Short communication: Little change takes place in Camembert-type cheese water activities throughout ripening in terms of relative humidity and salt

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ABSTRACT

Water activity (a_w) affects the growth and activity of ripening microorganisms. Moreover, it is generally accepted that a_w depends on relative humidity (RH) and salt content; these 3 variables were usually measured on a given day in a cheese without the microorganism layer and without accounting for a distinction between the rind, the underrind, and the core. However, a_w dynamics have never been thoroughly studied throughout cheese ripening. Experimental Camembert cheeses were ripened under controlled and aseptic conditions (temperature, gaseous atmosphere, and RH) for 14 d. In this study, only RH was varied. Samples were taken from the cheese (microorganism layer)–air interface, the rind, and the core. The a_w of the cheese–air interface did not change over ripening when RH varied between 91 and 92% or between 97 and 98%. However, on d 5, we observed a small but significant increase in a_w , which coincided with the beginning of growth of *Penicillium camemberti* mycelia. After d 3, no significant differences were found between the a_w of the cheese–air interface, the rind, and the core. From d 0 to 3, cheese rind a_w increased from 0.94 to 0.97, which was probably due to the diffusion of salt from the rind to the core: NaCl content in the rind decreased from 3.7 to 1.6% and NaCl content in the core increased from 0.0 to 1.6%. Nevertheless, a_w did not significantly vary in the core, raising questions about the real effect of salt on a_w .

Key words: water activity, relative humidity, salt content, Camembert ripening

Short Communication

The development and growth of cheese surface microorganisms, which play a key role in the ripening of soft cheeses, are strongly influenced by external conditions such as temperature, gaseous atmosphere, and relative humidity (**RH**) in the ripening room (Bonaïti et al.,

2004; Leclercq-Perlat et al., 2006, 2012). They are also influenced by internal physicochemical properties such as pH and water activity (a_w) and by concentrations of protein, fat, and salt (Walstra et al., 1999). Among these latter properties, a_w has a large influence on the growth of ripening microorganisms, as well as on the inhibition of spoilage microorganisms (Schvartzman et al., 2011). Indeed, most cheese molds and yeasts grow when a_w is >0.85 , and most ripening bacteria grow when a_w is >0.90 (Pérez Elortondo et al., 1999; Saurel et al., 2004). Moreover, according to Ludemann et al. (2004), a_w controls the proteolytic activities of *Penicillium* strains, and according to Ayyash and Shah (2011), with microbial changes, a_w influences the physicochemical properties of cheeses during ripening (water loss, texture, and pH).

For those reasons, it seems important to efficiently monitor a_w . Among the factors that influence a_w , Guinee (2004) and Hardy et al. (2000) highlighted the role of salting in cheese ripening: (1) the salt concentration diffusion in a soft cheese is connected to salting type (brining or dry salting), and in the case of brining, to salt content, salt type, static or stirred brining, pH, and cheese texture; (2) after brining, the a_w of the cheese surface is a function of brining time, salt content, stirring, and environmental conditions. According to the literature, the a_w of Camembert-type cheeses on market day varies between 0.93 (Lenoir et al., 1985) and 0.98 (Hardy et al., 2000). Moreover, some linear relationships can be established between a_w from ash and the soluble nitrogen content of cheese (Esteban and Marcos, 1990; Marcos et al., 1990). According to Saurel et al. (2004), (1) local a_w profiles during brining and ripening periods are highly dependent on the local distribution of NaCl content; (2) an accurate correlation expresses the water content in relation to NaCl concentration during brining; and (3) an overall correlation expresses a_w as a function of water, NaCl, and free NH_2 concentrations during ripening. When studying the effects of temperature and RH on the microbial, biochemical, textural, and sensory properties of Serra cheese on market day, Macedo et al. (1997) showed that salting and ripening RH had significant effects on these properties; that

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salt addition on the surface of the fresh cheese reduced microbial growth, a_w , moisture content, proteolysis, lipolysis, cheese flavor, and softness; and, finally, that an increase in RH during ripening increased all these properties.

To our knowledge, the methods used to measure a_w in these studies were difficult and time consuming (e.g., isotherm sorption), which explains why only a few ripening times (on market days) were studied. We conclude, therefore, that the evolution of a_w in Camembert-type cheeses throughout ripening has not been studied in detail, which is what we proposed to do in this study.

Soft mold surface-ripened cheeses (50 cheeses per run; each 30 ± 1 mm high and 107 mm in diameter, with each cheese weighing approximately 300 g) were made and ripened (Leclercq-Perlat et al., 2006, 2012). Briefly, 100-L vats of pasteurized milk were seeded with lactic acid bacteria (CHN11, Chr. Hansen, St-Germain-les-Arpajon, France; 15 mL/L of 16-h-old culture in sterile milk) and ripening cultures consisting of *Kluyveromyces marxianus* (strain 44s; GMPA collection, UNR GMPA, Thiverval-Grignon, France; 4×10^3 cfu/mL of milk), *Geotichum candidum* (strain D; Cargill, La Ferté-sous-Jouarre, France; 10^2 cfu/mL), *Penicillium camemberti* (strain R, Cargill; 8.1×10^3 spores/mL), and *Brevibacterium aurantiacum* (9175; American Type Culture Collection, Manassas, VA; 4×10^4 cfu/mL). After renneting, cheeses were molded and drained for 24 h. After 24 h of draining, the a_w values of the rind and the core were 0.995 ± 0.005 and 0.977 ± 0.001 , respectively. The cheeses were then brined in an unstirred bath (NaCl, 27%; pH = 5.5; 25 min; 14°C). After brining, cheeses were transferred to the ripening room under sterile conditions. This point in time was designated as the initial ripening time (d 0). Ripening took place at $13 \pm 1^\circ\text{C}$ under a periodically renewed atmosphere (Picque et al., 2006). Relative humidity in the ripening room was controlled at $85 \pm 1\%$. As soon as the cheeses were put in the ripening room, the RH increased immediately to $91 \pm 1\%$, although some water was trapped by cold point system (described in detail by Picque et al., 2006; Figure 1). This sudden increase in RH could be explained by water evaporation from cheese surfaces (Mirade et al., 2004). The RH was maintained at 91% RH until d 1.5. On d 1.5, the RH was set to $97 \pm 1\%$ but it took 0.5 d for the room to reach $97 \pm 1\%$ RH. From d 2.0 to 3.5, the RH in the ripening room remained constant at $97 \pm 1\%$. On d 3.5, the RH regulation was lowered to $92 \pm 1\%$ to simulate the opening of the ripening room door to allow the cheese-makers to conduct surface treatments on the cheeses. From d 4.3 to 5.0, the RH was $93 \pm 1\%$; from d 5.8 to 7.5, RH remained stable at $96 \pm 1\%$; from d 7.8 to 11.5, the RH value remained at $93 \pm 1\%$; from d 12.0 to 13.5, the RH was at $97 \pm$

1%; finally, from d 13.8 to the end of ripening, the RH remained stable at $93 \pm 1\%$. After each RH change, the RH stabilization in the ripening room lasted between 3 (after d 7) and 12 h (after the RH change on d 3.5). The range and length of these RH variations are unusual, but they were performed to study how RH could influence a_w . Indeed, Camembert-type cheeses are typically ripened between 90 and 98% RH according to industrial practices. However, RH fluctuations throughout the ripening in room are common because of the difficulties of keeping a ripening room at constant RH (Pajonk, 2001; Mirade et al., 2004). Water activity was measured at 14°C with a FA-st lab water activity meter (GBX, Romans-sur-Isère, France), using condensation point technology based on the dew point principle. The calibration was done before each series with K_2SO_4 salt to which a few drops of water were added ($a_w = 0.979$ at 14°C). Two types of cheese sampling and measurement were used in relation to ripening time. The first was a direct daily nondestructive a_w measurement on the 2 faces (top and underside) of the cheese. To do this, 3 cheeses were removed from the ripening room for 45 min, and 3 replicate measurements per face, lasting approximately 5 min each, were done. This measurement gave the a_w value of the cheese-air interface. The second sampling was a daily cheese sampling in which the a_w value of 2 parts (rind and core) of the cheeses and their salt contents were determined (destructive method). To do this, approximately 10 g of ground rind (2 mm high for the 2 cheese surfaces) or core (the rest of the cheese, close to 26 mm high) was placed in a dish and the a_w measured at 14°C. Three repetitions per cheese part were done. A nonparametric Wilcoxon statistical test was used to demonstrate differences between a_w values over time (Siegel and Castellan, 1988). The hypothesis of the equality of all values was tested on the ranges defined by the salt diffusion phase in the rind (from d 0 to 3, and from d 4 to 15).

The AFNOR NF V 04-288 reference method was used to determine the salt (NaCl) concentration (Amariglio, 1986). Its principle consists of destroying the OM of the ground cheese sample (2 g) in the presence of silver nitrate (0.1 M) using nitric acid (50%) and potassium permanganate in a saturated solution. The excess of silver nitrate is immediately titrated with ammonium thiocyanate (0.1 M) in the presence of a colored indicator until a red-brown colored suspension remains for 30 s. The salt content of each of the 3 repetitions was determined by calculation for each part (rind and core) of the cheese samples. The NaCl content value was the arithmetic average of these 3 analyses.

Figure 1 illustrates the comparison between a_w values of the cheese-air interface and those of the ground rind and core throughout ripening, as well as the evolution

Figure 1. Comparison between water activity values of the cheese–atmosphere interface and of the ground cheese rind and core, as well as changes in relative humidity (%) in the ripening chamber in relation to ripening time. ■ = mean a_w (error bars indicate SE) on the 2 faces (3 measurements per face) of the cheese (3 cheeses); ▲ and △ = a_w values in the rind and core of the cheese sample, respectively (SE = 0.005); solid curve = relative humidity in the ripening room. Ripening took place at $13 \pm 1^\circ\text{C}$ under a partially renewed atmosphere.

of RH in relation to ripening time. From the nonparametric Wilcoxon statistical test, the interface and core a_w values did not differ statistically ($P > 0.01$) from d 0 to 14; a_w values remained constant at 0.976 ± 0.005 . No significant differences ($P > 0.01$) in a_w were observed between the values of the cheese–air interface and those of the rind, except from d 0 to 2. From d 0 to 2, the a_w values of the rind were significantly lower than those of the cheese–air interface ($P < 0.1$). The interface a_w (mean $\pm$ SE) increased from 0.971 ± 0.002 to 0.981 ± 0.002 on d 5. This small increase was significant ($P < 0.01$) with a Bonferroni one-factor ANOVA (ripening time).

Figure 2 shows the changes in salt concentration and a_w of the rind and core throughout ripening. Immediately after salting, salt was concentrated in the cheese rind and its content was approximately 3.5%. This content decreased from d 0 to 4. The salt content of the cheese core increased from d 0 ($0.12 \pm 0.02\%$) to d 4 ($1.41 \pm 0.06\%$). Regardless of the cheese part sampled, salt content remained constant at $1.63 \pm 0.08\%$ after d 5 and until d 14. On d 0, rind a_w was approximately 0.94. From d 1 to d 3, it increased to 0.970 and remained close to 0.975 from d 3 to 14.

To our knowledge, most studies in the literature have provided a_w values of Camembert-type cheese on market days and, generally, for a ground cheese without the rind. However, cheese evolution is highly dependent

on the conditions used throughout ripening, even after wrapping (Picque et al., 2006; Leclercq-Perlat et al., 2012). Before the 1990s, Camembert cheeses were ripened under a RH of between 85 and 90%, inducing an a_w in the cheese core close to 0.93 on wrapping day (Lenoir et al., 1985). After the 1990s, RH generally varied between 90 and 95%, and the a_w was close to 0.97 (Macedo et al., 1997; Hardy et al., 2000; Simal et al., 2001; Hélias et al., 2007). In the current study, the core a_w value was of same order of magnitude (0.975 ± 0.005) on d 14 (wrapping day) as it was throughout ripening (d 0 to 14). This indicates that salt diffusion from the rind to the core (from d 0 to 4) and the salt concentration obtained on d 3 (1.2% NaCl) did not greatly influence the a_w , if the salt content was low enough. Indeed, except for salt content, no significant changes in the biochemical and physicochemical composition of the core were observed during this period in previous studies (Leclercq-Perlat et al., 2006, 2012).

Throughout ripening, the a_w of the top of the cheese did not differ significantly from that of the underside, which was in contact with the netting. Air mixing and gaseous atmosphere in the ripening room did not affect a_w . This can be explained by a very low air circulation rate (approximately 0.1 m/s; Hélias et al., 2007) and a short air renewal time (once a day for 5 min, $6 \text{ m}^3/\text{h}$). Moreover, when fresh cheese samples are placed in 27% NaCl brine, the high external salt concentration rapidly results in removal of water from the rind to the brine

Figure 2. Salt (NaCl) content (● and ○) and water activity (▲ and △) changes in the rind (solid symbols) and core (open symbols) of cheeses throughout ripening; error bars indicate SE. Ripening took place at $13 \pm 1^\circ\text{C}$ under a partially renewed atmosphere.

to reduce the rind a_w to 0.94 (2 mm in depth) while the cheese surface is “brined” and the a_w and concentration in salt in the core remain unaffected (Macedo et al., 1997). Indeed, 2 diffusion phenomena occur in the rind: (1) diffusion of water from the surface and, consequently, from the rind (2 mm depth) to the brine; and (2) diffusion of salt from the surface to the core. Therefore, a lesser concentration of water and a higher salt level can also explain why the a_w of the rind was 0.94 just after brining. Water activity in the core did not differ after brining compared with before in the current study because the short time between brining and the time of a_w analysis was insufficient to allow diffusion of salt from the rind to the core.

From d 4 to 14, when salt content was almost in equilibrium (NaCl content approximately 1.7%), the a_w values of the cheese–air interface, the rind, and the core were statistically significant ($P < 0.01$). From d 0 to 3, the a_w values of the cheese–air interface were significantly higher than those of the rind. This can be explained as follows: first, the a_w of the cheese–air interface was mainly due to the microorganism layer, whereas the a_w of the rind was mainly caused by cheese composition, which changed over time (e.g., salt, proteolysis). These 2 a_w values became statistically equal when salt diffusion was restricted. Second, the local pH of the microbial layer remained constant (approximately 7.7), whereas that of the rind changed in relation to variations in composition (Walstra et al., 1999; Saurel et al., 2004). Indeed, the yeasts (*Kluyveromyces* spp.) began to grow as soon as they were seeded in the milk. Then, they continued to expand until d 4, almost reaching their maximum count (5×10^7 cfu/g) on d 4. In parallel, *G. candidum* (filamentous strain) was in the growth phase, and its count reached 4×10^6 cfu/g (Leclercq-Perlat et al., 2012). Leclercq-Perlat et al. (2013) showed that (1) under 97 to 98% RH, the *P. camemberti* mycelia measured by quantitative PCR in the rind (2 mm depth) increased from d 1 (approximately 0.003 mg of dried *P. camemberti* per cm² of cheese surface), but was not visible to the naked eye, and (2) on d 4, *P. camemberti* mycelia became visible to the naked eye on the Camembert surface and the biomass reached approximately 0.35 mg of dried *P. camemberti* per cm² of cheese surface. Moreover, from d 0 to 3, the a_w of the rind was significantly lower than that of the core, although its biochemical and physicochemical composition did not change greatly. This was in accordance with Guo et al. (2012), who showed that when salt content increased, a_w decreased, especially when the salt content exceeded 2%.

After temperature, RH was the second main factor affecting ripening because of its actions on moisture and surface a_w (Choisy et al., 2000; Ramet, 2000). These

authors have also highlighted that the choice of RH is made (1) in relation to the microorganism sensitivity to a_w (required or banned microorganisms), and (2) the type of cheeses made: for soft mold-ripened cheeses, RH is maintained at between 85 and 95%. However, in this study, a_w changes did not correlate with RH variations between 91 and 97% (results not shown). This may indicate that above a minimum RH of approximately 90%, its influence on the interface a_w value was more restricted. Moreover, Hélias et al. (2007) showed that water transfer from the room or cheese–air interface onto the rind does not exist. On the contrary, they demonstrated the existence of water diffusion phenomena from the surface to the atmosphere. In fact, cheese mass loss rates were positive in the study of Hélias et al. (2007) as well as the current study, revealing this diffusion phenomenon. In addition, due to a RH lower than 100%, a “drying” surface developed, as shown by Leclercq-Perlat et al. (2012). Water diffusion from the core to the rind was a consequence of diffusion of water from the cheese surface to the atmosphere. Water transfer from the air to cheeses could not occur. Indeed, Mirade et al. (2004) showed that in a ripening room in which the conditions were close to that of this study (airflow rate <0.1 m/s, RH between 92 and 98% at 14°C, and soft cheese models with the a_w between 0.95 and 1.00), water transfer is only due to evaporation of water from the cheese surface to the air, leading to the cheese water losses observed by Hélias et al. (2007). This water evaporation from the surface to the atmosphere is also highlighted by Marcos (1993) and Ramet (2000).

Salt begins to diffuse from the cheese surface to the core when brining begins (Hardy et al., 2000; Guinee, 2004). From d 0 to 3, the evolution of the salt concentration (Figure 2) due to the diffusion phenomena of salt from surface to core was in accordance with the results of Hardy et al. (2000), even if the final salt content in the current study (1.7% NaCl) was lower than the one used by Hardy et al. (2000; around 2.2% NaCl). According to Hardy et al. (2000) and Ramet (2000), salt diffusion is linked to the migration of water from the core to the surface and positively linked to cheese pH, brine concentration, temperature, and the surface:volume ratio. During the first 3 d, rind a_w was strongly correlated with salt concentration of the rind. The migration of salt from the surface to the core is a slow process, and salt equilibrium was only reached on d 4. Cheese rind a_w was low at the end of brining ($a_w \approx 0.94$ or less) and increased to a maximum ($a_w \approx 0.975$) on d 3. After d 3, the small variations in a_w observed between the core and the rind could be due to changes in internal and local physicochemical characteristics (Walstra et al., 1999; Saurel et al., 2004).

On d 5, the small increase of interface a_w observed was probably caused by the beginning of *P. camemberti* mycelium development on the cheese surface (Leclercq-Perlat et al., 2006). Indeed, on d 5, *P. camemberti* uses carbon substrates in the presence of oxygen for mycelium growth and produces CO_2 and water. This was in accordance with respiratory dynamics observed for Camembert ripening (Picque et al., 2006). Moreover, a rapid increase in cheese surface pH was also observed on d 5. Indeed, pH is one of the main factors that controls the a_w of the cheese surface (Walstra et al., 1999), and the pH increase from 4.8 to 7.7 occurred at the same time (Leclercq-Perlat et al., 2006, 2012).

In conclusion, we describe detailed a_w dynamics in relation to salt content and RH changes in Camembert throughout ripening for 14 d. Under the conditions of this study, no differences were found in a_w between the 2 faces (top and underside) of the same cheese, indicating that surface a_w did not change when RH varied within the range studied. On d 5, the small increase in a_w observed might be due to the growth of *P. camemberti* mycelium and an increase in pH. For ground cheese samples, rind a_w increased from 0.94 (d 0) to 0.97 (d 3) when salt diffused from the rind (3.5% NaCl on d 0) to the core (1.6% on d 4). Cheese core a_w did not vary significantly, although the salt content increased from 0.1 to 1.6%. The difference observed between the cheese–air interface and the ground rind could be caused by differences in the composition of these layers.

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