7$ CFU/g (results not shown). These two descriptors were independent to ripening conditions. This can explain 1) *K. marxianus* exponential growth began from the ripening microorganism seeding in the milk (Leclercq-Perlat et al., 2004), 2) K. marxianus continues to growth after the transfer of Camembert cheeses in the ripening room even if its growth rate was lower than during the cheese-making day, and 3) whatever the run, on d0, the ripening conditions were the same (first drying phase).

This yeast is able to grow in liquid media as well as on a surface of a cheese whatever the ripening RH higher than 85 % (Guéguen and Schmidt, 1992). This was probably explained that RH did not influence on *K. marxianus* growth or death. These authors have also shown that the yeast growth rate was strongly linked to the temperature. The highest growth rate was observed at 16°C; this can be explained because this temperature is the most close to the optimal temperature of growth (around 25°C) (Barnett et al., 1990). *K. marxianus* death rate was highly linked with temperature. This was in accordance to the results of Walker and O'Neil (1990) that have shown that 1) *K. marxianus* cells died when the lactose concentrations became negligible in the medium and 2) an acceleration of *K. marxianus* death was observed with an increasing of the culture temperature.

Influence of ripening temperature and RH on Geotrichum candidum (GC) growth.

Temperature had significant quadratic effects on μ_{GC} while neither temperature nor RH had an effect on $t_{\mu GC}$ (4.0 ± 0.2 d) (Table 4a). The maximum values of μ_{GC} corresponding to a generation time of 0.38 d (≈ 9 h) were obtained at 16 °C whatever the RH. This also allowed showing the major impact of temperature on *G. candidum* growth. Like *K. marxianus*, *G. candidum* is not affected by the ripening RH when RH is higher than 80 % (Guéguen and Schmidt 1992, Lesage-Meessen and Cahagnier 1998). More the temperature was close to *G. candidum* optimum (25 °C; Barnett et al., 1990) more the specific growth rate was higher.

The maximal viable *G. candidum* concentration was independent to ripening conditions: whatever the runs, it was ranged from $2.0x10^7$ to $3.9x10^7$ CFU per g (results not shown). Moreover, these two yeast developments lead a rapid decreasing of the cheese acidity which involves a really setting up of the acid-sensitive bacterial flora. Temperature affected the growth of both *K. marxianus* and *G. candidum* while RH did not influence it. According to Lenoir et al. (1985) and Guéguen et al. (1992), this can be due to the ability of these two yeasts to growth equally in a liquid medium and on the surface of cheeses when the RH was higher than 85 %. These authors have highlighted that, in a surface of a cheese, the yeast growth was limited by the steric overcrowding of the microorganism layer. This can explain that whatever the run, the maximal viable concentrations of *K. marxianus* and *G. candidum* had the same value.

Influence of temperature and RH on Brevibacterium aurantiacum (BA) growth.

The three-dimensional response surface of μ_{BA} is given in Figure 5C. This Figure shows a strong increase of μ_{BA} for a combined increase of temperature and relative humidity. The effect of each factor is higher for the highest value of the other factor, denoting a positive interaction. Moreover, the equation associated allowed confirmed that temperature and RH had significant linear effects and an interactive effect on μ_{BA} (Table 4a). At 8°C, whatever the RH, μ_{BA} remained constant while at 16°C μ_{BA} was twice higher when RH increased from 88 to 98 %. Temperature had a significant influence (linear and quadratic terms) on $t_{\mu BA}$ (Table 4a). More the temperature was high more $t_{\mu BA}$ was lower.

B. aurantiacum grows better when the temperature is the most close to its optimal growth temperature (25 °C) and the RH is close to 98 % (Bergère and Tourneur, 1992, Bonaïti et al., 2004). However, this species sprays out and forms the main part of the flora of smear cheeses for which the ripening occurs between 8 to 16 °C under a RH higher than 96 %. Indeed, *B. aurantiacum* is greatly affected by the RH lower than 90 % (Reps, 1993). At 8°C under 88 % RH , its growth was restricted and from d0 to d40 its concentration increasing was small (less than 80 CFU/ g) while under RH 98 %, *B. aurantiacum* growth was more furthered and its concentration increase reached near 10^3 CFU/g. At 16°C, under 88 % RH,

B. aurantiacum grew better and its concentration increases throughout the ripening reached 10^4 CFU/g while under 98 % RH, its growth was the highest and its concentration increase reached more than 10^5 CFU.g^{-1}. Even if temperature seemed to have the main influence, these results showed the importance of interaction between temperature and RH on *B. aurantiacum* growth.

***Influence of ripening temperature and RH on Penicillium camemberti* (PC) sporulation.** After wrapping, *P. camemberti* spore concentrations remained constant whatever the ripening conditions. The three-dimensional response surface of μ_{PC} is shown in Figure 5D. Figure 5D shows a strong increase in specific sporulation rate of *P. camemberti* (μ_{PC}) for a combined increase of temperature and relative humidity. The effect of each factor is higher for the highest value of the other factor, denoting a positive interaction. This equation associated to this descriptor was related to temperature and RH (linear and interactive terms) (Table 4a). The time associated to μ_{PC} was modeled by an interactive term of θ and RH (Table 4a).

These two descriptors highlighted the importance of the temperature and RH interactiveness on *P. camemberti* sporulation. Whatever the temperature under RH of 88 % and whatever the RH at 8°C, μ_{PC} values were very low. This could be explained by *P. camemberti* sporulation negligible. The spore concentration increase from d5 to d14 was lower than 0.7 Log. At 12°C or 16°C under RH of 92% or 98%, μ_{PC} increased (Table 1) and its maximal spore concentration remained constant (≈ 10^5 spores/g WC) after d14. At 16°C under RH of 98%, μ_{PC}, corresponding to a generation time of 5.25 h, was the highest, pointing out a significant sporulation of *P. camemberti*; its concentration reaching 1.6 x 10^6 spores per g, was corresponded to 16 times higher than the ones of all other runs (results not shown).

Influence of ripening temperature and RH on rind pH and carbon substrate evolutions. *pH of the rind*. Whatever the run, the evolution of pH in the cheese rind is given in Figure 2. This figure allowed separating the runs into three groups, in relation to temperature. This allowed directly showing the preponderance of temperature over the pH dynamics. The values of the pH kinetic descriptors in rind (V_{pH} in unit per d and t_{VpH} in d) in relation to

temperature and RH are given in Table 2. The response surface of V_{pH} is given in Figure 5E. In the considered range, temperature had a strong positive effect on V_{pH}, while relative humidity had a slight negative influence. The equation of V_{pH} associated to this figure was linked to temperature (quadratic term) and its interactive term with RH while t_{VpH} was only linked with temperature (individual and quadratic terms) (Table 4b).

It is generally admitted that pH of rind depends on the lactate consumption and the alkaline product accumulation. Indeed, Gori et al. (2007) have shown that during its growth most cheese yeasts produce ammonia. Leclercq-Perlat et al. (2004) have shown that the lactate consumption is related to *K. marxianus* when any lactose is available in the rind, *G. candidum* growth and *P. camemberti* mycelium development. Therefore, both *K. marxianus* and *G. candidum* growth descriptors were depended on temperature while *P. camemberti* mycelium development was related to temperature and RH interactive term. According to Hardy et al. (2000), the enzymatic reactions involved in the lactate consumption and the ammonium production as well as the phenomena of diffusion occurred in the aqueous phase of the cheese. This could be explained the influence of RH on V_{pH} observed. Indeed, more the RH was low more the cheese was dry and more the aqueous phase of the cheese became restricted.

Carbon substrates. The values of the kinetic descriptors of lactose and lactate concentrations throughout the ripening (d0 – d40) in the rind and in the core are given in Table 2. The evolutions of lactose and lactate in the rind of cheese, in relation to ripening time, are given in Figure 3. This allowed showing that lactose and lactate evolutions were depended on mainly temperature and in the case of lactate an interaction of RH.

Lactose (LO) changes. In the rind, the three-dimensional response surface of its consumption rate (C_{LO} in g.kg $WC^{-1}.d^{-1}$) is given in Figure 5F. This surface, showing an increase only along the temperature axis, confirmed the preponderance of temperature effect for the maximal consumption rate of lactose. C_{LO} and t_{CLO} were related to temperature (quadratic and linear terms, respectively) (Table 4b). Whatever the runs, the decreasing of lactose began on d0 and took place during *K. marxianus* growth (Leclercq-Perlat et al.,

2004). Among ripening microorganisms, in the rind, only *K. marxianus* metabolize lactose into alcohols, water and CO_2. Its growth was only dependent on temperature, explaining the dependence of lactose consumption in the rind with temperature.

In the core, lactose consumption rate (D_{LO} in g .kg $WC^{-1}.d^{-1}$) and its associated time (t_{DLO} in d) were modeled by a function of temperature (linear term) for the both descriptors plus a quadratic term for t_{DLO} (Table 4b). The lactose concentration in the core is mainly linked to the one of the rind due to the importance of lactose diffusion from the core to the rind (Leclercq-Perlat et al., 2004). This can be explained the connection between lactose decreasing in the core and the temperature.

Lactate (LA) changes. In the rind, the three-dimensional response surface of lactate consumption rate (C_{LA} in g.kg $WC^{-1}.d^{-1}$) is given in Figure 5G. In the considered range, for low temperatures, the relative humidity had a positive effect on maximal consumption rate of lactate (C_{LA}), but this effect was reversed for high temperatures. Similarly, at low RH temperature increase raised C_{LA} but had almost no effect at high RH. This behavior denoted a strong negative interaction between T and RH.

C_{LA} was related to temperature (linear term), RH (quadratic term) and temperature and RH (interactive term) while t_{CLA} was only depended on the temperature (individual and quadratic terms). The time for which the end of post-acidification phase occurred also depended on the temperature: it began on d3 for runs carried out at 16°C and on d6 for the ones done at 8°C.

In the rind, all ripening microorganisms consume lactate (Choisy et al., 2000; Leclercq-Perlat et al., 2004). *K. marxianus* uses it only when the lactose concentration begins negligible. The Lactate concentration decreasing is related to *G. candidum* growth and to *P. camemberti* mycelium development (visually observed). This can explain the dependence of lactate decreasing rate with temperature. Indeed, temperature is one of the main factors influencing the microorganism growth and their enzymatic activities (Choisy et al., 2000). Moreover, the enzymatic activities take place into the aqueous cheese phase which is related to the water activity (a_w), another factor influencing the microorganism growths and their enzymatic activities (Hardy et al., 2000). According to these authors, a_w is depended on cheese

humidity (determined by dry matter), salting level and the RH used in the ripening room. In this study, only ripening RH varied. However, when RH is lower than 90 %, the water evaporation from the rind to the atmosphere becomes higher, driving a significant rind drying (Ramet, 2000). This involves a diminution of enzymatic activities and therefore a decreasing of C_{LA}. This explains the great dependence of C_{LA} with RH (Fig. 5G).

In the core, the maximal lactate decreasing rate (D_{LA}) was related to temperature and RH (quadratic terms), and their interactive term while t_{DLA} was only modeled by temperature (individual and quadratic terms).

According to Leclercq-Perlat et al. (2004), the lactate concentration in the core is mainly related to the one of the rind. Indeed, these authors have shown the importance of lactate diffusion phenomenon from the core to the rind. This can be explained the correlation between lactate decreasing in the core and the ripening conditions under study.

Influence of temperature and RH on cheese water mass losses.

The values of the cheese water mass loss (L_{H2O} in g/d) throughout the ripening in room (d0 – d14), in relation to temperature and RH, are given in Table 3.

The water mass loss rate (L_{H2O}) was modeled by RH (linear term) and RH and temperature (interactive term) (Table 4b). Its response surface is shown in Figure 5H. In the considered range, RH had a strong negative effect on L_{H2O} while temperature seemed have a slight positive interactive effect. Weigh loss was strongly related to a low RH, which led to much dehydratation of cheese rind. Indeed, water forms the main part of weight loss and it was both vaporized from surface to the atmosphere and diffused from the cheese core to the surface (Simal et al., 2001). In opposite, high RH led to lower weigh losses. However, RH alone did not explain cheese weight loss; it can exit a combination of external and internal conditions which induced the drying rate (Bonaïti et al., 2004). Temperature had also a lesser effect on the cheese weight loss rate than RH: for each RH the water losses were lower when ripening room temperature diminished (Fig. 5H).

The effect on RH on water mass loss rate has been previously shown for a smear cheese (Bonaïti et al., 2004). Indeed, whatever the temperature, under the lowest RH (< 88 %) a

significant drying of the cheeses occurred which limited the flora growth. Consequently, the water mass loss rate was the highest and around 30 % of initial cheese mass was lost. However, to our knowledge, the influence of RH and temperature interaction on this phenomenon did not clearly be shown previously.

Influence of ripening temperature and RH on underrind thickness evolution.

The cheese underrind thickness descriptors values throughout the ripening (d0 – d40) are given in Table 3. Temperature had an effect on time for which the underrind began visible ($t_{UR,I}$ in d). This time was 9.0 d, 7.0 d and 5.0 d at 8 °C, 12 °C and 16°C, respectively. The three-dimensional response surface of underrind thickness rate (V_{TUR}) is given in Figure 5I. This figure shows a strong increase of maximal increase rate of underrind thickness (V_{TUR}) for a combined increase of temperature and relative humidity. The effect of each factor is higher for the highest value of the other factors, denoting a positive interaction. V_{TUR} was related to temperature (individual and quadratic terms) and temperature x RH (interactive term). Its associated time was dependent on temperature (quadratic term) and RH (quadratic term) as well as their interactive term (Table 4b).

The underrind thickness gives information about overall proteolysis and lipolysis of the cheeses (Bonaïti et al., 2004). The temperature and RH increases had an effect on underrind thickness. Indeed, whatever the runs, the underrind began to grow when proteins and lipids began to be used by ripening microorganisms near the surface, i.e. when the lactate concentrations in the rind became restricted near the colonies. According to Lenoir et al. (1985) and Leclercq-Perlat et al. (2007), this was mainly related to temperature: more the temperature was higher more *G. candidum*, *P. camemberti*, and *B. aurantiacum* growths were fast, and more the lactate concentrations decreased fast, and earlier the organic matters were used by micro-organisms. Moreover, the enzymatic activities are mainly dependent on the water activity, and consequently, of RH, and a diminution of RH involves a slowdown of enzymatic activities (Choisy et al., 2000). Noomen (1983) had highlighted that the enzymes did not migrated from the rind to the core. This explains that the underrind of cheeses ripened under lower RH (< 90 %) was dried and grew slower than the other ripening

conditions. On the contrary, under RH 98 % and 16°C, the underrind increased quickly and on d14 its thickness reached more than 6 mm per face (Figure 4) while on d40 the cheeses were completely runny.

CONCLUSION

Whatever the temperature, a RH of 88 % affected negatively microorganism growths, pH increase, carbon substrate consumptions, and underrind thickness throughout cheese ripening. Whatever the RH, a temperature of 16 °C induced an acceleration of the cheese ripening process. Indeed, microorganism growths and all enzymatic reactions started earlier than under the other temperatures. In fact, the best ripening conditions to obtain an optimum between microbiological and biochemical characteristics appear to be 13°C and 94 % RH.

In general, a fast installation and growth of yeasts and *Penicillium* is sought to ensure a dense coating of Camembert cheeses, to speed up the ripening and to avoid potential spoilage contamination. Such a study will provide main elements for a better and rational control of the Camembert-type cheese ripening process.

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REFERENCES

Barnett, J.A., R.W. Payne, and D. Yarrow. 1990. A guide to identifying and classifyibg yeasts. 4th ed. Cambridge University Press, Cambridge, UK.

Bergère, J.L., and C. Tourneur. 1992. The smear of the cheese surface. Pages 127–163 *in* Les Groupes Microbiens d'Intérêt Laitier. J. Hermier, J. Lenoir, and W. Weber, ed. CEPIL, Paris, France.

Bonaiti, C., M.-N. Leclercq-Perlat, E. Latrille, and G. Corrieu. 2004. Deacidification by *D. hansenii* of smear soft cheeses ripened under controlled conditions: relative humidity and temperature influences. J. Dairy Sci. 87:3976–3988.

Callon, C., D. Picque, G. Corrieu, and M.C. Montel. 2011. Ripening conditions: A tool for the control of *Listeria monocytogenes* in uncooked pressed type cheese. Food Control 22: 1911-1919.

Choisy, C., M. Desmazeaud, J.C. Gripon, G. Lamberet, and J. Lenoir. 2000. The biochemistry of ripening. Pages 82-151 in Cheesemaking: from science to quality assurance. A. Eck and J.C. Gillis, ed. Lavoisier Publishing, Paris, France.

Cohen, J. 1992. A power primer. Psychological Bull. 112:115-119.

Gori, K, H.D. Mortensen, N. Arneborg, L. Jespersen. 2007. Ammonia production and its possible role as mediator of communication for *Debaryomyces hansenii* and other cheese-relevant yeast species. J. Dairy Sci. 90: 5032-5041.

Gripon, A. 1993. Mould-ripened cheeses. In Cheese: Chemistry, Physics and Microbiology, Vol. 2, pp. 111–136 (Ed. PF Fox). London, United Kingdom: Chapman & Hall.

Guéguen, M., and J.L. Schmidt. 1992. Levures et *Geotrichum candidum*. Pages 165–196 *in* Les Groupes Microbiens d'Intérêt Laitier. J. Hermier, J. Lenoir, and W. Weber, ed. CEPIL, Paris, France.

Hardy, J., J. Scher, H. E. Spinnler, E. Guichard, and J. C. Gripon. 2000. Physical and sensory properties of cheese. Pages 447–473 in Cheesemaking: From Science to Quality Assurance. A. Eck and J. C. Gillis, ed. Intercept Limited, Andover, UK.

Hemme D, and J. Richard. 1986. Utilization of L-Methionine and production of methanethiol by bacteria isolated from raw milk Camembert cheese. Lait 66 :135–142.

Leclercq-Perlat, M.-N., F. Buono, H.-E. D. Lambert, H.E. Spinnler, and G. Corrieu. 2004. Controlled Production of Camembert-Type Cheeses: Part I. Microbiological and physicochemical evolutions. J. Dairy Res.71:346-354.

Leclercq-Perlat, M.-N., D. Picque, H. Riahi, G. Corrieu. 2006. Microbiological and biochemical aspects of Camembert-type cheeses depend on atmospheric composition in the ripening chamber. J. Dairy Sci. 89:3260-3273.

Leclercq-Perlat, M.-N., G. Corrieu, and H.-E. Spinnler. 2007. Controlled production of Camembert-type cheeses: Part III. Free fatty acid evolutions. J. Dairy Res.74:218-225.

Lenoir, J., G. Lamberet, J. L. Schmidt, and C. Tourneur. 1985. La maîtrise du bioréacteur Fromage. Biofutur 41:23–50.

Lesage-Meessen, L., and B. Cahagnier. 1998. Mécanismes d'adaptation des micromycètes aux activités de l'eau réduite. Pages 19–35 in Moisissures des aliments peu hydratés. B. Cahagnier, ed. Sci & Tech. Agro-Alimentaires. Lavoisier Tec & Doc, Paris, France.

Malcata, F.X., A.M.P. Gomes, M.L. da Costa. 1995. 'Probiotic' goat cheese: Effect of ripening temperature and relative humidity on proteolysis and lipolysis. J. Dairy Sci. 78(Suppl. 1):99.

Mirade, P.-S., T. Rougier, A. Kondjoyan, J.-D. Daudin, D. Picque, and G. Corrieu. 2004. Caractérisation expérimentale de l'aéraulique d'un hâloir de fromagerie et des échanges air - produit. Lait 84:483-500.

Noomen, A. 1983. The role of the surface flora in the softening of cheeses with a low initial pH. Netherlands Milk & Dairy Journal 37: 229–232

Nunez, M., A. M. Guillen, M. A. Rodriguez-Marin, A. M. Marcilla, P. Gaya, and M. Medina. 1991. Accelerated ripening of ewes' milk Manchego cheese: The effect of neutral proteinases. J. Dairy Sci. 74:4108–4118.

Picque, D., M.-N. Leclercq-Perlat, and G. Corrieu. 2006. Effects of atmospheric composition on respiratory behavior, weight loss, and appearance of Camembert-type cheeses during Chamber Ripening. J. Dairy Sci. 89:3250-3259.

Ramet, J. P. 2000. Comparing ripening technology of the various types of cheese. Pages 418–446 in Cheesemaking: From science to quality assurance. A. Eck and J. C. Gillis, ed. Lavoisier Publishing, Paris, France.

Reps, A. 1993. Bacterial Surface-ripened cheeses. Pages 137–172 *in* Cheese: Chemistry, physics and microbiology. Vol. 1. General Aspects. 2nd ed. P. F. Fox, ed. Chapman & Hall, London, UK.

Schepers, A. W., J. Thibault, and C. Lacroix. 2000. Comparison of simple neural networks and nonlinear regression models for descriptive modelling of *Lactobacillus helveticus* growth in pH controlled batch cultures. Enzym. Microb. Technol. 26:431–445.

Simal, S., E.S. Sanchez, J. Bon, A. Femenia, and C. Rossello. 2001. Water and salt diffusion during cheese ripening: Effect of the external and internal resistances to mass transfer. J. Food Eng. 48:269-275.

Sihufe, G.A., S.E. Zorilla, D.J. Mercanti, M.C. Perotti, C.A. Zalazar, and A.C. Rubiolo. 2007. The influence of ripening temperature and sampling site on the lipolysis in Reggianito Argentino Cheese. Food Res. Int. 40: 1220-1226.

Sihufe, G.A., S.E. Zorilla, and A.C. Rubiolo. 2010a. The influence of ripening temperature and sampling site on the proteolysis in Reggianito Argentino Cheese. Food Sci. Tech. 43: 247-253.

Sihufe, G.A., S.E. Zorilla, M.C. Perotti, I.V. Wolf, C.A. Zalazar, N.G. Sabbag, and A.C. Rubiolo. 2010b. Acceleration of cheese ripening at elevated temperature. An estimation of the optimal ripening time of a traditional Argentinean hard cheese. Food Chem. 119: 101-107.

van den Tempel, T., and M. Jakobsen. 2000. The technological characteristics of *Debaryomyces hansenii* and *Yarrowia lipolytica* and their potential as starter cultures for production of Danablu. Int. Dairy J. 10:263–270.

Von Weissenfluh, A., and Z. Puhan. 1987. The effect of environmental conditions in the ripening room on the growth of *Penicillium camemberti* and the quality of Camembert cheese. Schweizer. Milchwirtsch. Forsch. 16:37-44.

Walker, G.M., and J.D. O'Neil. 1990. Morphological and metabolic changes in the yeast *Kluyveromyces marxianus* var. *Marxianus* NRRLY2415 during fermentation of lactose. J. Chem. Technol. Biotechnol. 49 (1): 75-89.

Table 1. Left: Presentation of the 2-factor, 3 level complete factorial experimental design (3^2). Right: Values of kinetic descriptors of *K marxianus* (KM), *G. candidum* (GC), *B. aurantiacum* (BA), and *P. camemberti* (PC) obtained for each temperature (θ) and relative humidity (RH) used during Camembert-type cheese ripening in chamber.

N°	Ripening conditions		*K. marxianus*			*G. candidum*		*B. aurantiacum*		*P. camemberti*	
			Growth phase	Death phase		Growth phase		1rst Growth phase		Sporulation phase	
	θ	RH	$\mu_{KM} \pm s$	$D_{KM} \pm s$	t_{DKM}	$\mu_{GC} \pm s$	$t_{\mu GC}$	$\mu_{BA} \pm s$	$t_{\mu BA}$	$\mu_{PC} \pm s$ (j^{-1})	$t_{\mu PC}$
1	8	88	0.24 ± 0.07	0.044 ± 0.006	33.3	0.72 ± 0.02	4.5	0.26 ± 0.06	34.5	0.04 ± 0.05	33.7
2	8	92	0.22 ± 0.05	0.043 ± 0.009	30.6	0.62 ± 0.05	4.4	0.24 ± 0.05	35.6	0.14 ± 0.03	19.3
3	8	98	0.22 ± 0.05	0.044 ± 0.006	30.3	0.72 ± 0.03	4.5	0.27 ± 0.03	34.1	0.13 ± 0.03	19.8
4	8	98	0.21 ± 0.07	0.038 ± 0.008	29.9	0.68 ± 0.07	4.5	0.23 ± 0.04	34.5	0.12 ± 0.05	19.6
3 & 4	**8**	**98**	**0.21 ± 0.06**	**0.041 ± 0.008**	**30.1**	**0.70 ± 0.05**	**4.5**	**0.25 ± 0.05**	**34.3**	**0.12 ± 0.5**	**19.7**
5	12	88	0.32 ± 0.04	0.049 ± 0.008	29.8	0.96 ± 0.03	3.8	0.5 ± 0.2	16.2	0.07 ± 0.02	20.5
6	12	92	0.50 ± 0.08	0.050 ± 0.009	30.3	1.04 ± 0.05	3.7	0.57 ± 0.02	15.0	0.60 ± 0.2	9.2
7	12	92	0.48 ± 0.07	0.049 ± 0.009	30.2	1.04 ± 0.03	3.9	0.57 ± 0.04	15.1	0.5 ± 0.1	9.5
8	12	92	0.50 ± 0.07	0.049 ± 0.008	29.5	0.91 ± 0.06	3.7	0.58 ± 0.09	15.0	0.5 ± 0.1	10.0
9	12	92	0.46 ± 0.07	0.052 ± 0.008	29.6	0.9 ± 0.1	3.8	0.60 ± 0.09	14.9	0.55 ± 0.07	9.9
10	12	92	0.56 ± 0.09	0.049 ± 0.008	29.4	1.0 ± 0.1	3.6	0.59 ± 0.09	14.9	0.5 ± 0.1	9.8
6 to 10	**12**	**92**	**0.50 ± 0.07**	**0.050 ± 0.007**	**29.8**	**1.0 ± 0.1**	**3.7**	**0.58 ± 0.09**	**15.0**	**0.53 ± 0.1**	**9.7**
11	12	98	0.58 ± 0.07	0.053 ± 0.008	30.7	0.96 ± 0.03	3.8	0.69 ± 0.09	13.0	0.7 ± 0.1	12.8
12	16	88	0.9 ± 0.1	0.070 ± 0.006	27.9	1.57 ± 0.07	4.2	0.69 ± 0.09	12.5	0.03 ± 0.04	10.7
13	16	88	0.94 ± 0.07	0.072 ± 0.006	27.8	1.6 ± 0.1	4.1	0.65 ± 0.08	12.7	0.03 ± 0.03	10.7
12 & 13	**16**	**88**	**0.92 ± 0.08**	**0.071 ± 0.006**	**27.9**	**1.59 ± 0.08**	**4.1**	**0.67 ± 0.09**	**12.6**	**0.03 ± 0.04**	**10.7**
14	16	92	0.98 ± 0.06	0.066 ± 0.009	27.9	1.8 ± 0.1	3.8	0.9 ± 0.1	11.8	0.32 ± 0.06	10.8
15	16	98	0.9 ± 0.1	0.069 ± 0.005	25.1	1.7 ± 0.1	4.2	1.4 ± 0.2	12.4	3.2 ± 0.6	8.7

μ_{KM}, D_{KM}, μ_{GC}, μ_{BA}, and μ_{PC}, maximal growth and death rate of *K. marxianus,* maximal growth rates of *G. candidum* and *B. aurantiacum*, and maximal sporulation rate of *P. camemberti* (in d^{-1}), respectively. These rates were determined from Weibull models. s, standard deviation.

t_{DKM}, $t_{\mu GC,}$ $t_{\mu BA}$, and $t_{\mu PC}$, times associated to D_{KM}, $\mu_{GC,}$ μ_{BA}, and μ_{PC} and given with a standard deviation of 0.1 d. Each Weibull model had a determination coefficient higher than 0.98 and is given with a *p-value* lower than 0.01. The degrees of freedom of μ_{KM}, D_{KM}, μ_{GC}, μ_{BA}, and μ_{PC} determinations are equal to 45, 17, 51, 36, and 51, respectively.

Table 2. Values of kinetic descriptors of pH of the rind, lactose and lactate concentrations in the rind and in the core of Camembert-type cheeses throughout the ripening obtained for each temperature (θ, °C) and relative humidity (RH, %) used during the Camembert-cheese ripening in room.

					Lactose				Lactate			
N° Run	Ripening conditions		pH of the rind		Consumption in the rind		Decreasing in the core		Consumption in the rind		Decreasing in the core	
	θ	RH	V_{pH}	t_{VpH}	C_{LO}	t_{CLO}	D_{LO}	t_{DLO}	C_{LA}	t_{CLA}	D_{LA}	t_{DLA}
1	8	88	1.2	9.5	1.59	5.5	0.83	5.6	1.97	7.5	1.01	10.9
2	8	92	0.9	9.4	2.00	5.0	1.07	5.5	2.70	7.5	1.12	10.9
3	*8*	*98*	1.1	9.4	1.80	4.8	0.77	5.5	3.45	7.4	0.88	10.8
4	*8*	*98*	1.1	9.4	1.80	4.9	0.77	5.5	3.42	7.5	0.85	10.7
3 & 4	**8**	**98**	**1.1**	**9.4**	**1.80**	**4.9**	**0.77**	**5.5**	**3.44**	**7.5**	**0.86**	**10.8**
5	12	88	1.4	6.6	2.09	2.6	1.71	3.9	3.24	6.9	2.73	7.4
6	*12*	*92*	1.3	6.6	3.00	2.6	1.53	3.9	3.11	6.8	1.88	7.6
7	*12*	*92*	1.3	6.6	3.01	2.6	1.58	3.9	3.11	6.8	1.79	7.6
8	*12*	*92*	1.3	6.7	3.09	2.6	1.57	3.9	3.08	6.8	1.83	7.7
9	*12*	*92*	1.3	6.6	3.10	2.7	1.54	3.9	3.12	6.8	1.78	7.7
10	*12*	*92*	1.3	6.6	3.05	2.6	1.55	4.0	3.14	6.8	1.82	7.6
6 to 10	**12**	**92**	**1.3**	**6.6**	**3.05**	**2.6**	**1.55**	**3.9**	**3.11**	**6.8**	**1.82**	**7.6**
11	12	98	1.3	6.6	2.27	2.4	1.35	3.8	4.45	6.4	1.36	7.5
12	*16*	*88*	4.8	5.1	5.40	1.1	1.83	3.3	5.86	5.0	1.98	5.4
13	*16*	*88*	4.9	5.1	5.50	1.1	1.71	3.3	5.80	5.0	2.04	5.4
12 & 13	**16**	**88**	**4.8**	**5.1**	**5.45**	**1.1**	**1.77**	**3.3**	**5.83**	**5.0**	**2.02**	**5.4**
14	16	92	2.0	5.2	8.10	1.2	1.82	3.3	3.97	5.0	1.84	5.5
15	16	98	2.1	5.1	7.12	1.3	1.93	3.4	2.24	5.00	1.65	5.5

V_{pH}, maximal increasing rate of pH in the rind (pH unit/d) determined from the slope of Weibull modeling and given with standard deviation of 0.1 unit. t_{VpH}, time associated to V_{pH} (d) and given with a standard deviation of 0.1 d.

C_{LO}, and D_{LO}, maximal consumption or decreasing rates of lactose (LO) in the rind and in the core respectively (g lactose/kg/d). C_{LA}, and D_{LA} maximal consumption or decreasing rates of lactate (LA) in the rind and in the core (g lactate/kg/d). These rates are determined from the slope of Weibull modeling and given with standard deviation of 0.05.

t_{CLO}, t_{DLO}, t_{CLA}, and t_{DLA}, (in d) times for which C_{LO}, D_{LO}, C_{LA}, and D_{LA} are obtained, respectively, and they are given with standard deviation of 0.1 d.

Whatever the Weibull model, the coefficient of determination is higher than 0.99 and the probability *p-value* is lower than 0.01. The degrees of freedom for Weibull models are equal to 203 for pH of the rind, 34 for lactose, and 26 for lactate.

Table 3. Values of descriptors of cheese water mass loss (L_{H2O}) between d14 and d0 and underrind thickness obtained for each temperature (θ, °C) and relative humidity (RH, %) used during the Camembert-cheese ripening in room.

N°	Ripening conditions		Underrind thickness			
Run	Θ (°C)	RH (%)	L_{H2O} ± s ($g.d^{-1}$)	$t_{UR,i}$ (d)	V_{TUR} ± s ($mm.d^{-1}$)	t_{VTUR}
1	8	88	1.2 ± 0.3	9.0	0.50 ± 0.05	30.4
2	8	92	1.0 ± 0.1	9.0	0.46 ± 0.07	29.8
3	8	98	0.7 ± 0.1	9.0	0.59 ± 0.06	29.4
4	8	98	0.6 ± 0.1	9.0	0.57 ± 0.06	29.4
3 & 4	**8**	**98**	**0.6 ± 0.1**	**9.0**	**0.58 ± 0.06**	**29.4**
5	12	88	2.1 ± 0.2	7.0	0.62 ± 0.04	29.3
6	12	92	1.7 ± 0.3	7.0	0.63 ± 0.05	29.5
7	12	92	1.5 ± 0.3	7.0	0.59 ± 0.04	29.6
8	12	92	1.7 ± 0.3	7,0	0.58 ± 0.07	29.5
9	12	92	1.6 ± 0.3	7.0	0.59 ± 0.05	29.5
10	12	92	1.6 ± 0.3	7.0	0.62 ± 0.05	29.5
6 to 10	**12**	**92**	**1.6 ± 0.3**	**7.0**	**0.60 ± 0.06**	**29.5**
11	12	98	1.2 ± 0.2	7.0	0.85 ± 0.04	29.2
12	16	88	2.5 ± 0.3	5.0	0.80 ± 0.07	20.8
13	16	88	2.6 ± 0.3	5.0	0.86 ± 0.06	20.7
12 & 13	**16**	**88**	**2.6 ± 0.3**	**5.0**	**0.83 ± 0.07**	**20.8**
14	16	92	2.2 ± 0.1	5.0	1.14 ± 0.07	20.5
15	16	98	1.8 ± 0.2	5.0	1.18 ± 0.06	20.5

L_{H2O}, water loss rate (g/d), calculated from 20 cheeses per run and between d0 and d14;
$t_{UR,i}$, time for which the underrind become observable (in d).
V_{TUR}, maximal increasing rate of underrind thickness (in mm/d) determined from the slope of Weibull model. s, standard deviation.
t_{VTUR}, time for which V_{TUR} is obtained and given with a standard deviation of 0.1 d.
The Weibull models are given with a *p-value* lower than 0.01 and with a determination coefficient higher than 0.98. The degree of freedom of these Weibull models is 203.

Table 4a. Best-fit equations for the effects of temperature and relative humidity on microbial dynamic descriptors during Camembert-type cheese ripening. All factors of the equations for temperature (θ, °C) and relative humidity (RH, %) are given:*, p-value value lesser than 0.01 or **, p- value lesser than 0.001. R, determinant coefficient. df, degree of freedom. SE, standard error. *p-value* relation.

Descriptors	Equation	R	df	SE	p-value relation
μ_{KM}	0.0037 (± 0.0002)** x θ²	0.97	13	0.063	$< 10^{-7}$
$t_{\mu KM}$	1.5 ± 0.1 d whatever θ and RH used				
D_{KM}	0.031 (± 0.002)** + 0.00014 (± 0.00001)** x θ²	0.97	13	0.003	$< 10^{-7}$
t_{DKM}	35 (± 1)** – 0.48 (± 0.07)** x θ	0.83	13	0.856	$1.47x10^{-4}$
μ_{GC}	0.29 (± 0.05)** + 0.0052 (± 0.0004)** x θ²	0.97	13	0.097	$< 10^{-7}$
$t_{\mu GC}$	NS				
μ_{BA}	6.1 (± 0.9)** – 0.71 (± 0.08)** x θ – 0.07 (± 0.01)** x RH+ 0.0086 (± 0.0008)** x θ x RH	0.97	11	0.041	$< 10^{-7}$
$t_{\mu BA}$	126 (± 3)** – 15.8 (± 0.6)** x θ + 0.54 (± 0.02)** x θ²	0.99	11	0.641	$< 10^{-7}$
μ_{PC}	27 (±7)* – 3.2 (± 0.6)* x θ – 0.31 (± 0.08)* x RH + 0.036 (± 0.007)** x θ x RH	0.92	12	0.319	$3.3x10^{-5}$
$t_{\mu PC}$	36 (± 5)** – 0.019 (± 0.005)** x θ x RH	0.95	11	0.867	$< 10^{-7}$
t_{PCi}	36 (±3)**–3.0 (±0.3)**xθ–0.09 (±0.02)*xRH+0.09 (±0.01)** x θ²	0.99	11	0.394	$< 10^{-7}$

μ, maximum growth rate (per d) and t_{μ}, day for which μ is obtained (d).
D_{KM}, maximum death rate of KM (per d) and t_{DKM}, day for which D_{KM} is obtained (d)
$t_{PC\,i}$, day on which the first mycelia of PC were observed (d).
NS, none significant.
Indexes: KM for *Kluyveromyces marxianus*, GC for *Geotrichum candidum*, BA for *Brevibacterium aurantiacum*, and PC for *Penicillium camemberti*.

Table 4b. Best-fit equations for the effects of temperature and relative humidity on physicochemical and biological kinetic descriptors during Camembert-type cheese ripening. All factors of the equations for temperature (θ, °C) and relative humidity (RH, %) are given:*, p-value value lesser than 0.01 or **, p- value lesser than 0.001. R, determinant coefficient. df, degree of freedom. SE, standard error. p-value relation.

Descriptors	Equation	R	df	SE	p relation
V_{pH}	6 (± 1)* + 0.050 (± 0.006)** x θ² – 0.011 (± 0.003)* x θ x RH	0.91	12	0.339	9.78×10^{-6}
$t_{VpH,}$	18.9 (± 0.3)** – 1.51 (± 0.05)** x θ + 0.040 (± 0.002)** x θ²	0.99	12	0.064	$< 10^{-7}$
C_{LO}	0.025 (± 0.003)** x θ²	0.90	13	0.875	$< 10^{-7}$
t_{CLO}	8.6 (± 0.4)** – 0.48 (± 0.03)** x θ	0.98	13	0.324	$< 10^{-7}$
D_{LO}	0.12 (± 0.01)** x θ	0.92	13	0.155	1.52×10^{-6}
t_{DLO}	11.6 (± 0.3)** – 1.00 (± 0.04)** x θ + 0.030 (± 0.002)** x θ²	0.99	12	0.069	$< 10^{-7}$
C_{LA}	– 32 (± 6)** + 6.0 (± 0.9)** x θ + 0.0038 (± 0.0007)** x RH² – 0.06 (± 0.01)** x θ x RH	0.92	11	0.504	1.12×10^{-4}
t_{CLA}	5.8 (± 0.5)** + 0.47 (± 0.09)** x θ – 0.032 (± 0.004)** x θ²	0.99	12	0.119	$< 10^{-7}$
D_{LA}	2.9 (± 0.5)** – 0.016 (± 0.004)* x θ² – 0.00054(± 0.00008)** x RH² + 0.005 (± 0.001)** x θ x RH	0.94	11	0.144	$5.0\ 10^{-6}$
t_{DLA}	20.7 (± 0.4)** – 1.53 (± 0.07)** x θ + 0.036 (± 0.003)** x θ²	0.99	12	0.089	$< 10^{-7}$
L_{H2O}	8.2 (± 0.6)** – 0.091 (± 0.007)** x RH + 0.0016 (± 0.0001)** x θ x RH	0.99	12	0.098	$< 10^{-7}$
V_{TUR}	1.2 (± 0.3)* – 0.38 (± 0.06)**x θ + 0.009 (± 0.002)* x θ² + 0.0025 (± 0.0004)** x θ x RH	0.99	11	0.187	$< 10^{-7}$
t_{VTUR}	44 (± 8)** – 0.25 (± 0.06)* x θ² – 0.005 (± 0.001)* x RH² + 0.06 (± 0.02)* x θ x RH	0.85	11	2.335	2.1×10^{-4}

V, maximal increasing rate (unit/d) and t_V, time for which the maximal increasing rate V is obtained (d).
C_{LO}, lactose maximal consumption rate in the rind (g/kg/d) and t_{CLO}, time for which C_{LO} is obtained (d).
D_{LO}, lactose maximal decreasing rate in the core (g/kg/d) and t_{DLO}, time for which D_{LO} is obtained (d).
C_{LA}, lactate maximal consumption rate in the rind (g/kg/d) and t_{CLA}, time for which C_{LA} is obtained (d).
D_{LA}, lactate maximal decreasing rate in the core (g/kg/d) and t_{DLA}, time for which D_{LA} is obtained (d).
Indexes: pH for pH and T_{UR} for cheese underrind thickness.

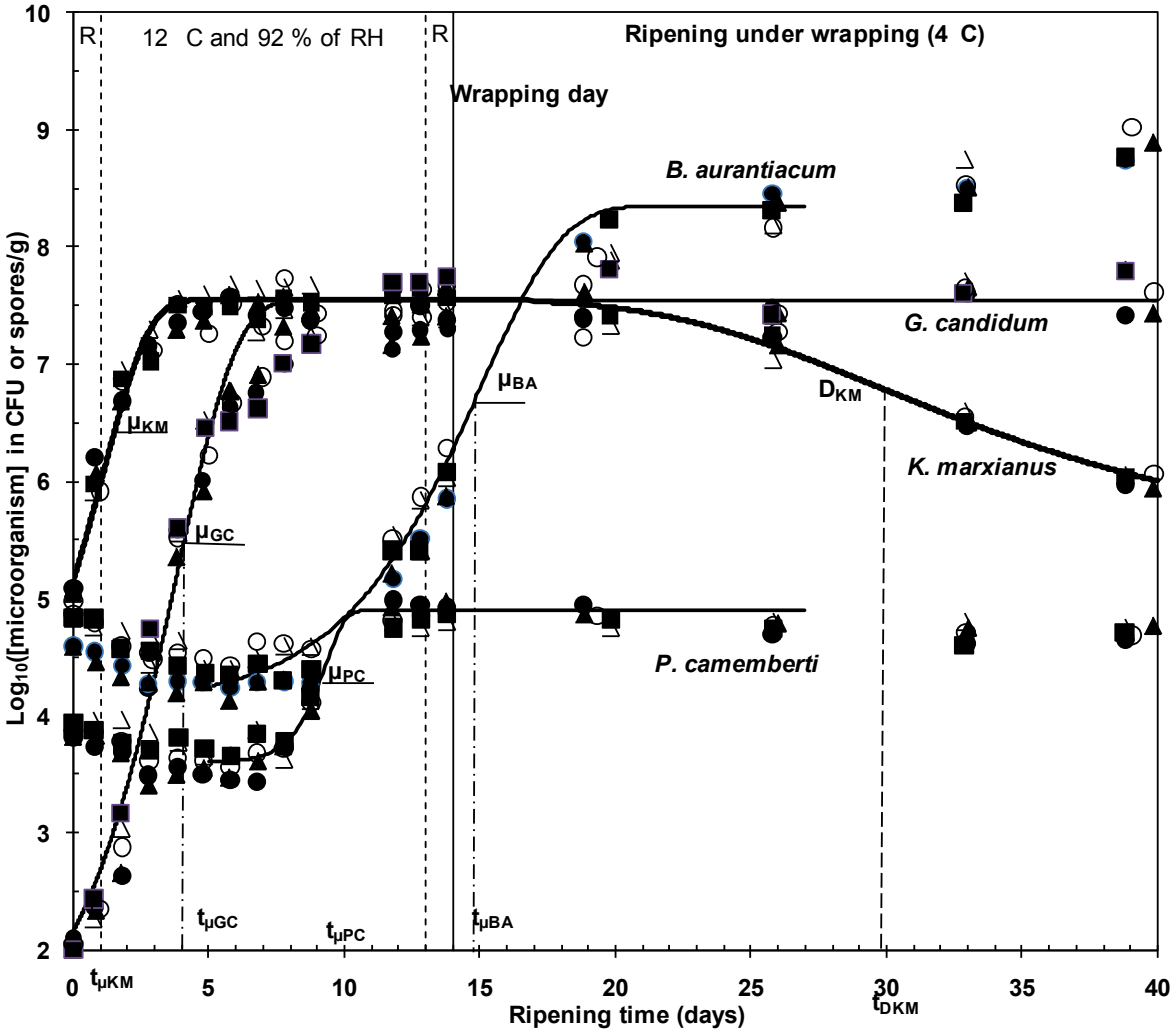

Figure 1: Evolution of the decimal logarithm of *K. marxianus*, *G. candidum*, and *B. aurantiacum* viable cell concentrations as well as the one of *P. camemberti* spore concentrations, in relation to ripening time for runs 6 (●), 7 (▲), 8 (Δ), 9 (■), and 10 (○) carried out under standard ripening conditions (12°C; 92 % RH). R, surface drying phases carried out under 12°C and 85 % of RH. μ_{KM}, μ_{GC}, μ_{BA}, and μ_{PC}, maximal growth rate of KM, GC, BA, and PC (d^{-1}), respectively, were obtained by calculating the slope of the first Weibull model (—). $t_{\mu KM}$, $t_{\mu GC}$, $t_{\mu BA}$, and $t_{\mu PC}$, the days for which μ_{KM}, μ_{GC}, μ_{BA}, and μ_{PC} were obtained. D_{KM}, maximal death rate of *K. marxianus* (d^{-1}) is obtained by calculating the slope of death phase from Weibull model (—). t_{DKM}, the day for which D_{KM} is obtained. KM, *Kluyveromyces marxianus*; GC, *Geotrichum candidum*; BA, *Brevibacterium aurantiacum*; PC, *Penicillium camemberti*.

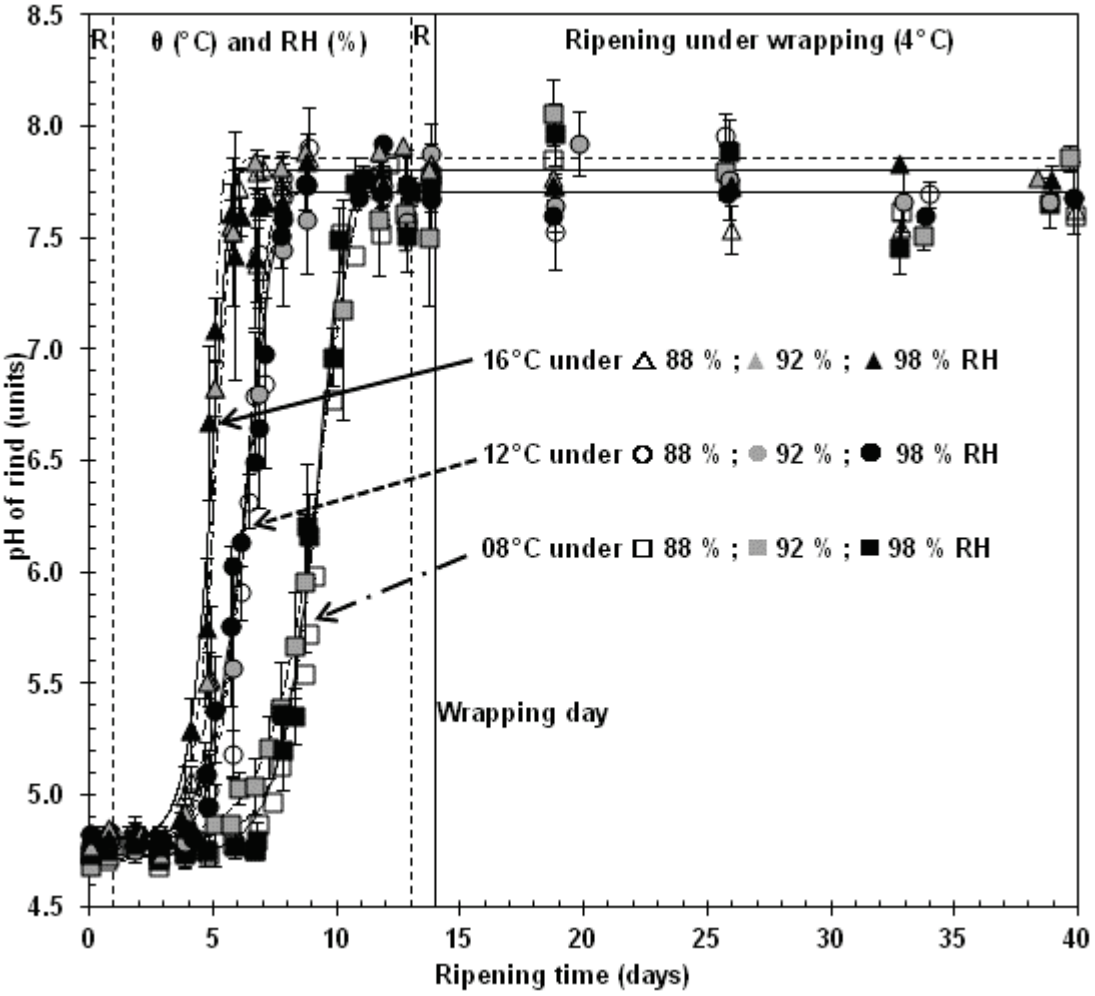

Figure 2: Increase of the pH in the rind of a Camembert-type cheese, in relation to ripening time for the nine ripening conditions of temperature and RH and determination of their kinetic descriptors from Weibull model: — - —. – – –, and —— for the run carried out at 8, 12 and 16°C, respectively. R, surface drying phases carried out under 12°C and 85% of RH. The central point conditions are given as the average of the five runs carried out under 12°C and 92 % of RH. For each experimental point, upright line represents standard deviation.

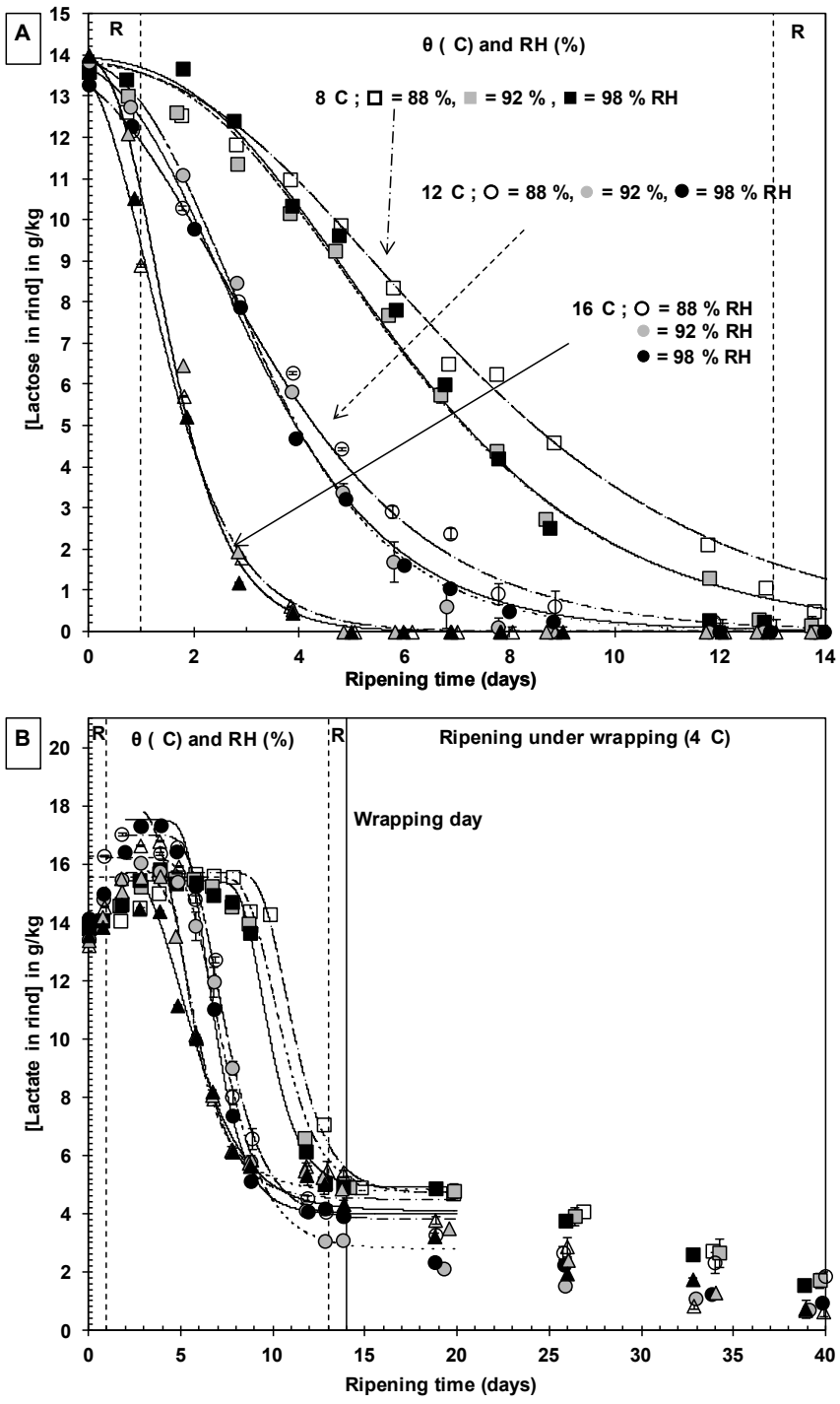

Figure 3: Evolution of lactose (A) and lactate (B) concentrations of the rind, in relation to ripening time for the nine ripening conditions of temperature and RH and determination of their kinetic descriptors from Weibull model: **— -, – –**, and **—** for the run carried out at 8, 12 and 16°C, respectively. △, ▲, and ▲, runs carried out at 16°C under 88, 92 and 98 % of RH, respectively. ○, ●, and ●, runs carried out at 12°C under 88, 92 and 98 % of RH, respectively. □, ■, and ■, runs carried out at 8°C under 88, 92 and 98 % of RH, respectively. R, surface drying phases carried out under 12°C and 85% of RH. The central point conditions are given as the average of the five runs carried out under 12°C and 92 % of RH.

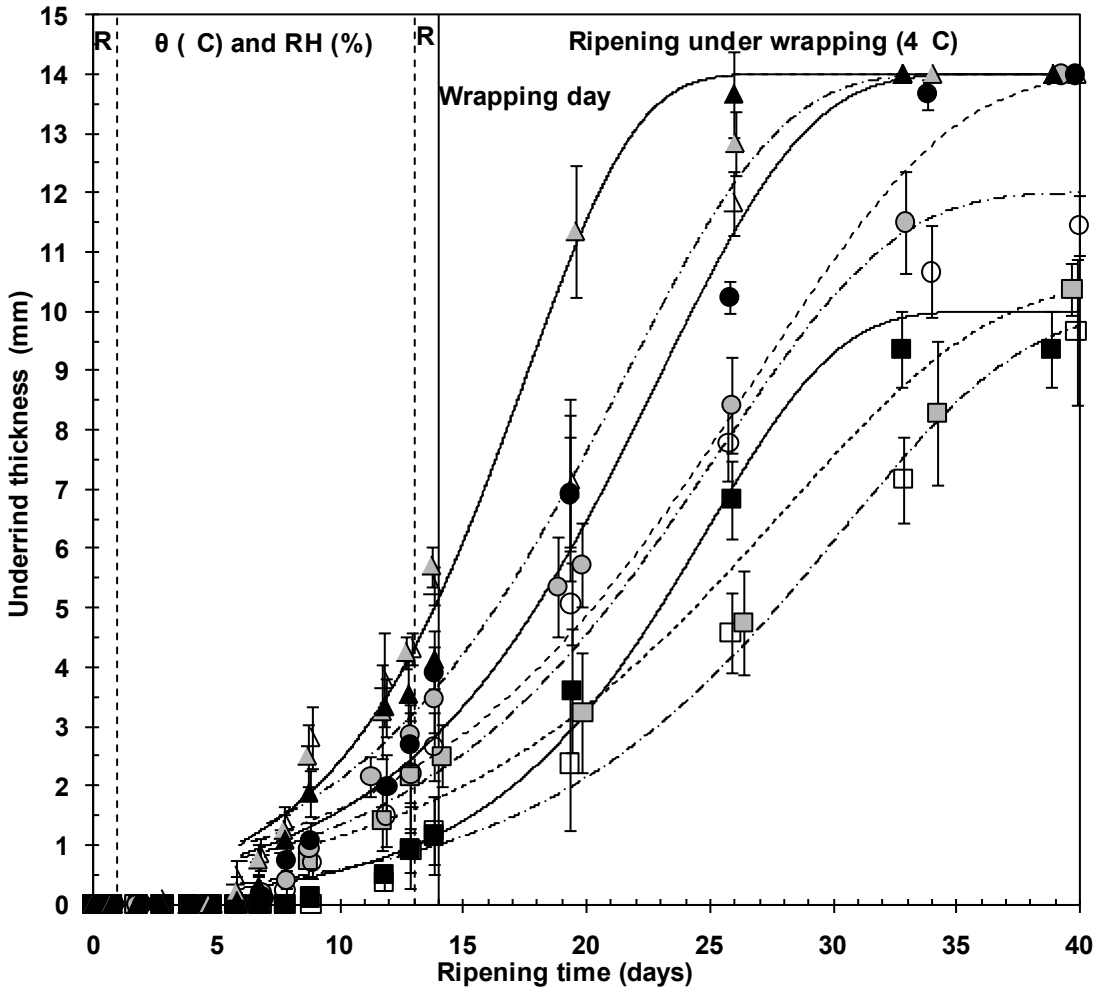

Figure 4: Evolution of underrind thickness (mm), in relation to ripening time for the nine ripening conditions of temperature and RH and determination of their kinetic descriptors from Weibull model: — - —. – – –, and —— for the run carried out at 8, 12 and 16°C, respectively. △, ▲, and ▲, runs carried out at 16°C under 88, 92 and 98 % of RH, respectively. ○, ●, and ●, runs carried out at 12°C under 88, 92 and 98 % of RH, respectively. □, ■, and ■, runs carried out at 8°C under 88, 92 and 98 % of RH, respectively. R, surface drying phases carried out under 12°C and 85% of RH. The central point conditions are given as the average of the five runs carried out under 12°C and 92 % of RH.

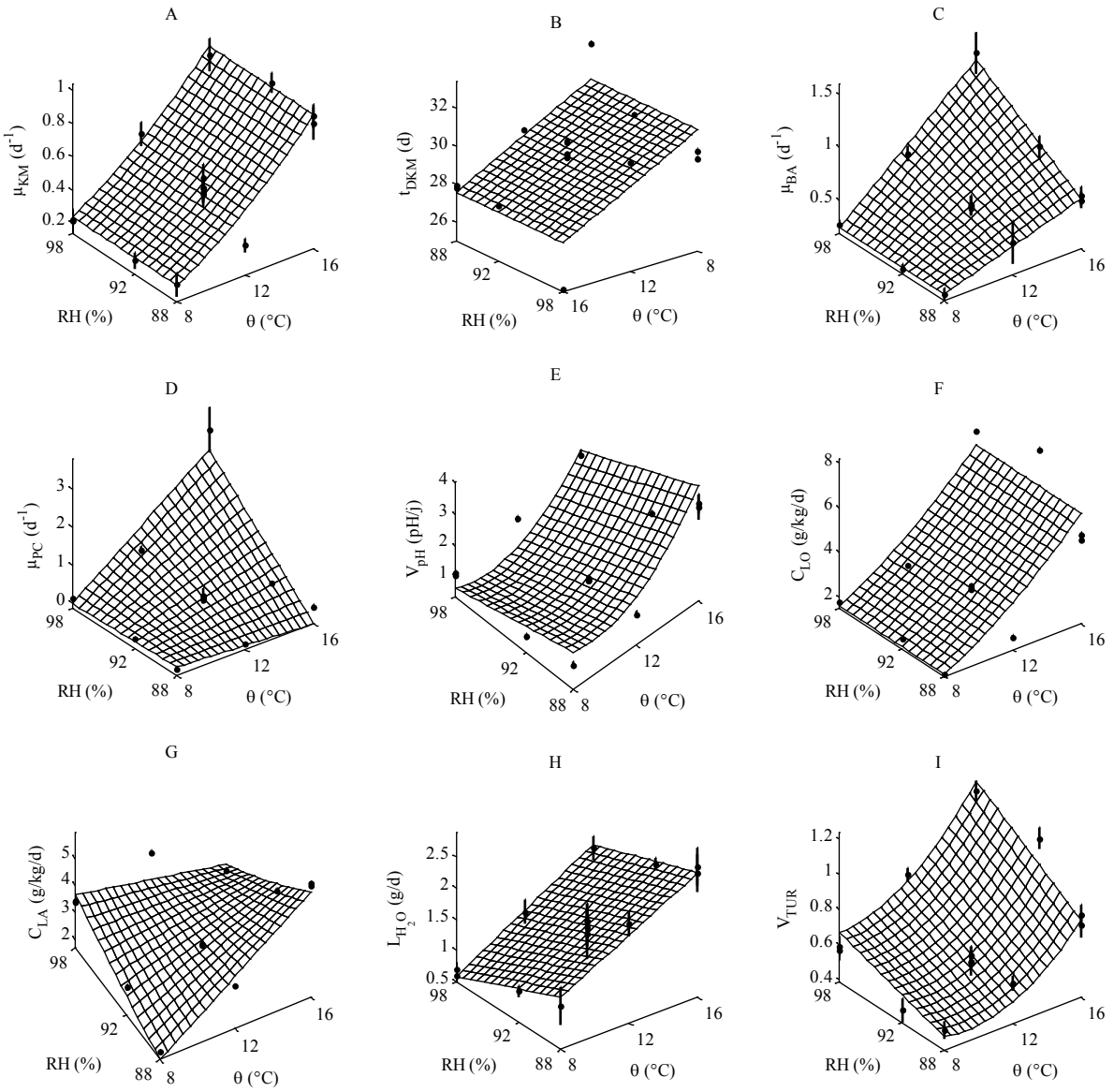

Figure 5: Some main estimated response surface plots of dynamic descriptors as a function of temperature (θ, °C) and RH (%) and representative to the different shapes of plots Observed.
A- μ_{KM}, maximal growth rate of *K. marxianus* (d^{-1}); B- t_{DKM}, time associated to D_{KM} (d); C- μ_{BA}, maximal growth rate of *B. aurantiacum* (d^{-1}); D- μ_{PC}, maximal sporulation rate of PC (d^{-1}); E- V_{pH}, increasing rate of pH in the rind; F- C_{LO}, maximal lactose consumption rate in the rind (g/kg/d); G- C_{LA}, maximal lactate consumption rate in the rind (g/kg/d); H- L_{H2O}, cheese mass loss rate ($g.d^{-1}$); I- V_{UR}, maximal increasing rate of underrind thickness (mm per d).