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► **To cite this version:**

Marie Noelle Leclercq-Perlat, Mariette Sicard, Nathalie Perrot, Ioan-Cristian Trelea, Daniel Picque, et al.. Temperature and relative humidity influence the ripening descriptors of Camembert-type cheeses throughout ripening. *Journal of Dairy Science*, 2015, 98 (2), pp.1325 - 1335. 10.3168/jds.2014-8916 . hal-01195508

HAL Id: hal-01195508

<https://hal.science/hal-01195508>

Submitted on 11 Jul 2017

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**Temperature and relative humidity influence the sensorial properties of Camembert-type
cheeses throughout the ripening.**

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Running title: Θ and RH effects on ripening descriptors

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1 **ABSTRACT:** Sensorial qualities are main factors that determine consumers' preference. Six
2 descriptors were defined to represent the sensorial changes in Camembert cheeses: *Penicillium*
3 *camemberti* appearance, rind odor and color, underrind thickness, underrind consistency, and
4 core hardness. To evaluate the effects of the main process parameters on these descriptors,
5 Camembert cheeses were ripening under different temperature (8, 12, and 16 °C) and RH (88,
6 92, and 98 %).

7 This study underlined that the sensorial descriptors were highly dependent on temperature and
8 RH used throughout ripening in chamber. All sensorial descriptor changes can be explained by
9 microorganism growth and activities, pH, carbon substrate diffusion and assimilation, cheese
10 moisture. On d40, at 8°C under RH 88%, all sensorial descriptors scored the worst: the cheese
11 was too dry, its odor and its color were similar to the ones of the cheeses without ripening, the
12 underrind was the driest, and the core was the hardest. At 16 °C under RH 98 %, the odor was
13 strong ammoniac and the color was dark brown, the underrind represented all the thickness of
14 the cheeses but it was completely runny, noting an over-ripened cheese. From the response
15 surface analyses, it can show that the best ripening conditions to achieve an optimum between
16 sensorial qualities and marketability were 13 ± 1 °C and $94 \pm 1\%$ RH.

17

18 **Keywords:** descriptors of cheese ripening, temperature, relative humidity.

19

20 **Abbreviation keys:** θ , ripening temperature (°C). d14 for 14 days of ripening and d40 for 40
21 days of ripening. di, ith day of ripening. RH, relative humidity (%). T_{UR} , underrind thickness
22 (mm). N_{PC} , *Penicillium camemberti* appearance notation. C_{UR} , underrind consistency notation.
23 DM, dry matter (g of dry cheese per 100 g of fresh cheese)

24

1 **Interpretative summary:**

2 We evaluated the effects of temperature and relative humidity (RH) on the sensorial properties
3 of Camembert ripened under different temperatures and RH.

4 On d14 and d40, all sensorial descriptors were highly dependent on temperature and RH. Their
5 changes can be explained by microorganism growth, and by the evolutions of pH, carbon
6 substrates and cheese moisture throughout the ripening. At 8°C under RH 88 %, on d40, all
7 sensorial descriptors scored the worst while at 16°C under RH 98 %, the cheeses were over-
8 ripened. Statistical analysis showed that the best ripening conditions to achieve an optimum
9 between sensorial qualities and consumer's acceptability were close to 13°C and 94% RH.

10

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INTRODUCTION

12 Among environmental parameters affecting the Camembert-type cheese ripening, temperature,
13 relative humidity (RH), and gaseous atmosphere composition of ripening chambers have the most
14 effects on sensorial properties (Choisy et al., 2000; Ramet, 2000; Weissenfluh and Puhan, 1987).

15 These authors have highlighted the predominant role of temperature and have reported that low
16 increase of temperature can accelerate cheese ripening but it can also produce off-flavor
17 components or inconsumable products. This is also in agreement with the studies about Cheddar
18 cheeses by Hannon et al. (2005) and Reggianito Argentino cheeses by Sihufe et al. (2010). To our
19 knowledge, any systematic works about ripening conditions of Camembert-type cheeses have
20 been realized on the influence of ripening temperature and RH on the cheese sensorial
21 qualities. Consequently, it is very difficult to have a trend about the influence of temperature and
22 RH on the sensorial qualities of Camembert-type cheeses throughout their ripening. However, it
23 is admitted that sensorial properties like surface coating appearance, surface color or odor, are
24 basic because they determine the choice and preferences of consumers (Dufossé et al., 2005).

25 According to Trystram (1996), managing sensory properties at the manufacturing stage with the
26 aim of controlling them is particularly difficult due to a few numbers of sensors available.
27 Knowledges about the links between microbiological and physicochemical phenomena and the

1 changes in cheese sensorial properties are still lacking. According to Perrot et al. (2004) and
2 Sicard et al. (2011), operators often play an important role and, in practice, interact with
3 automatic systems to assess the sensorial properties of the soft cheeses and to adjust the
4 process. According to Lemoine (2001), operator assessment and reasoning still play a major
5 role in the cheese ripening process, especially concerning sensorial property control. In
6 factories, the cheese-makers may use to instrumental measurements and empirical sensorial
7 perceptions at the same time (Perrot et al., 2004; Picque et al., 2006). Moreover, Bonaïti et al.
8 (2004) have shown that temperature and RH had also an influence on creamy underrind
9 thickness and on its consistency. Indeed, at 16°C under 100 % RH the underrind of a smear
10 cheese became completely runny after 20 days of ripening. Recently, Sicard et al. (2011) have
11 shown that it is possible to study Camembert ripening process by macroscopic evolutions of
12 cheeses evaluated from an expert's point of view. These authors established a correlation of
13 76 % between the microbiological, physicochemical, and biochemical data and the sensory
14 phases measured according to expert knowledge.

15 The aim of this study was to evaluate the effects of temperature and relative humidity (RH) on
16 developments of sensorial properties in the Camembert-type cheeses throughout their ripening
17 (from d0 to d40) for the same set of cheese-making runs. These descriptors were studied in
18 association to the microbial and physicochemical evolutions occurring throughout ripening.

19

20

MATERIAL AND METHODS

Camembert cheese-making and ripening

22 The microorganisms used (*Kluyveromyces marxianus*, *Geotrichum candidum*, *Penicillium*
23 *camemberti*, and *Brevibacterium aurantiacum*) and the cheese-making have been described in
24 detail previously by Leclercq-Perlat et al. (2012). Surface mould soft cheeses (100 cheeses per
25 cheese-making run, each weighing 300 ± 20 g) were manufactured in a sterile environment. The
26 cheeses were aseptically transferred to a previously sterilized ripening chamber. After 24 h at
27 12 °C and 85 ± 1% RH, they were kept at 8, 12 or 16 °C and 88, 92 or 98 % RH, with the same

1 periodically renewed atmosphere. To that end, on d0, the ripening chamber, which contained air
 2 (CO₂ = 0%), was sealed. Given that during respiration the CO₂ concentration increased and
 3 when the CO₂ concentration reached 0.5%), it was automatically decreased to 0.1% by injecting
 4 humid sterile air (Picque et al., 2006). The cheeses were turned on the fifth day. On d13, they
 5 were kept for 24 h under 12 °C and 85 % RH. On d14, the cheeses were wrapped into a
 6 reference wrap (CdL, Amcor Flexibles, Barbézieux, France) and ripened at 4°C until d40.

7 **Sensorial analyses performed on the cheeses**

8 The sensorial analyses were performed on each cheese sample under the same light and the
 9 same temperature (14 ± 1 °C). For that, each cheese was put into a glass box with a cover and the
 10 sensory evaluation by the assessors was made immediately.

11 According to Sicard et al. (2011) methodology, five expert assessors for analyzing each cheese
 12 were sufficient. Each expert assessed each cheese, taking into account five descriptors
 13 generally used by the cheese-makers, and scored on a scale from 1 to 5 (Table 1). Six sessions
 14 were devoted to training and evaluation before the validation of panel's performance (Sicard et
 15 al. 2011). To determine the progress of Camembert-type cheese ripening, the appearance of
 16 the cheese (*P. camemberti* coating density and its surface covering) was assessed as
 17 previously described by Bonaïti et al. (2004) and Sicard et al. (2011). *P. camemberti*
 18 appearance was assessed in terms of mycelium density and uniformity, varying from 1 (less
 19 than 10 % of cheese surface were covered by *P. camemberti* mycelium) to 5 (more than 90 %
 20 of surface were covered by *P. camemberti*). In addition, for each cheese sampling, the overall
 21 odor, rind color under *P. camemberti* coating, underrind consistency (noted C_{UR}), and core
 22 hardness (noted H_{core}) on d14 and on d40 were considered. The higher differences of each
 23 sensorial descriptor between two panelists were lower than 0.2. The overall cheese odor varied
 24 from fresh cheese (score 1) to ammoniac (score 5). The typical odor of Camembert
 25 corresponded to the score of 3. The cheese color under the *P. camemberti* coating varied from
 26 white (score 1) to dark brown (score 5). The typical color (cream) of a Camembert corresponded
 27 to a score of 3. The creamy underrind thickness is a main factor allowing Camembert ripening
 28 according to Bonaïti et al. (2004) and Leclercq-Perlat et al (2004a, 2006, 2012), but this

1 descriptor does not allow cheese-makers to define completely the quality of ripening due to the
 2 destruction of cheeses. Indeed, in practice, cheese-makers use another descriptor: the
 3 underrind consistency (C_{UR}) which characterizes the texture of ripened part of cheese as
 4 highlighted by Sicard et al. (2011). The creamy underrind thickness (T_{UR} , mm) was measured
 5 with a decimeter with an accuracy of 0.2 mm. The underrind consistency (Picque et al., 2011)
 6 varied from dried (score 1) to very runny (score 5). The core hardness, characterizing the
 7 texture of the core part, was estimated by the ease with which the cheese was ground with a
 8 mortar and pestle. This hardness varied from a very soft (score 1) to very hard (score 5).
 9 To simplify the analysis, two characteristic days of ripening were only considered: the wrapping
 10 day (on d14) from which the cheeses were marketed and the end of ripening (on d40)
 11 corresponded to the end of shelf-life of the cheeses.

12 ***Experimental design***

13 The effects of temperature and RH on the cheese sensory descriptors were examined using a
 14 two-factor, three-level complete factorial experimental design (3^2) as described by Leclercq-
 15 Perlat et al. (2012). The 9 combinations of temperature and RH are shown in Tables 2 and 3.
 16 The levels of each factor used were 8, 12, and 16 °C for temperature and 88, 92, and 98 % for
 17 RH. These levels were chosen according to the range of temperature and RH generally used
 18 for Camembert cheese ripening. The 9 combinations of temperature and RH are shown in
 19 Tables 2 and 3 (left part). Because the experiment took place over a lengthy period, the runs
 20 corresponding to the central point of the experimental design (12°C, 92 % RH) were quintupled
 21 (run 6 to 10). The runs under 8°C and 98 % RH (runs 3 and 4) and those under 16°C and 88 %
 22 RH (runs 12 and 13) were simply duplicated. The remaining runs were done just once.

23 ***Statistical analyses***

24 For the runs made under the same conditions (runs 3 and 4; runs 6 to 10, and runs 12 and 13),
 25 sensorial descriptors were compared by a 2 factor (time and trial) analysis of variance
 26 (ANOVA). The hypotheses examined were the equality of the trials and the absence of any
 27 interactions. This test was significant for the risk α ($\alpha = 1 - p(F_{obs} < F_{crit}) \leq 0.01$). To control the
 28 equality of trials, the test power ($1-\beta$), representing the risk of a false interpretation, was

1 determined according to Mann and Whitney's method (Cohen, 1992). This power risk must be
2 higher than 0.9 to consider that the interpretation of this test is accurate.

3 The general linear model within Statistica Software was performed to calculate quadratic models
4 and to determine the influence of the two factors (θ , RH) on each sensorial descriptor. The non-
5 significant terms were omitted one by one, using the procedure Stepwise Backwards (Statistica),
6 consequently only the terms significant at 99 % of confidence level (p -value < 0.01) were
7 considered. The three-dimensional response surfaces of each descriptor in relation to θ and RH
8 factors were plotted to illustrate the main and interactive effects. These effects can be linear,
9 quadratic and/or interactive.

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11

RESULTS AND DISCUSSION

12 Whatever the run, on d0, *P. camemberti* appearance (absence of visual mycelium), overall odor
13 (fresh cheese) and rind color (white) scored 1. Core hardness scored 3 (medium). For these
14 descriptors, the standard deviations were less than 0.2. The underrind was non-existent.

15 **Description of ripening under 12°C and 92 % RH**

16 ***Statistical run reproducibility.***

17 The reproducibility of cheese ripening descriptors was studied for runs 6 to 10 carried out under
18 RH 92% and 12°C (Table 2). The hypotheses of equality of the means were greatly satisfactory
19 ($1 - \alpha = 0.99$ and the test power $(1 - \beta) = 0.94$). Although the run period had lasted for three
20 years, the runs carried out in the same conditions were statistically identical.

21 The time (t_{PCm} in d), for which the first mycelia were observed in the rind or edge of cheeses,
22 defined the growth of *P. camemberti* mycelium and the appearance of *P. camemberti* (noted
23 N_{PC}) covering was defined as mycelium density and uniformity. Whatever the central point runs
24 (6 to 10), the *P. camemberti* mycelia became visible on d5. From d14 to d40, all the cheeses
25 were covered with a dense and white thick mycelium, giving the highest notation (Table 2).

26 On d14, the overall odor and the rind color were, respectively, "camembert" and cream while on
27 d40 they scored, respectively, "camembert-ammoniac" and dark cream (Table 3). From d14 to

1 d40, the core hardness was medium and the underrind consistency was qualified to soft. The
 2 underrind thickness (T_{UR}) was close to 3.3 mm on d14 and formed all the cheese thickness on
 3 d40 (Table 3). For the 5 runs carried out under the standard ripening, all the descriptors were
 4 statistically identical with 99 % confidence levels.

5 **Influence of temperature and RH on ripening descriptors**

6 The scores of ripening descriptors are reported in Table 2 for *P. camemberti* appearance and
 7 Table 3 for the other descriptors.

8 ***Appearance of Penicillium camemberti***

9 From the descriptors previously defined (t_{PCm} and N_{PC}), t_{PCm} was independent to RH and only
 10 related to temperature (Table 2). On d14, the appearance of *P. camemberti* mycelium (N_{PC14})
 11 was a function of temperature (linear and quadratic terms), RH (linear term) and temperature
 12 and RH (interactive term), and on d40 (N_{PC40}) it was related to temperature and RH (linear and
 13 quadratic terms) (Table 4). The three-dimensional response surfaces of appearance of
 14 *P. camemberti* (N_{PC}) on d14 and d40 are given in Figures 1A and 1B. The N_{PC14} surface
 15 response (Fig. 1A) shows a main effect of temperature. Indeed, from 8°C up to about 12°C,
 16 N_{PC14} increased with temperature and decreased afterwards (quadratic effect). The relative
 17 humidity effect was relatively smaller and depended on the temperature, pointing out an
 18 interactive effect: N_{PC14} increased with RH at low temperature and decreased at high
 19 temperature. The N_{PC40} surface response (Fig. 1B) shows the importance of the quadratic
 20 effects of temperature and RH: N_{PC40} reached its maximum value for a temperature near 12°C
 21 and a RH near 93%.

22 Ripening in chamber at 16°C under 98 % of RH promoted *P. camemberti* sporulation as
 23 previously shown by Leclercq-Perlat et al. (2012). Lenoir et al. (1985) have pointed out that the
 24 optimal mycelium growth is obtained under 90 – 94 % RH for a temperature between 10 and
 25 14°C. Moreover, *P. camemberti* mycelium is not fond of higher RH (Ramet, 2000). Indeed,
 26 white and down coating of the surface of Camembert disappears when the RH of ripening room
 27 is higher than 95 % for a temperature of 12-14°C even if these conditions last only 2 or 3 days.
 28 However, neither *P. camemberti* visual notation nor *P. camemberti* spore counts give a real idea

1 for mycelium growth and development, the first due to the limitation of human eyes (saturation
 2 when *P. camemberti* coating was complete) and the second due to the destruction of mycelium.
 3 Indeed, *P. camemberti* sporulation was accelerated throughout the ripening by the temperature
 4 (Leclercq-Perlat et al., 2012, 2013). Higher the temperature was, earlier its growth occurred and
 5 earlier its concentration became maximal. Moreover, at 16 °C and 98 % RH, *P. camemberti*
 6 mycelium appeared damaged due to disruption of the equilibrium between this mold and
 7 *G. candidum* in favor to this yeast while *P. camemberti* spore concentration was higher.

8 ***Influence of temperature and RH on ripening descriptors***

9 **Rind odor.** On d14, the rind odor was musty at 8°C, Camembert at 12°C and Camembert–
 10 ammonia at 16°C (Table 3). This descriptor was related to temperature (individual and quadratic
 11 terms), RH (individual term), and temperature and RH (interactive term) (Table 4). In addition,
 12 the odor uniformly increased with temperature between 8 and 16°C while the RH effect was
 13 almost negligible at 8°C and positive at 16°C (Fig. 1C). On d40, the rind odor remained musty at
 14 8°C under 88 and 92 % but became “camembert” under 98%. It was “camembert–ammoniac” at
 15 12°C whatever the RH and at 16°C and 88 %. It was “ammoniac” at 16°C under 92 and 98 %.
 16 This descriptor was a function of temperature (individual and quadratic terms), and RH (linear
 17 term) (Table 4). The odor increased with temperature and RH had a constant positive linear
 18 effect whatever the temperature (Fig. 1D).

19 An increasing of the temperature induced a better growth of the ripening microorganisms,
 20 mainly *G. candidum* and *P. camemberti* mycelium and when the temperature was higher than
 21 10°C *B. aurantiacum* maximal exponential growth rate took place on d15 (12°C and 92 % RH,
 22 just after the wrapping) or on d12.6 at 16°C and 88 % RH (Leclercq-Perlat et al., 2012). A better
 23 expression of these enzymatic activities can also be induced by RH (Choisy et al., 2000).
 24 Moreover, an increasing of temperature and RH can allow an increasing of aroma compound
 25 production and an increasing in the variety of flavor compounds (Le Quéré, 2011; McSweeney
 26 and Sousa, 2000; Sablé and Cottreau, 1999).

27 **Rind color.** On d14, the rind color increased with the temperature from white, corresponding to
 28 a cheese with a very little ripening (8°C under 88 and 92 % or 12°C under 88 %) to brown (16°C

1 under 92 and 98 %). On d40, this color rose from white (8°C under 88 or 92 %) to dark brown,
 2 corresponding to an unacceptable color for a Camembert cheese (16°C under 92 or 98 %). On
 3 d14, rind color was a function of temperature and RH (individual and quadratic terms) while on
 4 d40, it was only related to temperature and RH (individual terms) (Table 4). The fig. 1E shows
 5 the importance of quadratic terms on the color on d14 while the fig. 1F shows that on 40 the
 6 color uniformly increased with temperature and RH (linear effects).

7 The rind color depends directly on production of pigments by *Brevibacterium species* (Dufossé
 8 et al., 2001). At 8°C whatever the RH or at 12°C and 88 % RH, a delay to the yeast installation
 9 and growths (Leclercq-Perlat et al., 2012) and *P. camemberti* mycelium was observed as shown
 10 by t_{PCm} (Table 2). These main consequences were a delay to rind deacidification and to
 11 *B. aurantiacum* growth (Leclercq-Perlat et al., 2012). Indeed, on d40, these authors showed that
 12 1) at 8°C under 92 or 98 %, the bacterium concentrations were the weakest (between 6×10^6 and
 13 2×10^8 CFU per g). On the contrary, at 16°C under 92 or 98 %, the rind deacidification became
 14 earlier (from d2.5) and *B. aurantiacum* grew better, reaching 8×10^9 CFU/g; and 2) at 16°C under
 15 98 % RH, *P. camemberti* mycelium disappeared after d8 (Leclercq-perlat et al., 2013) while the
 16 mold sporulation increased. These results highlighted the dependence of RH on rind color due
 17 to a better development of *B. aurantiacum* and a bad down coating of *P. camemberti* mycelium
 18 when the RH was higher than 95 %. Moreover, indirect interactions between yeasts, molds and
 19 *B. aurantiacum* can allow a better development of color (Leclercq-Perlat et al., 2004b; Lecocq et
 20 al., 1996), this could be due to an important proteolysis (Bockelmann et al., 1997).

21 **Underrind thickness.** On d14, $T_{UR,14}$ rose around 50 % when the temperature increased from
 22 8 °C to 12°C while it rose between 32 % and 40 % when temperature increased from 12°C to
 23 16°C. $T_{UR,14}$ increased from 10 % (8-12°C) to 15 % (16°C) when the RH increased from 88 to
 24 92 % while it rose from 9 % to 17 % when the RH increased from 92 to 98 %. These results are
 25 in agreement with the ones of Gomes et al. (1998) and Gomes and Malcata (1998). Indeed,
 26 these authors have shown that temperature had a more important effect than RH for different
 27 proteolysis levels. $T_{UR,14}$ (in mm) was related to temperature (linear term) and temperature and
 28 RH (interactive term) (Table 4) and $T_{UR,14}$ increased along the temperature axis from 8 to 16°C

1 and the temperature effect was stronger at higher RH (Fig. 2A).

2 On d40, at 8°C whatever the RH, the underrind thickness ($T_{UR,40}$ in mm) reached around one-
 3 third of cheese thickness (Table 3). This can be explained by a slowdown of all ripening
 4 microorganism growths and as a result by their enzymatic reactions due to the excessive drying
 5 of cheese (Leclercq-Perlat et al., 2012). Indeed, Lesage-Meessen and Cahagnier (1998) have
 6 highlighted that when the water activity, strongly linked up to RH during ripening (Ramet, 2000),
 7 is progressively lowered the microbial growth is repressed. Whatever the temperature, for the
 8 ripening carried out under 88 % RH the underrind thickness did not increase as well as the
 9 ripening made under 92 or 98 % RH. For the other ripening conditions, all the thickness of
 10 cheese was ripened. This descriptor was related to temperature (quadratic term) and RH (linear
 11 and quadratic term) and their interactive term. These effects were shown by Fig. 2B which
 12 exhibited a maximum towards $14 \pm 1^\circ\text{C}$ and $93 \pm 1\%$.

13 **Underrind consistency and core hardness**

14 *Underrind consistency* (C_{UR}). On d14 or d40 at 8 °C and 88 % RH (run 1) the underrind was
 15 very dried (Table 3). Whatever the temperature under 88 % RH the score of underrind
 16 consistency was lower than the ones obtained for the two other RH. Only the central points
 17 (runs 6 to 10) had a soft and creamy underrind typical of a Camembert cheese (Table 3).
 18 Whatever the RH at 16°C the cheeses did not have an underrind consistency acceptable
 19 because the underrind had runny edges (88 % RH) or completely runny (92 and 98 % RH). The
 20 underrind consistency which showed almost linear increases along the temperature and RH
 21 axes in the considered range (prevailing linear effects without interaction), confirmed the
 22 influences of temperature and RH an d14, (Fig. 2C) as well as on d40 (Fig. 2D). Moreover, the
 23 temperature effect was relatively stronger, especially on d14. Whatever the ripening time, the
 24 underrind consistency was correlated with temperature and RH (linear terms).

25 *Core hardness*. On d14 under 88 % RH the core was hard (at 8°C) or very hard (at 12 °C or
 26 16 °C) (Table 3). Whatever the temperature under 92 % or 98 %, the hardness remained
 27 medium On d40, under 88 % RH, it remained hard at 8 °C or very hard at 12 °C but it scored
 28 medium at 16°C. At 12°C or 16°C under 92 or 98 %, all the cheese thickness was ripened and

1 the core hardness cannot be scored by the assessors.

2 On d14, the core hardness was a function of temperature (individual term) and RH (interactive
3 term) (Fig. 2E). This descriptor reached a minimum for a temperature that depended on the RH
4 (interaction): it was around 13°C for 98 % RH and around 11°C for 88% RH as shown in fig. 1K.
5 Core hardness decreased with RH increase and the RH effect was slightly stronger for higher
6 temperatures. On d40, it was impossible to determine a relation between core hardness and the
7 ripening conditions because all the cheese was ripened.

8 The underrind consistency and the core hardness are two descriptors drawing the texture of
9 cheeses. The cheese texture mainly depends on its pH, the entirety of caseins and lipids, and
10 the moisture (Lawrence et al. 1987). Indeed, whatever the ripening conditions, the pH of
11 underrind was close to the one of rind (Leclercq-Perlat et al., 2012). This can be explained by
12 the lactate and ammonium concentration gradients which exist between the core and the rind
13 (Leclercq-Perlat et al., 2004a; Vassal et al., 1986). The cheese pH is also defined by the
14 production of microbial enzymes and their activities (Ramet, 2000). Moreover, whatever the
15 temperature under 88 % RH, there was no soft and creamy underrind due to 1) an excessive
16 drying of cheese, 2) a delay of *K. marxianus* and *G. candidum* growths, 3) a lower growth of
17 *P. camemberti* and *B. aurantiacum* (Leclercq-Perlat et al., 2012, 2013), and 4) lower enzymatic
18 activities as shown by overall rind odor. Indeed, at 16°C and 92 % RH and under 98 % RH at 12
19 or 16 °C, the cheese underrind was completely runny, noticed an over-ripening. With the rapid
20 increase of pH and its consequence on the texture, the underrind “liquefaction” can be
21 explained by the casein breakdown and enzymatic activities associated due to a better and swift
22 microorganism growths (Leclercq-Perlat et al., 2012; 2013). Indeed, the hydrolyze reactions
23 which take place in cheese involve the water fixation and in a cheese core, the breakdown
24 products of caseins are largely water soluble and proteolytic activities in cheese is mainly
25 determined by the level of moisture, temperature of ripening, and changes in pH throughout the
26 ripening (Ramet, 2000; Lawrence et al., 1987). Moreover, the changes in underrind consistency
27 can be explained by enzymatic activities of surface ripening flora though the enzymes remained
28 near their production place, i.e. the rind (Noomen, 1983). *K. marxianus* has only an endocellular

1 proteolytic activity (Klein et al. 2002), *G. candidum* strongly contributes to the proteolysis by its
 2 huge enzymatic system (Boutrou et Guéguen, 2005; Boutrou et al., 2006), *P. camemberti* also
 3 owns a complex proteolytic system which is more active when the rind pH is up to 6.0 (Choisy et
 4 al., 2000; Lenoir et al., 2005), and *B. aurantiacum* is also well-known for its important proteolytic
 5 activities (Lecocq et al., 1996; Rattray and Fox, 1999). Under 98 % RH at 12°C and under 92 or
 6 98 % RH at 16°C, Leclercq-Perlat et al. (2012) have shown that 1) the death of *K. marxianus*
 7 cells began earlier and was more intensive than under the other ripening conditions; 2)
 8 *G. candidum* growth also began earlier (1-2 days), reaching its maximal concentration faster; 3)
 9 *P. camemberti* spore concentrations were the highest, reaching around 5.5×10^6 spores per g on
 10 d40 at 16°C; and 4) under 98 % RH at 12 or 16 °C, *B. aurantiacum* also grew quickly, reaching
 11 a concentration higher than 1×10^9 CFU per g. Moreover, a synergy has been found between *G.*
 12 *candidum* and *P. camemberti*, the first allowed an easier assimilation of peptides and amino
 13 acids as carbon sources by *P. camemberti*, involving a more intense proteolysis in the underrind
 14 of cheeses (Aziza et al., 2005; Aziza and Amrane, 2006). The underrind consistency is also
 15 related to lipid breakdown mainly due to *G. candidum* and *P. camemberti* (Lenoir et al., 1985)
 16 while *K. marxianus* and *B. aurantiacum* have no significant lipolytic activities (Leclercq-Perlat et
 17 al., 2007). Lipolytic enzyme activities mainly depend on temperature and cheese moisture which
 18 is dependent on RH (Choisy et al., 2000; Leclercq-Perlat et al., 2012; Ramet, 2000). Indeed, a
 19 low RH led to much dehydration of the cheese rind by water losses Water was vaporized from
 20 the surface toward the atmosphere, while at the same time water diffused from the core toward
 21 the surface (Simal et al., 2001; Bonaïti et al., 2004). This dehydration increased with the
 22 temperature: under 88 % RH at 16°C, the dry matter (DM) on d14 and on d40 was the most
 23 important, 47 % and 53 ± 1 % respectively, leading the stopping of ripening. However, whatever
 24 the RH at 8°C, the core remained hard on d40 although its DM (42–44 %) did not differ between
 25 the other ripening conditions. This could be explained by a slowdown of enzymatic reactions
 26 occurring in the cheese probably due to the lower temperature. By consequence of higher
 27 enzymatic activities and a lower dehydration of cheese, under 98 % RH at 12°C or 16°C, the
 28 underrind lost its softening to become liquid. These two factors involved a cheese moisture

1 variable in relation to conditions of the ripening. Whatever the temperature under 88 % RH, the
2 water cheese mass losses and the DM of the core were the highest (Leclercq-Perlat et al.,
3 2012). Under the same RH, the water mass loss increased from 54 % at 8 °C (88 % RH) and
4 68 % at 16 °C (92 or 98 % RH) when the temperature increased from 8°C to 16°C. This showed
5 cheese moisture importance and, by consequence, the importance of RH on core hardness.

6

7

CONCLUSIONS

8 Because of all cheeses were wrapped with the same film and ripened under wrapping in the
9 same conditions, the conditions of ripening in room govern the Camembert-type cheeses
10 happening during their wrapping. In this work, the cheese properties assessed by cheese
11 ripening experts were highly dependent on temperature and relative humidity used throughout
12 the ripening. All sensorial descriptor changes (rind odor and color, underrind thickness,
13 underrind consistency, and core harness) can be explained by microorganism growth, pH,
14 carbon substrate diffusion and assimilation, cheese moisture as well as microbial enzymatic
15 activities. All these factors were also linked to temperature and RH used throughout the
16 ripening. Under 88% RH at 8°C, on d40, all sensorial descriptors scored the worst by the
17 assessors: the cheese was too dry, its odor and its color were similar to the ones of the cheeses
18 defectively ripened, the underrind was the driest, and the core was the hardest. On the contrary,
19 under 98 % RH at 16 °C, the odor and the color of the cheeses were ammonia and dark brown,
20 the underrind represented all the thickness of the cheeses but it was completely runny. These
21 descriptors noticed an over-ripened cheese. As previously shown for microbial and
22 physicochemical kinetic descriptors (Leclercq-Perlat et al. 2012), the best ripening conditions to
23 obtain an optimum between sensorial qualities and cheese ripening properties appear to be
24 13 °C and 94 % RH. Such a study will provide main elements 1) to develop a mechanistic
25 model allowing monitoring the Camembert-type cheese ripening process in relation to the
26 ripening conditions, 2) to slow down or to accelerate the ripening process in relation to market
27 demand and, 3) to correct some flaws.

1 **ACKNOWLEDGMENTS**

2 This work was supported by the French Research Ministry through a contract of National
3 Program of Agronomic Research (PNRA). The authors are deeply grateful to F. Lecornué and
4 T. Cattenoz (UMR GMPA) for their technical assistance and they are gratefully acknowledged
5 the five assessors for their expertise.

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- 26

1 **Table 1.** Definition and score of the descriptors used to evaluate the Camembert-type cheeses
 2 ripening progress.

Descriptor	Notation	Scale and reference				
		1	2	3	4	5
PC appearance	N _{PC}	< 10 %	25 %	50 %	75 %	> 90 %
Overall odor	Odor	Fresh cheese	Musty	Camembert	Camembert ammoniac	Ammoniac
Rind color	Color	White	Cream	Dark cream	Light brown	Dark brown
Underrind consistency	C _{UR}	Very dried	Dried	Soft	Soft with runny edge	Runny
Core hardness	H _{core}	Very soft	Soft	Medium	Hard	Very hard

3
 4
 5 **Table 2.** Descriptors of *P. camemberti* appearance obtained for each temperature (θ, °C) and
 6 relative humidity (RH, %) used during Camembert-type cheese ripening.

N°	θ (°C)	RH (%)	t _{PCm}	N _{PC14}	N _{PC40}
1	8	88	10	2	2
2	8	92	10	2	3
3	8	98	9	3	2
4	8	98	9	3	2
3 & 4	8	98	9	3	2
5	12	88	6	4	5
6	12	92	4.5	5	5
7	12	92	5	5	5
8	12	92	5	5	5
9	12	92	4.5	5	5
10	12	92	5	5	5
6 to 10	12	92	5	5	5
11	12	98	4	4	3
12	16	88	3	4	2
13	16	88	3	4	2
12 & 13	16	88	3	4	2
14	16	92	3	3	3
15	16	98	3	2	1

7
 8 t_{PCm}, day for which *P. camemberti* mycelium is visually detected by the panel (standard
 9 deviation = 0.5 d). N_{PC14} and N_{PC40}, the appearance notation on d14 (wrapping) and on d40 (end
 10 of ripening), respectively.

11

1 **Table 3.** Scores of descriptors of Camembert-type cheese ripening on d14 (before wrapping)
 2 and on d40 (end of ripening) obtained for each temperature (θ, °C) and relative humidity (RH,
 3 %).

4

Run	θ (°C)	RH (%)	On d14 (before wrapping)					On d40 (at the end of ripening)				
			Odor	Color	T _{UR}	C _{UR}	H _{core}	Odor	Color	T _{UR}	C _{UR}	H _{core}
1	8	88	2	1	1.3	1	4	2	1	9.5	1	4
2	8	92	2	1	1.8	1	4	2	1	10.5	2	4
3	8	98	2	1.5	2.0	2	3	3	2	10	3	4
4	8	98	2	1.5	2.0	2	3	3	2	10	3	4
3 & 4	8	98	2	1.5	2.0	2	3	3	2	10	3	4
5	12	88	3	1	2.7	2	5	4	2	11	2	5
6	12	92	3.5	2	3.3	3	3	4	3	14	3	–
7	12	92	3.5	2	3.3	3	3	4	3	14	3	–
8	12	92	3.5	2	3.5	3	3	4	3	14	3	–
9	12	92	3.5	2	3.4	3	3	4	3	14	3	–
10	12	92	3.5	2	3.3	3	3	4	3	14	3	–
6 to 10	12	92	3.5	2	3.3	3	3	4	3	14	3	–
11	12	98	3.5	2	4.1	3	2	4.5	3	14	4	–
12	16	88	4	3	3.9	4	5	4	4	12.5	4	3
13	16	88	4	3	3.9	4	5	4	4	12	4	3
12 & 13	16	88	4	3	3.9	4	5	4	4	12	4	3
14	16	92	5	4	5.7	5	4	5	5	14	5	–
15	16	98	5	4	6.0	5	3	5	5	14	5	–

5

6 T_{UR}, underrind thickness in mm (standard deviation = 0.5 mm).
 7 The overall cheese odor varied from fresh cheese (score 1) to ammoniac (score 5). The cheese
 8 color under the *P. camemberti* coating varied from white (score of 1) to dark brown (score 5).
 9 The underrind consistency (noted C_{UR}) varied from a dried (score 1) to a very runny underrind
 10 (score 5). The cheese core hardness (noted H) was estimated by the ease with which the
 11 cheese can be ground with a mortar and pestle and varied from very hard (score of 1) to very
 12 soft (score 5). –, the core was totally ripened.

Table 4. Best-fit equations for the effects of temperature and relative humidity on Camembert-type cheese ripening descriptors. 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 for the relation.

Descriptors	Equation	R	df	SE	p-value
On wrapping day (d14)					
N _{PC14}	$-54 (\pm 10)^{**} + 0.4 (\pm 0.1)^{**} \times \theta + 0.4 (\pm 0.1)^* \times RH - 0.13 (\pm 0.01)^{**} \times \theta^2 - 0.039 (\pm 0.008)^{**} \times \theta \times RH$	0.94	10	0.393	2.00×10^{-5}
Rind odor	$-103 (\pm 23)^* - 0.8 (\pm 0.2)^* \times \theta + 2.3 (\pm 0.5)^{**} \times RH - 0.013 (\pm 0.003)^{**} \times \theta^2 + 0.012 (\pm 0.002)^{**} \times \theta \times RH$	0.99	10	0.114	$< 10^{-7}$
Rind color	$-154 (\pm 41)^* - 0.7 (\pm 0.02)^* \times \theta + 3.3 (\pm 0.9)^* \times RH + 0.042 (\pm 0.007)^{**} \times \theta^2 - 0.017 (\pm 0.005)^* \times RH^2$	0.98	10	0.195	1.16×10^{-7}
T _{UR} (mm)	$-1.8 (\pm 0.3)^{**} - 0.7 (\pm 0.1)^{**} \times \theta + 0.012 (\pm 0.001)^{**} \times \theta \times RH$	0.98	12	0.273	$< 10^{-7}$
C _{UR}	$-11 (\pm 2)^{**} + 0.41 (\pm 0.05)^{**} \times \theta + 0.10 (\pm 0.02)^{**} \times RH$	0.97	12	0.280	$< 10^{-7}$
H _{core}	$-10 (\pm 1)^* + 0.057 (\pm 0.007)^{**} \times \theta^2 - 0.014 (\pm 0.002)^{**} \times RH \times \theta$	0.91	12	0.384	1.17×10^{-5}
At the end of ripening (on d 40)					
N _{PC40}	$-365 (\pm 76)^{**} + 3.1 (\pm 0.3)^{**} \times \theta + 8.0 (\pm 0.9)^{**} \times RH - 0.13 (\pm 0.01)^{**} \times \theta^2 - 0.042 (\pm 0.009)^{**} \times RH^2$	0.97	10	0.363	$< 10^{-7}$
Rind odor	$-15 (\pm 2)^* + 1.0 (\pm 0.2)^{**} \times \theta + 0.12 (\pm 0.002)^{**} \times RH - 0.030 (\pm 0.008)^* \times \theta^2$	0.97	11	0.241	2.87×10^{-7}
Rind color	$-11 (\pm 1)^{**} + 0.41 (\pm 0.02)^{**} \times \theta + 0.10 (\pm 0.02)^{**} \times RH$	0.97	12	0.280	$< 10^{-7}$
T _{UR} (mm)	$-472 (\pm 97)^{**} + 10 (\pm 2)^{**} \times RH - 0.08 (\pm 0.02)^{**} \times \theta^2 - 0.06 (\pm 0.01)^{**} \times RH^2 + 0.025 (\pm 0.004)^{**} \times \theta \times RH$	0.97	10	0.453	1.01×10^{-6}
C _{UR}	$-16 (\pm 2)^{**} + 0.33 (\pm 0.02)^{**} \times \theta + 0.17 (\pm 0.02)^{**} \times RH$	0.96	12	0.294	$< 10^{-7}$
H _{core}	NS				

N_{PC}, appearance notation of *Penicillium camemberti*. Overall rind cheese odor varied from fresh cheese (score 1) to ammoniac (score 5).

The cheese color under the *P. camemberti* coating varied from white (score 1) to dark brown (score 5). The cheese core hardness (Hard_{core}) was estimated by the ease with which the cheese could be ground with a mortar and pestle: from very soft (score 1) to very hard (score 5). T_{UR}, underrind thickness (mm). The underrind consistency (C_{UR}) varied from a dried (score 1) to a very runny underrind (score 5). NS, none significant. 14 for d14, 40 for d40 and core for the cheese core.

Θ and RH effects on ripening descriptors

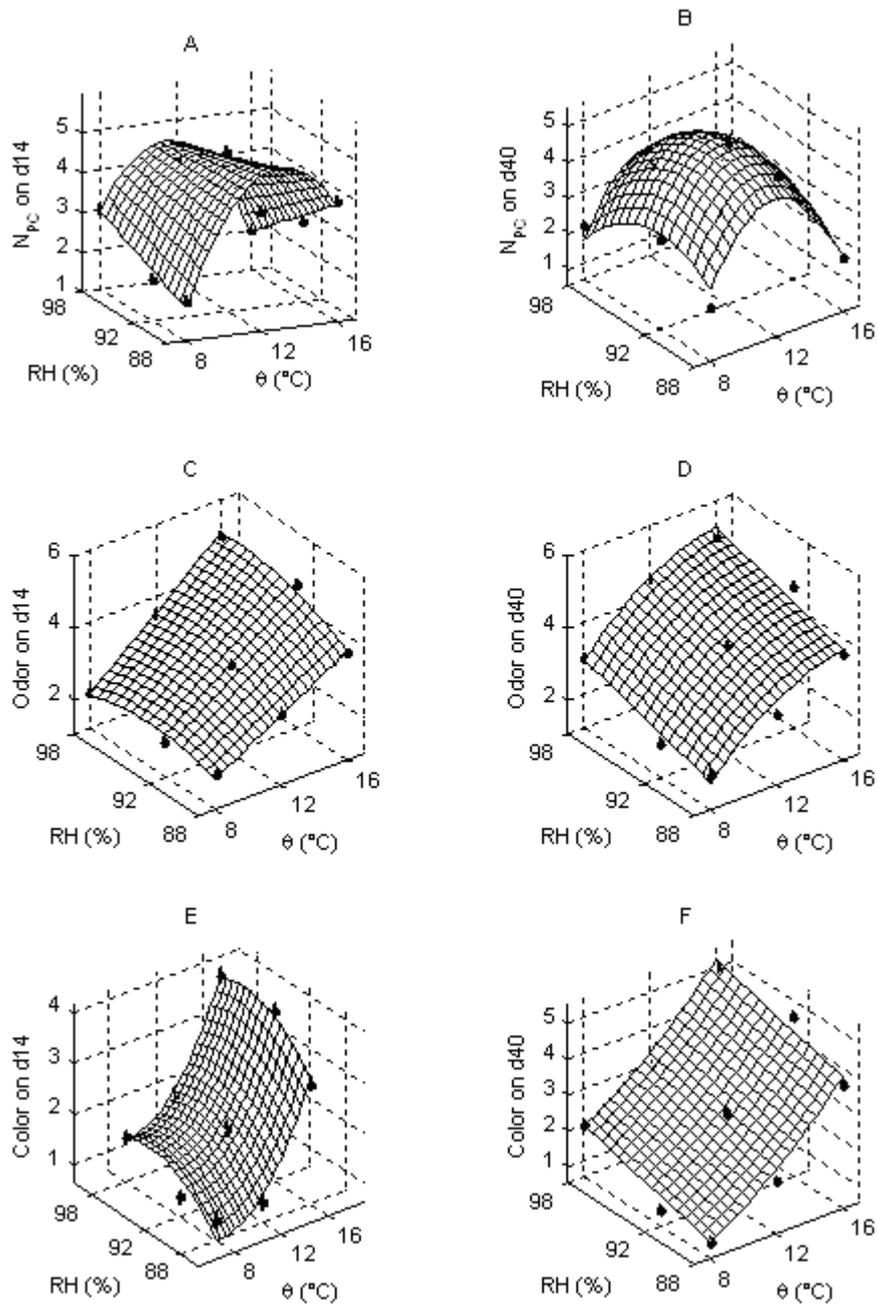


Figure 1: Estimated response surface plots of ripening descriptors as a function of temperature (θ , °C) and RH (%). A) PC appearance notation on d14; B) PC appearance notation on d40; C) Odor on d14; D) Odor on d40; E) Color on d14; F) Color on d40.

Θ and RH effects on ripening descriptors

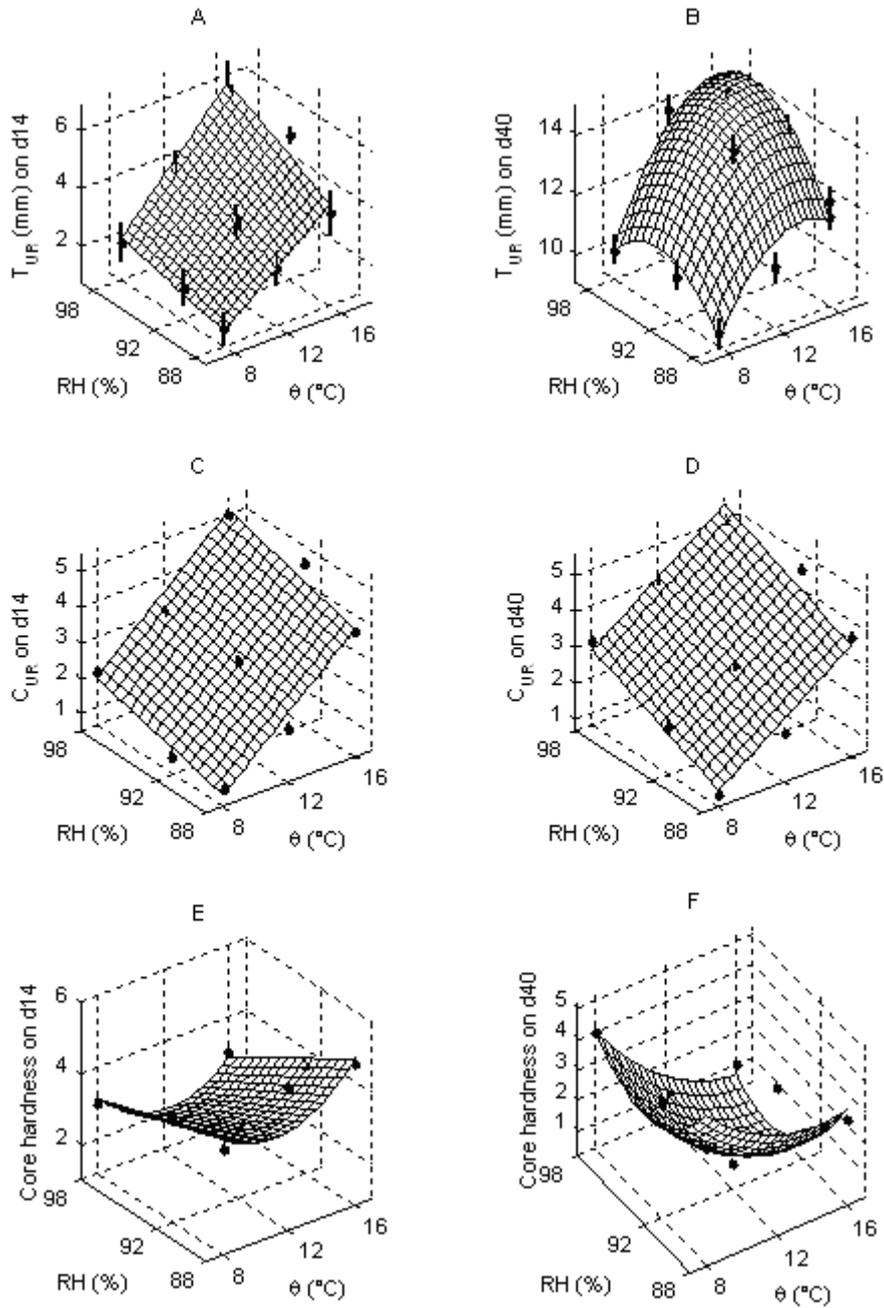


Figure 2: Estimated response surface plots of ripening descriptors as a function of temperature (θ , °C) and RH (%). A) Underrind thickness (T_{UR}) on d14 (mm); B) Underrind thickness on d40; C) Underrind consistency (C_{UR}) on d14; D) Underrind consistency on d40; E) Core hardness on d14; F) Core hardness on d40.