

Microbiological, Physico-Chemical, and Biochemical Changes During the Ripening of a Camembert Cheese Made from Pasteurized Cow's Milk

N. Guizani*, M. Al-Mugheiry, and M. Al-Ruzeiki

Department of Food Science and Nutrition,
College of Agriculture, Sultan Qaboos University,
P.O. Box 34, Al-Khod 123, Sultanate of Oman

التغيرات في الخصائص الميكروبية و الفيزيوكيميائية و الكيموحيوية أثناء نضاج جبنة الكمبرت المصنوعة من حليب بقرى مبستر

المخلص: صنعت جبنة الكمبرت من حليب بقرى مبستر بالطريقة التقليدية بغرض تحديد التغيرات في المحتوى الميكروبي و الخصائص الفيزيوكيميائية و الكيموحيوية خلال فترة الإنضاج لمدة ٣٠ يوماً. كان العد البكتيري الكلي مرتفعاً في الجبن طوال فترة الإنضاج، وشكلت بكتيريا حمض اللاكتيك مجموعة البكتيريا الرئيسية على سطح و مركز الخثرة معاً. لكن النشاط الميكروبي شكل أهمية أكبر على السطح منه في المركز. لوحظ وجود تباين نموذجي لكل مجموعة ميكروبية أثناء الإنضاج على السطح و في المركز. أحدث المحتوى البكتيري الكلي الخارجي الغير متجانس مع وجود نمو عالٍ للفطريات و الخمائر و الفطريات (أهمها: بنسيليوم كمبرتي) و البكتيريا المحبة للملحة أحدث معدلاً كلياً للتحلل البروتيني و الدهني يعادل حوالي ١,٥ مرة أكبر على السطح منه في مركز الخثرة في نهاية فترة الإنضاج. وصلت عملية إنتقال الملح من الخثرة إلى مرحلة التوازن بعد ٢٣ يوماً من الإنضاج. لوحظ وجود إنخفاض سريع في الرقم الهيدروجيني على السطح، وظلت درجة الإنحدار في الرقم الهيدروجيني بين السطح و المركز ثابتة أثناء مدة الإنضاج.

ABSTRACT: Camembert cheese was manufactured from pasteurized cow's milk by the traditional method in order to determine the changes in the microflora, physico-chemical, and biochemical characteristics over 30-day ripening period. The total bacteria counts were high in cheese throughout ripening with lactic acid bacteria being the main microbial group both on the surface as well as in the center of the curd. However, the microbial activity is more important on the surface than in the center. Each group of microorganisms showed a typical variation during ripening on the surface and in the center. External heterogenous microflora, with high population of yeast, molds (mainly *P. camemberti*), and halophilic bacteria, induced a total rate of proteolysis and lipolysis about 1.5 times greater on the surface than in the curd at the end of ripening. Migration of salt from the curd reached equilibration after 23 days of ripening. A faster decrease in the pH of the surface was observed and a gradient of pH between the surface and the center was maintained during the ripening period.

The purpose of aging in cheese is to develop specific flavor, body, and texture qualities. These characteristics result from the activity of microorganisms and enzymes. For such development to take place, the cheeses must be maintained under the conditions favorable to the desired growth and activity. The aging conditions can also result in objectionable changes if the original milk is contaminated with undesirable

microorganisms, or if improper manufacturing procedures are used.

The control of the ripening process of cheese needs the precise knowledge of the development of the main physico-chemical, biochemical, and microbiological characteristics at different stages of ripening.

The effects of ripening on the chemical and physical characteristics of cheese have been studied by numerous

* Corresponding author.

scientists. However, most of this research has focused on hard cheeses, and limited work has been done on soft cheeses.

Soft cheeses, such as Camembert, ripen very quickly because of their high moisture content and the rapid growth of surface mold. The action of the mold protease, in addition to the proteolytic actions of the coagulant and the protease from the starter culture, transforms the insoluble casein into acid-soluble N fractions.

Studies related to the ripening of French traditional Camembert obtained from raw cow's milk have been the subject of numerous publications (Lenoir, 1963a, 1963b; Schmidt and Lenoir, 1978; Richard and Zadi, 1983; Le Graet *et al.*, 1983; Richard, 1984; Vassal *et al.*, 1986). These researchers showed that the development of microbial flora and the main physico-chemical and biochemical characteristics during ripening of Camembert were not the same on the surface and the center of the cheese.

Considering that very little work has been done on Camembert made from pasteurized cow's milk, this study dealt with the quantitative changes of the microbial flora (total flora, mesophilic lactic bacteria, molds and yeasts, halotolerant bacteria, total coliforms, and enterobacteria) and the main physico-chemical characteristics (pH, total solids, sodium chloride, fat, and proteins) and biochemical (proteolysis and lipolysis) during ripening of Camembert made from pasteurized cow's milk.

Materials and Methods

CHEESE MANUFACTURE AND SAMPLING: Camembert cheese was manufactured following the method of Kosikowski (1982). Whole milk from Sultan Qaboos University Agricultural Experiment Station was pasteurized (85°C/15s) and cooled to 32°C. The milk was inoculated at 2.5% with a mixed lactic starter culture (a combination of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) and 0.01% *Penicillium caseicolum* spore powder (Chr. Hansen's Lab, Denmark). Calf rennet extract was added at a ratio of 4.5-6 ml single strength rennet per 45 kg of milk. When the curd was formed, it was held 3 times the length of coagulation period and then cut. The curd was maintained at 32°C for 1 hour and then loaded into plastic cheese molds. The molds were maintained for about 3 hours to allow whey separation and build strength and pliability to the curd. A fine mist of *P. camemberti* was then sprayed over the full curd. After 30 min, the curd was dry-salted and left to air-dry for 1-2 days at 14°C. The cheeses were then held in a curing chamber set at 14°C and about 85% RH. The cheeses were turned every 2 to 3 days and wrapped at 14 days.

The cheeses were approximately 130 mm in diameter and 60 mm high. Three samples were taken randomly from the surface and the center (inner part) of the curd for analysis at regular intervals during the ripening process (30 days in this study). The center samples were taken by slicing of the cheese block. The analyses were run in duplicates.

MICROBIOLOGICAL ANALYSES: The change of the micro-flora during ripening was studied on the surface as well as in the center of the cheese. After separating the surface from the center of the curd, each was homogenized in a blender, and 10 g was dissolved in 90 ml of sterile 2% sodium citrate solution heated at 45°C (Schmidt and Lenoir, 1978). The appropriate dilutions prepared were then incubated in duplicates. Plate counts were carried out of total aerobic organisms using plate count agar (30°C for 48 h) (Harold, 1976); mesophilic lactic acid bacteria using Elliker medium (30°C for 48h) (Leveau *et al.*, 1991); halophilic flora using MSA agar (30°C for 48 h); enterobacteria using violet red bile agar (37°C for 24-48 h) (Beerens and Luquet, 1987); coliform bacteria using brilliant green bile agar (37°C for 24-48); and molds and yeasts using potato dextrose agar acidified with tartaric acid to pH 3.5 (30°C for 3 days) (Harold, 1976).

PHYSICAL AND CHEMICAL ANALYSES: The development of the physico-chemical and biochemical characteristics during ripening was measured on the surface and the center of the curd. Dry matter was determined by oven drying at 105°C (IDF-FIL, 1982). The total and soluble nitrogen at pH 4.6 in 0.5 M trisodium citrate with a pH of 7.0 were measured by the method described by Gripon *et al.* (1975). The kinetics of global proteolysis was followed by the ratio of soluble nitrogen (SN) to total nitrogen (TN). Fat was determined by the Gerber-Van Gulik method (Kleter, 1976; Trujillo *et al.*, 1999). Free fatty acids was determined using the rapid method of Gallois and Langlois (1990). Sodium chloride analysis was carried out using the International Dairy Federation (IDF-FIL, 1979). The pH was measured with a Beckman pH-meter by immersing the electrode into a blend of 10 g of grated cheese sample with 50 ml of distilled water.

Results and Discussion

QUANTITATIVE DEVELOPMENT OF THE DIFFERENT MICROBIAL GROUPS DURING RIPENING: The development of the main microbial groups involved in the ripening of the cheese is shown in Table 1. It is apparent from the results that each group of microorganisms underwent a characteristic development on the surface and the center of the cheese. At the beginning

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TABLE 1
Main microbial groups during ripening of Camembert cheese.

Type of flora (log cfu.g ⁻¹)		Cheese ripening time (days)					
		1	6	11	17	23	30
Total flora	S	7.72 ± 0.33	7.77 ± 0.76	7.86 ± 0.17	7.94 ± 0.38	7.93 ± 0.27	7.91 ± 0.19
	C	7.70 ± 0.82	7.68 ± 0.63	7.66 ± 0.91	7.63 ± 0.52	7.67 ± 0.46	7.57 ± 0.28
Lactic Mesophilic flora	S	7.61 ± 1.03	7.71 ± 1.24	7.72 ± 0.95	7.69 ± 0.79	7.63 ± 0.85	7.43 ± 0.28
	C	7.61 ± 0.87	7.34 ± 1.13	7.08 ± 1.32	7.00 ± 1.19	7.04 ± 0.89	7.28 ± 0.88
Halotolerant flora	S	4.80 ± 0.46	4.96 ± 0.72	5.34 ± 0.63	5.56 ± 1.01	7.04 ± 0.94	7.18 ± 1.15
	C	4.47 ± 0.29	4.74 ± 0.76	4.54 ± 1.12	4.52 ± 1.31	4.57 ± 1.64	4.51 ± 1.23
Coliforms	S	3.56 ± 1.04	3.56 ± 1.16	3.40 ± 0.99	3.45 ± 1.65	3.53 ± 1.93	3.49 ± 1.27
	C	3.58 ± 0.93	3.18 ± 1.42	3.08 ± 1.15	3.15 ± 0.95	2.99 ± 1.74	3.18 ± 1.33
Enterobacteria	S	3.14 ± 1.03	3.00 ± 1.06	2.98 ± 1.54	2.94 ± 1.60	2.88 ± 1.09	2.79 ± 1.24
	C	2.61 ± 1.56	2.38 ± 1.27	2.28 ± 1.92	2.11 ± 1.85	1.96 ± 1.25	1.84 ± 1.86
Yeasts and molds	S	4.67 ± 1.52	5.96 ± 1.75	5.95 ± 2.02	5.92 ± 1.78	5.85 ± 2.36	5.85 ± 2.16
	C	4.51 ± 0.98	4.63 ± 1.05	4.76 ± 1.60	4.61 ± 1.45	4.53 ± 1.94	4.54 ± 1.43

S - Surface. C - Center.

of ripening, the total microflora count was the same on the surface and in the center of the curd. After eleven days, however, it became more important on the surface (7.3×10^7 cfu/g) compared to that of the center (4.6×10^7 cfu/g). This numerical trend persisted until the end of ripening. The difference may be explained by the progressive neutralization of the cheese surface by the mold (*P. caseicolum*), thus allowing the rapid implantation of a halotolerant and acid-sensitive flora that is maintained at a high level during the ripening stage (Hassouna *et al.*, 1996).

Mesophilic lactic flora, responsible for the degradation of residual lactose into lactic acid, constitutes the dominant microflora of the surface and the center during the first 23 days of ripening. They represent about 50% of the total flora. However, their relative importance decreased as ripening progresses, accounting for only 33% of the total flora at the end of ripening. The decrease may be the result of the competition of a halophilic flora comprised mainly of micrococci, coryneform bacteria, and fecal streptococci (Lenoir, 1963a; Richard and Zadi, 1983; Richard, 1984). Their number reached 1.5×10^7 cfu/g of cheese at the end of the ripening period, representing 19% of the total flora on the surface.

In the ripening of Camembert cheese, the development of the halotolerant flora is very typical. Being aerobic and acid-sensitive, they grow actively on the surface (their initial surface population of 6.3×10^4 increased to 1.5×10^7 cfu/g at the end of ripening). In the center of the curd, the proliferation of halotolerant bacteria is relatively low since the maximum measured population on day 23 of ripening amounted to 3.7×10^4 cfu/g. Because of the dominance of lactic acid bacteria,

the center of the curd remained acidic during ripening, making it difficult for halotolerant and acid-sensitive bacteria to compete.

Besides their well-known role in the development of aroma (and flavor) and on the modification of the texture during ripening of Camembert, yeasts and molds (mainly *P. camemberti*) also act as the agents responsible for the progressive surface neutralization of the cheese. They metabolize residual lactic acid, thus allowing the surface proliferation of micrococci whose number at the end of ripening was a hundred times more than that of yeasts and molds.

With respect to the contaminating flora (coliforms and enterobacteria in general), their number was higher on the surface than in the center at all ripening stages. Lenoir (1963a) reported that the number of coliforms varies significantly from one Camembert to another and even from one sample to another for the same lot of Camembert.

QUANTITATIVE DEVELOPMENT OF MAIN PHYSICO-CHEMICAL AND BIOCHEMICAL PARAMETERS DURING RIPENING: The results of the development of the main physico-chemical parameters of Camembert during ripening, on the surface and the center of the cheese, are shown in Table 2.

The total solids, both on the surface and the center of the cheese, increased continuously during the ripening period. The increase was due to surface evaporation and the exchange of volatile products (water, ammonia, fatty acids, and other volatile compounds) between the cheese and its environment (Hassouna *et al.*, 1996). At the end of ripening, the total solids reached the mean value of 52.86 g/100g of

TABLE 2

Development of main physico-chemical characteristics in the surface(S) and center (C) during ripening of Camembert cheese.

Physico-chemical parameter		Cheese ripening time (days)					
		1	6	11	17	23	30
pH	S	4.81 ± 0.09	4.89 ± 0.12	5.37 ± 0.13	5.96 ± 0.13	6.22 ± 0.13	6.38 ± 0.11
	C	4.76 ± 0.10	4.82 ± 0.10	5.04 ± 0.12	5.20 ± 0.15	5.20 ± 0.15	5.60 ± 0.14
Total solids ¹	S	47.20 ± 1.08	49.36 ± 1.24	50.62 ± 1.38	51.28 ± 1.65	52.63 ± 1.58	53.80 ± 1.72
	C	47.13 ± 1.23	47.98 ± 1.01	48.72 ± 1.24	49.51 ± 1.41	51.49 ± 1.33	51.92 ± 1.51
Total nitrogen ¹	S	2.93 ± 0.06	3.01 ± 0.08	3.12 ± 0.08	3.48 ± 0.10	3.76 ± 0.07	4.08 ± 0.08
	C	2.93 ± 0.07	2.95 ± 0.09	3.08 ± 0.09	3.27 ± 0.12	3.71 ± 0.09	3.89 ± 0.11
Soluble nitrogen ¹	S	0.18 ± 0.12	0.29 ± 0.09	0.41 ± 0.12	0.61 ± 0.25	0.93 ± 0.23	1.05 ± 0.07
	C	0.21 ± 0.14	0.21 ± 0.18	0.32 ± 0.14	0.56 ± 0.16	0.79 ± 0.19	0.88 ± 0.05
Degreee of proteolysis ²	S	6.14 ± 1.41	9.63 ± 1.05	13.14 ± 2.72	17.53 ± 1.90	24.74 ± 2.36	25.92 ± 2.05
	C	7.16 ± 1.80	7.12 ± 1.40	10.39 ± 1.95	13.70 ± 1.58	21.29 ± 1.89	22.62 ± 1.86
NaCl ¹	S	0.41 ± 0.28	4.22 ± 0.46	2.35 ± 0.38	2.19 ± 0.35	2.28 ± 0.45	2.26 ± 0.57
	C	0.22 ± 0.13	0.17 ± 0.29	1.47 ± 0.70	2.08 ± 0.46	2.24 ± 0.50	2.33 ± 0.34
Fat ³	S	48.15 ± 1.15	48.03 ± 1.30	47.92 ± 1.08	47.83 ± 1.31	47.27 ± 1.09	47.16 ± 1.12
	C	48.15 ± 0.98	48.12 ± 1.42	47.86 ± 1.17	47.75 ± 1.19	47.07 ± 1.35	47.10 ± 1.27
Total free fatty acids ⁴	S	8.79 ± 0.32	8.82 ± 0.37	14.65 ± 0.61	22.39 ± 0.53	25.90 ± 0.60	27.69 ± 0.61
	C	8.75 ± 0.26	8.80 ± 0.31	13.12 ± 0.48	17.25 ± 0.45	18.48 ± 0.55	19.51 ± 0.51

¹In g/100 g of cheese.

²In g/100 g of soluble nitrogen/100 g of total nitrogen.

³In g/100 g of solids.

⁴In meq/100 g of fat.

cheese, the end of ripening, the total solids reached the mean value of 52.86 g/100g of cheese, considered normal for this type of cheese. The total solids affect the texture of finished products (Vassal *et al.*, 1986; Hassouna and Guizani, 1995).

Figure 1 shows the concentration of sodium chloride on the surface and center of the curd during ripening. Immediately after salting, the concentration of salt on the surface and in the center were 4.22% and 0.71%, respectively. A progressive diffusion of salt from the surface to the center will take place thereafter due to the existence of concentration gradient of the salt between the two zones, that persisted until day 23 of ripening. It shows migration of water from the center to the surface

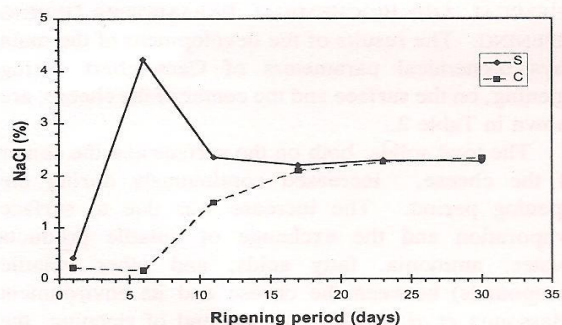


Figure 1. Changes in NaCl content during ripening of Camembert cheese (S - Surface and C - Center).

(Hardy, 1987). From day 23 to day 30, the salt/water ratio showed a stable value of approximately 49 g/kg for both zones of cheese.

The fat content, expressed on a dry-basis, varied relatively very little during the ripening process in both the surface and the center of the curd. The relative constancy of fat may be attributed to the weak lipolysis reaction that takes place in surface mold-ripened cheeses. In surface ripened cheeses such as Camembert, lipolysis touches only 3 to 5% of the total fat (Choisy *et al.*, 1987). In this study, the total reduction of fat during the entire ripening period was 0.99 and 1.05 g/100g of total solids for the surface and the center of the curd, respectively.

The pH of the cheese increased progressively on the surface and the center of the curd during the ripening process. This could be attributed to the assimilation of the lactic acid and the deamination of the amino acids by the mold (*P. camemberti*). Thus, as the mold neutralized the acidity of the cheese, the pH increased (Lenoir, 1984). It is worthy to note that deacidification was more pronounced on the surface than in the center of the cheese at all ripening stages. Thus, the pH increased 1.57 units on the surface whereas the overall increase was only 0.84-pH units in the center.

The variation of proteolysis, on the surface and the center of the cheese, during ripening is shown in Figure 2. At the beginning of ripening, the soluble nitrogen was 6.14% and 7.16% of the total nitrogen on the

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surface and center, respectively. This soluble nitrogen fraction is mainly the result of the proteolytic action of rennet at a pH of 4.6 (Lenoir, 1963a; Gripon *et al.*, 1975).

During maturation, a net difference in proteolytic activity appeared between the surface and the center of the cheese. On the surface, the soluble nitrogen increased after 6 days (from 6.14% to 9.63%) while the degree of proteolysis remained constant at the center. From day 6 to day 23, the solubilization of nitrogen became very fast on the surface (from 9.63% to 24.74%) and at the center (from 7.12% to 13.72%). After day 23, surface proteolysis was slowed down after achieving the maturation of the cheese. However, in the center of the cheese, proteolysis continued at a slightly higher rate than on the surface.

Besides the obvious action of chymosin on the degradation of casein during ripening, the kinetics and the nature of proteolysis in Camembert depend mainly on the action of proteases secreted by the *Penicillium* (Choisy *et al.*, 1987; Cerning *et al.*, 1987) and of *mesophilic streptococci* (Le Bars *et al.*, 1975; Desmazeaud *et al.*, 1976; Desmazeaud and Vassal, 1979). Many micrococci also possess a very important proteolytic power (Lenoir, 1963b; Richard and Zadi, 1983; Choisy *et al.*, 1987).

In this type of cheese, total proteolysis appears to be 1.2 to 1.5 times higher on the surface than in the center. The protein content at the end of ripening averaged the value of 500 g.kg⁻¹ of dry solids, considered normal for this type of cheese (Hassouna and Guizani, 1995). It is worthy noting that at the end of ripening, the average soluble protein content (23%) was relatively similar to that reported by Lenoir (1963b) for pasteurized Camembert aged 24 days but less than that reported for raw Camembert aged 34 days. Heat-treatment of milk could slow down the proteolysis of Camembert by modifying the total flora and the proteolytic system of raw milk. These were observed for pressed cooked-cheeses obtained from mildly heated milk (Berdaque *et*

al., 1990; Hassouna and Masrar, 1995).

Finally, a parallel is observed between the variation of soluble nitrogen and that of pH during ripening. This can be due to the double activity, proteolytic and deacidifying, of *P. camemberti* during its growth (Vassal *et al.*, 1986).

Conclusion

Quantitative microbial results showed that each group of microorganisms undergoes a characteristic variation on the surface as well as the center of the cheese at the different steps of ripening of Camembert from pasteurized cow's milk. Proteolysis and lipolysis were greater on the surface of the cheese than in the center. This was mainly due to the presence of a surface flora rich in yeasts and molds (*P. camemberti*) and in halotolerant bacteria equipped with an important proteolytic and lipolytic power. The neutralization of the surface of the cheese by yeasts led to the formation of a pH gradient between the surface and the center, which was maintained during all ripening process. In return, the gradient of salt concentration disappeared after about 3 weeks.

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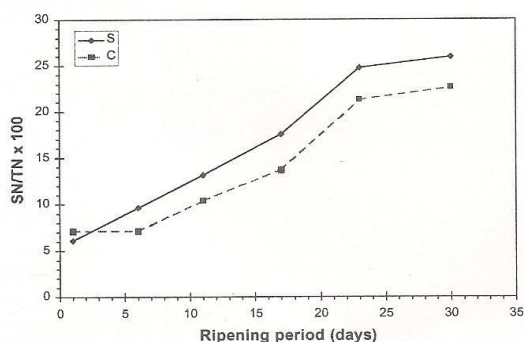


Figure 2. Development of proteolysis during ripening of Camembert cheese (S - Surface and C - Center).

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