ASSESSMENT OF PROTEOLYSIS AND LIPOLYSIS INTENSITY IN PECHERSKY CHEESE RIPENING IN THE PRESENCE OF *PENICILLIUM CAMEMBERTI* AND *PENICILLIUM ROQUEFORTI* MOLDS

Yu. T. Orlyuk and M. I. Stepanishchev*

Institute of Food Resources, National Academy of Sciences,
ul. M. Raskovoi 4A, Kiev, 02660 Ukraine,
*phone/fax: (38044) 517-08-92, e-mail: stepanischev@i.ua

(Received January 22, 2014; Accepted in revised form March 4, 2014)

Abstract: High intensity of proteolysis and lipolysis in the curd due to the activity of mold enzymes is characteristic of mold-ripened cheeses. The intensity of proteolysis and lipolysis in cheese curd during the ripening process of Pechersky cheese containing two types of mold has been investigated in order to delineate the optimal production parameters. The results showed that the intensity of enzymatic processes in Pechersky cheese was higher than in Roquefort and Camembert cheeses. This is due to the bidirectional ripening of Pechersky cheese, with mold *Penicillium roqueforti* mediating the ripening starting from the center of the block and the mold *Penicillium camemberti* mediating the ripening starting from the surface of the block. The data obtained allow for a reduction of the ripening time of Pechersky cheese to 21 days.

Keywords: *P. camemberti, P. roqueforti*, Pechersky cheese, proteolysis, lipolysis

UDC 637.3
DOI 10.12737/4128

INTRODUCTION

The consumption of mold-ripened cheeses in the CIS has shown a tendency to increase during the last ten years. Unfortunately, domestic companies produce a limited range of such cheeses and the demand for these cheese varieties is met only partially. Production of mold-ripened cheese is more profitable than that of hard cheeses due to the lower cost of raw materials per unit of final product. Soft cheeses, including mold-ripened cheese, account for up to 40% of cheese production volume in Western Europe [1]. The share of such cheeses in the total production volume in the world is increasing every year due to their high biological value and unique organoleptic characteristics. According to the experts' estimates, cheeses with white surface mold account for about (7–8)% of the total cheese production volume in Europe and for about (2-3)% of world production volume. French companies alone produce more than 300 000 tons of cheese with white surface mold per year.

Unstable quality parameters remain the main disadvantage of domestic mold-ripened cheeses. The use of foreign technologies for the production of mold-ripened cheese cannot provide for stable quality parameters, and therefore modifications taking the features of the domestic production facilities into account must be introduced into the technologies. Thus, improvement of the existing domestic technologies for the production of mold-ripened cheese and the development of new technologies are tasks of high priority.

The presence of mold microflora possessing high proteolytic and lipolytic activity is characteristic of mold-ripened cheese [2]. Biochemical processes that occur in cheese during ripening are associated with the development of microorganisms and their enzymatic activity that depends on many factors, such as active acidity, redox potential and water activity of the cheese mass, and ripening conditions (temperature, relative humidity, and intensity of air exchange in the maturation chamber) [3, 4, 5]. Proteolysis and lipolysis processes that determine the organoleptic characteristics of cheese can be controlled by selecting the technological parameters of cheese production and the regimen of cheese ripening [6, 7, 8]. Proteolysis is a critical process in the production of all kinds of cheeses, since it is responsible for the structural changes and has a significant effect on the formation of cheese taste and flavor. Intensity of proteolysis is much higher in mold-ripened cheeses than in other types of cheese; for example, the content of soluble nitrogen in blue cheese amounts to (50–65) mass % [9]. The level of proteolysis in cheese with white surface mold is rather high, although lower than that in blue cheese [4, 10]. The content of soluble nitrogen in the outer part of the block of mature Camembert cheese is 35% of the total nitrogen content, and that in the middle of the block is 25% [4, 10].

Free fatty acids are formed in the cheese mass during lipolysis, and the volatile fatty acids among them account for the flavor and taste of the cheese.

OBJECTS AND METHODS OF RESEARCH

Proteolysis in ripening cheese was evaluated by monitoring the content of nitrogen compounds (total nitrogen, total soluble nitrogen) according to the modified Kjeldahl method developed at VNIIMS (All-Union Research Institute of Butter and Cheese Production). Qualitative and quantitative composition of amino acids in cheese was determined by ion exchange

chromatography using the amino acid analyzer Biotronik LC 2000 (Germany). Samples for chromatography were prepared by hydrolyzing cheese samples with 6 n. hydrochloric acid at a temperature of (108 ± 2)ºC for 24 hours and further evaporation in vacuo at a temperature of 45ºC. Caseins and cleavage products thereof were detected by electrophoresis in polyacrylamide gels. Sample preparation included removal of fat by hexane extraction, drying, and dissolving the proteins obtained in a buffer (pH 8.3) with sodium dodecyl sulfate and β-mercaptoethanol. Spectrophotometry was used to quantitate the protein fractions. The densitograms obtained were processed using computer software Image Pro Gel Analyzer, Version 2.0, and Total Lab 1D.

Lipolysis intensity was assessed by measuring the amount of free fatty acids (FFA) and volatile fatty acids (VFA). VFAs were quantitated using distillation; namely, 30 ml sulfuric acid were added to a cheese sample (5 g), the mixture was distilled and titrated with 0.1 n. sodium hydroxide solution, and FFAs were analyzed by chromatography using a Kupol 55 device (Russia).

RESULTS AND DISCUSSION

Samples of cheese (Pechersky, Roquefort and Camembert) for the experiment were produced from normalized milk with a fat content (f.c.) of 3.2 %. The milk was pasteurized at (72 ± 2)°C for (15–20) s. The milk was cooled to fermentation temperature of (32 ± 1)°C, and calcium chloride together with milk-clotting enzyme was added to it. The curd was cut and kneaded for 30 min in the case of Camembert, 40 min in the case of Pechersky, and 60 min in the case of Roquefort, to obtain a granular curd. The duration of curd treatment varied due to the different requirements to water content (w.c.) in the cheese samples, namely, 60% by weight in Camembert, 50% in Pechersky, and 45% in Roquefort. The processed curd was placed into self-pressing forms. After self-pressing and the increase of active acidity in the cheese mass to (4.6 ± 0.1) pH units, cheese blocks were salted in brine during 80 min in the case of Camembert, 120 min in the case of Pechersky, and 180 min in the case of Roquefort, to obtain cheese curd with a salt content (s.c.) of 1, 2 and 3 mass. %, respectively. The salted cheese blocks were dried for (60–120) min. Holes of 3 mm in diameter were pierced in Roquefort and Pechersky cheeses to provide conditions for the development of *Penicillium roqueforti* mold. Cheese blocks were placed into maturation chambers with relative air humidity of (94–96)% and air temperature of (8 ± 0.5)°C for Roquefort, (10 ± 0.5)°C for Pechersky cheese, and (12 ± 0.5)°C for Camembert. *Penicillium roqueforti* mold (produced by the company Danisco) was added into the cheese mass during the molding of cheese blocks. *Penicillium camemberti* mold (produced by Danisco) was applied to the surface of the cheese block by spraying. Changes in proteolysis and lipolysis parameters of Pechersky cheese samples during ripening were assessed by comparing the parameters with those of Camembert and Roquefort cheeses produced under similar conditions.

Proteolysis during ripening of the test cheese samples was assessed by measuring the ratio of soluble nitrogen content to the total nitrogen content (Fig. 1).

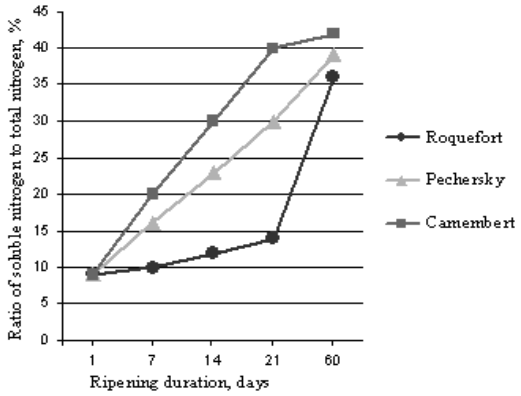

Fig. 1. Changes in the soluble nitrogen: total nitrogen ratio in test samples of cheese during ripening.

Graphic processing of the results showed that the above named ratio equaled 39% for Camembert cheese samples and 30% for Pechersky cheese samples on day 21 of ripening, this being more than twice higher than the respective parameter for Roquefort cheese (14%). Proteolysis intensity in test samples of cheese was also evaluated using information on the content and composition of free amino acids in these samples. The content of free amino acids in test samples of cheese on day 21 of ripening is illustrated by Fig. 2. Analysis of the results showed that total content of free amino acids in the samples of Pechersky cheese was 23% lower than in samples of Camembert cheese, with the most pronounced differences noted for aspartic acid, serine, glutamic acid, proline, alanine, phenylalanine, and lysine, and 19% higher than in Roquefort cheese, with the most pronounced differences noted for aspartic acid, valine, methionine, isoleucine, and leucine.

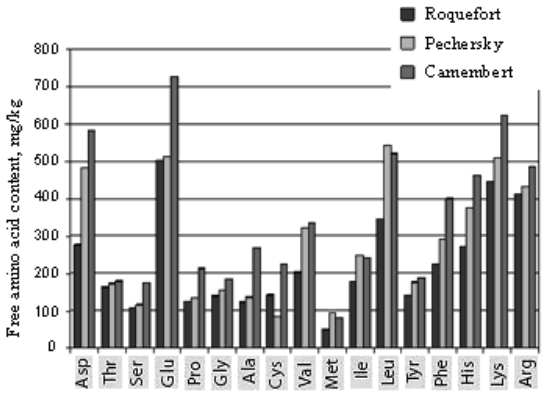

Fig. 2. Amino acid composition of experimental samples of cheese on day 21 of ripening.

Results of the analysis of fractional composition of proteins in cheese samples during ripening are shown in Table 1.

Table 1. Content of protein fractions in cheese during ripening

CHEESE SAMPLE	FRACTIONAL COMPOSITION OF PROTEINS, %					
	Peptides 120-70 kDa	α-casein	β- casein	Peptides 28-26 kDa	Peptides 20-18 kDa	Peptides 16-12 kDa
Roquefort						
After self-pressing	3.26	41.48	37.52	5.39	5.28	1.07
Day 21 of ripening	6.86	34.01	29.84	9.62	10.31	3.78
Camembert						
After self-pressing	3.41	40.85	36.21	5.64	6.37	1.74
Day 21 of ripening	12.24	19.20	27.19	16.85	15.57	4.35
Pechersky						
After self-pressing	3.88	41.02	36.73	5.24	5.93	1.52
Day 21 of ripening	12.21	25.02	28.57	12.72	12.37	3.56

Analysis of the results revealed a tendency to a decrease of the number of casein fractions in test samples during the ripening of cheese. The content of α-casein decreased by 18% in Roquefort cheese, by 53% in Camembert cheese, and by 39% in Pechersky cheese on day 21 of maturation. The content of β-casein decreased from 37.52 % to 29.84 % in Roquefort cheese, from 36.21 % to 27.19 % in Camembert cheese, and from 36.73 % to 28.57 % in Pechersky cheese.

The content of FFAs (mg per kg cheese) was measured to assess lipolysis during the ripening of test cheese samples. Graphic processing of the results showed that the content of FFAs in cheese on day 21 of ripening equaled 400 mg/kg for Camembert, 4500 mg/kg for Pechersky cheese, and 3000 mg/kg for Roquefort cheese (Fig. 3). Low FFA content in Camembert cheese is due to lower lipolytic activity of enzymes from the mold *P. camemberti*, as compared with those of the mold *P. roqueforti.*

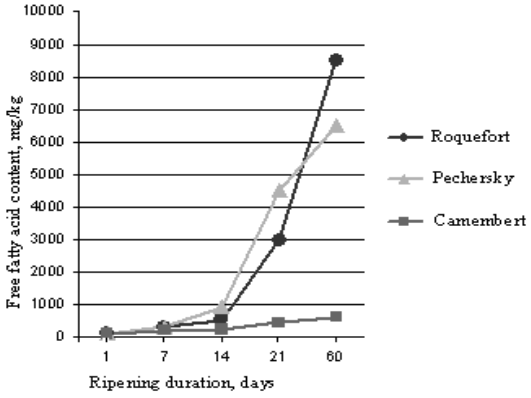

Fig. 3. Changes in the level of lipolysis in Roquefort, Pechersky, and Camembert cheese during ripening.

The fatty acid composition was determined on day 21 of ripening, in order to perform a more detailed assessment of lipolysis in test samples of cheese (Fig. 4).

The results revealed a high content of palmitic, stearic, oleic, linoleic, and linolenic acids in Roquefort and Pechersky cheeses. The latter three acids are essential, and their content in Roquefort and Pechersky cheeses amounts to 30% of the total FFA content.

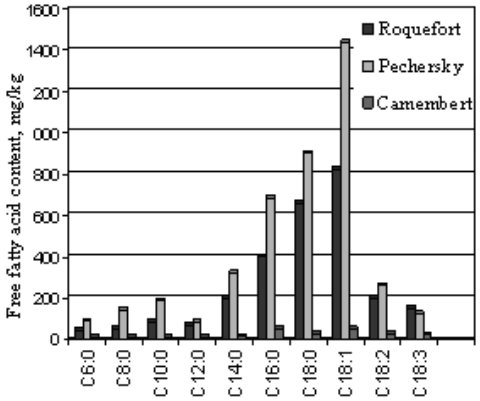

Fig. 4. Fatty acid composition of Roquefort, Camembert and Pechersky cheeses on day 21 of ripening.

The content of VFAs in test samples of cheese at different stages of ripening was assayed as well (Fig. 5).

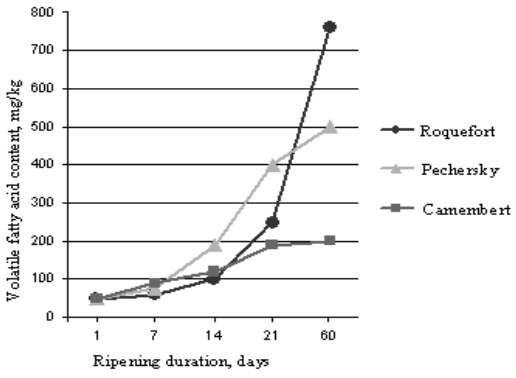

Fig. 5. Changes of the content of volatile fatty acids during ripening of the cheeses under investigation.

The results showed that VFAs accumulate in all test samples during ripening. A significant increase of

VFA content in Roquefort cheese (from 243 to 765 mg/kg) was observed between the 21-st and 60-th days of maturation, while in Camembert cheese it occurred between days 1 and 21 of maturation (from 44 to 178 mg/kg), and the increase of VFA content in Pechersky cheese was intense throughout the ripening period (from 44 mg/kg on day 1 to about 500 mg/kg on day 60). VFA content in Roquefort, Camembert and Pechersky cheese increased 6.1-, 4.2- and 8.6-fold, respectively, during the first 21 days of ripening, this being indicative of a higher enzyme activity of a combination of two types of mold (*P. camemberti* and *P. roqueforti*).

Pechersky cheese occupies an intermediate position between the blue cheeses of Roquefort type and white-mold cheeses of Camembert type. The studies performed allowed for the conclusion that the proteolysis level in Pechersky cheese ripened for 21 day reaches that of mature Camembert cheese (which usually ripens within 14 days), and the lipolysis level of Pechersky cheese ripened for 21 day is close to that of mature Roquefort cheese (ripened for 60 days). Simultaneous development of two types of mold during the ripening of Pechersky cheese allows for faster ripening and the production of cheese with unique organoleptic characteristics.

REFERENCES

1. Shergina, I.A., Klassifikatsiya i osobennosti proizvodstva myagkikh syrov (Classification of soft cheeses and specific features of their production), *Syrodeliye i maslodeliye* (*Cheesemaking and butter-making*), 2008, no. 4, pp. 8–9.
2. Singer, S., The History and Processing of Cheese, Charleston: Bazaar Press, 2011.
3. Tamime, A.Y., Processed Cheese and Analogues, New York: John Wiley and Sons, 2011.
4. Law, B.A. and Tamime, A.Y., Technology of Cheesemaking, New York: John Wiley and Sons, 2011.
5. Carroll, R. and Hobson, P., Making Cheese, Butter and Yogurt, North Adams: Storey publishing, 2012.
6. Pitt, J.I. and Hocking, A.D., Fungi and Food Spoilage, Berlin: Springer, 2009.
7. Heather, P., The Life of Cheese: Crafting Food and Value in America, University of California Press, 2012.
8. Langley Sammis, J., Cheese Making, Memphis: General books, 2012.
9. Lison, K., The Whole Fromage: Adventures in the Delectable World of French Cheese, New York: Crown Publishing group, 2013.
10. Chen, L.-S., Ding, Q.-B., Ma, Y., Chen, L.-G., and Maubois J.-L., The effect of yeast species from raw milk in China on proteolysis in Camembert-type cheese, *Food and Bioprocess Technology*, 2012, vol. 5, pp. 2548–2556.