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CHARACTERISTICS OF CAMEMBERT-TYPE CHEESE RIPENING PRODUCED FROM MILK IN WHICH COMPLEX BETWEEN CASEIN AND WHEY PROTEIN IS FORMED

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Abstract: The Camembert-type cheese was produced from milk in which complex between casein and whey protein is formed by heating at 87°C during 10 min. After cooling to 40°C, 0.35% yogurt culture, 400 mg/l $CaCl_2$, suspension of *Penicillium candidum* culture and rennet were added to milk. Cheese ripening occurred during 20 days in ripening room at 10°C and humidity of 90-95%.

The yield of cheese increased because of great total nitrogen matter utilisation due to the formation of co-aggregates, namely nitrogen matter content of whey was 0.0651%, which is significantly less related to traditional manufacturing.

The deepest changes during ripening were observed in milk proteins, as indicated by high value of ripening coefficient (maturity index). At the end of 20 days' ripening, the soluble nitrogen content was 83.97%, i.e. it was 8.76-fold greater than at the beginning of ripening. The pH of cheese showed permanent increase, it arose from 4.02 to 5.82 during investigated ripening period. Titratable acidity decreased during the first ripening stage (1-10 days), from 237.73°T to 146.18°T, due to protein breakdown induced by proteolytic system of *Penicillium Candidum* and lactic acid neutralisation. At the second stage of ripening, titratable acidy increased to 190.13°T at the end of ripening period.

The sensory characteristics of cheese (aroma, flavour and texture) were characteristic of this cheese type.

Key words: Camembert, casein-whey proteins complex, ripening, soluble nitrogen, acidity.

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Introduction

Camembert belongs to the group of cheeses with white moulds. For the manufacture of some soft and semi-soft cheese varieties, moulds are used with a role to enhance aroma and flavour, and to improve texture of cheese. The presence of moulds on cheese surface influence the specific appearance of cheese, while a great biochemical activity of moulds influence typical aroma and taste of cheese (Gripon, 1990). The white moulds, namely *Penicillium camemberti, Penicillium candidum and Penicillium caseicolum* are widely used for camembert-type cheese production.

There are a great number of investigations of cheese production from heat-treated milk (Law, 1981). Almost all of milk components undergo some transition during heat treatment of milk. The most investigated change is the formation of complex between casein and whey protein, which is formed when milk is heated above 85°C (Elfagm and Wheelock, 1977, Elfagm and Wheelock, 1978, Havea et al., 1998, Paulsson and Dejmek, 1990, Mottar et al, 1989, Parnell-Clunies et al., 1988). The formed complex is known as milk protein co-aggregates. The co-aggregates could be easily precipitated by organic and inorganic acids, and $CaCl_2$, respectively, or by their combination to form co-precipitates of milk protein (Maćej, 1983, Maćej et al., 1998, Maćej et al., 2000, Muller, 1971, Mulvihill and Donovan, 1987). Also, proteins from milk fat membranes react with whey proteins during heat treatments of milk (Kim and Jimenez-Flores, 1995, Corredig and Dalgleish, 1996, van Boekel and Folkerts, 1991). According to aforementioned, there is a clear explanation for the higher utilisation of total milk proteins and fat, and a greater yield of cheese (Maćej et al., 1996, Jovanović et al., 1996, Maćej and Jovanović, 1998, Maćej, 1992, Pudja et al., 1995, Ghosh et al., 1999) manufactured from heat treated milk.

There are a great number of problems during the manufacture of cheeses from milk in which milk protein co-aggregates are formed. Our earlier investigations (Maćej, 1989, Maćej, 1992, Maćej and Jovanović, 1999) show that the problems in this field are very complex and associated with the following issues:

a) The formation of milk protein co-aggregates during different milk heat treatment;

b) The influence of milk heat treatment on the enzymatic coagulation of milk during cheesemaking;

c) The requirements for the production of curd with a good rheological characteristics;

d) The changes during ripening.

In addition, during our earlier investigations we showed that quality white cheese in brine could be produced from milk in which co-aggregates of milk protein were formed. (Maćej, 1989, Maćej, 1992, Maćej and Jovanović, 1999). In these experiments, we investigate the possibility of using heat-treated milk for Camembert-type cheese production, with the following objectives:

a) To increase cheese yield and rentability related to traditional manufacture, due to higher milk protein utilisation;

b) To enhance cheese nutritive value because of whey protein as co-aggregates remain in cheese;

c) To improve cheese sensory characteristics;

d) To increase cheese variety on the market.

Materials and Methods

Milk used for production of Camembert-type cheese was obtained from the dairy "Beograd" PKB – Imlek.

The following analyses of milk were performed:

a) Standard drying method at 102 ± 2°C , (IDF Standard 21B:1987)

b) Milk fat determination – standard method according to Gerber, (Carić et al., 2000)

c) Total proteins content – photometric method by using "Promilk" instrument

d) Determination of titratable acidity according to Soxlet-Henkel, (Carić et al., 2000)

e) Determination of milk pH, with pH-meter Sentron 1001

f) For milk density determination lactodensimeter was used, (Carić et al., 2000)

Technological process of Camembert production from milk in which milk protein co-aggregates are formed:

The raw milk was heat treated at 87°C during 10 min, then cooled to 40°C, and 0.35% yogurt culture, 400 mg/l $CaCl_2$ and suspension of *Penicillium candidum* culture were added to milk. The amount of rennet, which is needed for coagulation to be finished during 60 min, was added to milk. When coagulation was finished, and gel achieved a desired strength, gel was cut into a cube 2-3 cm in size, and left for 20 min, the time needed for hardening of cubes. The curd was then fulfilled into linen percolator, to allow whey separation by self-pressing during 20 min, then to round molds, which were especially made for this kind of cheese. During moldings, cheese was salted with dry salt. Molds were rolled every 15 min during 3 hours, to allow further whey separation. After that, cheese was surface sprayed with suspension of *Penicillium candidum* culture and left at room temperature for the next 20 hours. Cheese was then flipped into ripening room where humidity was 90-95% and temperature 10°C. After 10 days, cheese was packed in PE (poly-ethilene) foil to prevent cheese drying.

Cheese was analyzed on 1^{st}, 10^{th} and 20^{th} day.

The following analyses of cheese were performed:

a) Titratable acidity according to Terner's method, (Carić et al., 2000)

b) Determination of pH during cheese ripening - with pH-meter Sentron 1001

c) Determination of total solids by standard drying method at 102 ± 2°C, (IDF Standard 4A:1982)

d) Determination of milk content according to Van Gulik method, (Carić et al., 2000)

e) Determination of NaCl content according to Mohr method, (Hojman, 1972).

f) Determination of total nitrogen matter by Kjeldahl method, (IDF Standard 20A:1986)

g) Determination of soluble nitrogen matter, (Kuchroo and Fox, 1982)

h) Ripening coefficient (maturity index) was calculated as percent of soluble nitrogen in total nitrogen matter

The following analyses of whey were performed:

a) Determination of total solids by standard drying method at 102 ± 2°C , (IDF Standard 4A:1982)

b) Determination of fat content – standard method according to Gerber, (Carić et al., 2000)

c) Determination of total nitrogen matter by Kjeldahl method, (IDF Standard 20A:1986)

d) Titratable acidity determination according to Soxlet-Henkel, (Carić et al., 2000)

e) Determination of pH with pH-meter Sentron 1001

f) For whey density determination lactodensimeter was used, (Carić et al., 2000)

Statistical analysis was performed. All data for investigated parameters are shown as mean values. Also, analyses of variance for all data were performed (Standard deviation and coefficient of variation).

Results and Discussion

The quality parameters of milk used for the production of Camembert are shown in Table 1. The quality parameters of whey are shown in Table 2.

T a b . 1. - The parameters of milk quality

Investigated parameters	Calculated parameters		
	$\bar{x}$ (n=6)	Sd	Cv
TS[1] (%)	12.07	0.3827	3.17
Fat (%)	3.41	0.4533	13.29
NFTS[2] (%)	8.66	0.1135	1.31
Proteins (%)	3.05	0.0935	3.07
Titratable acidity (°SH)	6.45	0.1658	2.57
pH	6.54	0.0303	0.4634
ρ[3]	1.0308	0.0007	0.07

[1] TS- total solid; [2] NFTS – non-fat total solid; [3] ρ - density

The investigations show, Table 2, greater utilisation of milk nitrogen matter when complex between casein and whey protein is formed. The gained whey, on average, contains 0.0651% nitrogen matter, which is significantly less related to traditional manufacture. The average fat content is 0.08%, and compared with data for fat content gained in traditional manner, it could be concluded that not only the greater utilisation of nitrogen matter occur but also milk fat. According to M a ć e j , 1992, M a ć e j and J o v a n o v i ć , 1999, when white cheeses are produced from heat-treated milk, the milk fat utilisation is by 64.71% greater compared with traditional way of production.

T a b . 2. - The parameters of whey quality

Investigated parameters	Calculated parameters		
	$\bar{x}$ (n=6)	Sd	Cv
TS[1] (%)	6.19	0.0274	0.44
Fat (%)	0.08	0.0250	31.25
Nitrogen mater (%)	0.0651	0.0008	1.27
Titratable acidity (°SH)	8.95	2.4632	27.52
pH	5.44	0.3773	6.93
ρ[2]	1.0268	0.0004	0.04

[1] TS- total solid; [2] ρ- density

G h o s h et al., 1999, produced Camembert-type cheeses in a traditional way and by using stronger heat treatment of milk (80°C during 3 min). These authors found significant increase of total solids (66.06%) and protein (91.07%) utilisation compared with traditional manner (61.06% for total solids and 84.54% for protein).

The changes of nitrogen matter during ripening

The most important changes occur in proteins during Camembert-type cheese ripening. Proteolytic processes that occur during Camembert-type cheese ripening are very complex, because many proteinases are involved (Trieu-Cuot and Gripon, 1982). According to Fox, 1981, proteinases which participate in ripening, could be arraged into three major groups:

a) The native (indigenous) milk proteinases (especially plasmin);

b) The endogenous proteinases coming from microorganisms, and

c) The exogenous proteinases, which are added to milk (e.g. milk-clotting enzymes).

The surface microflora, especially *Penicillium caseicolum* and *Penicillim camemberti*, play the essential proteolysis role. The most important proteinases from *Penicillium caseicolum* and *Penicillim camemberti* are aspartyl- and metallo-proteinases, which belong to the exocellular proteolytic system (Gripon, 1990, Trieu-Cuot and Gripon, 1982a). As a result of action of these proteinases on casein, protein fractions, which include protein fraction insoluble at pH 4.6, are formed, (Trieu-Cuot and Gripon, 1982a). Trieu-Cuot et al., 1982b, found that *Penicillium caseicolum* proteinases hydrolyse ß-casein into 5 main degradation products. The activity of metallo-proteinases is detectable immediately after the development of the moulds (7 day), while the aspartyl-proteinases activity is detectable 3 days later. In this period, the amount of peptides originating from the action of metallo-proteinases on ß-casein slowly decrease, while the amount of ß-asp fragments (98-209 amino acids residue of ß-casein) increase, as a consequence of the action of aspartyl-proteinases (Trieu-Cuot and Gripon, 1982a,b).

Lenoir et al., 1979, suggested that small peptides could migrate from surface to the center of the cheese. These authors observed the presence of ß-asp peptides in cheese center after 35 ripening days. The molecular weight of these peptides is 12.000-35.000.

The results of total nitrogen and soluble nitrogen matter dynamics during ripening are shown in Tables 3 and 4.

Tab. 3. - Dynamics of total nitrogen during ripening

Cheese age (days)	Total nitrogen (%)			Total nitrogen in cheese dry matter (%)		
	Calculated parameters					
	$\bar{x}$ (n=6)	Sd	Cv	$\bar{x}$ (n=6)	Sd	Cv
1.	2.211	0.1207	5.46	6.031	0.1756	2.91
10.	2.335	0.2760	11.82	5.943	0.2070	3.48
20.	2.854	0.2078	7.28	5.684	0.2001	3.52

T a b . 4. - Dynamics of soluble nitrogen matter during ripening

Cheese age (days)	Soluble nitrogen (%)			Soluble nitrogen in cheese moisture (%)		
	Calculated parameters					
	$\bar{x}$ (n=6)	Sd	Cv	$\bar{x}$ (n=6)	Sd	Cv
1.	0.2127	0.0298	13.99	0.3392	0.0652	19.23
10.	1.1567	0.0518	11.62	1.9277	0.2240	11.62
20.	2.3903	0.1464	6.13	4.8523	0.5631	11.60

Cheese contains some soluble nitrogen matter, mean being 0.2127%, as early as on the first ripening day. However, the amount of soluble nitrogen matter is manyfold greater on 10^{th} ripening day, which indicates high intensity of proteolytic processes in this period. Mean soluble nitrogen is 1.1567%, i.e. it is 5.44-fold greater. Such intensive proteolytic processes mainly results from the action of *P. candidum* proteinases, due to the intensive growth of *P. candidum* between 4^{th} and 10^{th} day. Between 10^{th} and 20^{th} days of ripening, there is very intensive increase of soluble nitrogen matter, mean being 2.3903, i.e. 2.07-fold greater compared with 10^{th} day and even 11.24-fold compared with 1^{st} day of ripening. Table 5 shows calculated data for the ripening coefficient.

T a b . 5. - Dynamics of ripening coefficient for Camembert-type cheese

Cheese age (days)	Ripening coefficient Calculated parameters		
	$\bar{x}$ (n=6)	Sd	Cv
1.	9.59	0.8795	9.17
10.	50.14	5.6378	11.24
20.	83.97	4.8328	5.75

It could be concluded that percent of soluble nitrogen matter in total nitrogen matter increase during ripening period. The ripening coefficient is 9.59 for 1^{st} day and rises 5.23-fold on 10^{th} day, and even 8.67-fold on 20^{th} day. Mean of ripening coefficient is 83.97% at the end of ripening stage.

Our results correlate well with the results of other authors. F u r t a d o et al., 1984, found that after three weeks of ripening Camembert contains about 70% of soluble nitrogen matter. They found by electrophoresis that 86% of αs1-casein and 35% of ß-casein were hydrolysed. K a t a o - K a et al., 1987, found the soluble nitrogen matter increase from 4.8 on to 25.7 mg/g cheese (i.e. 79% of total nitrogen matter) between week 2 and 4 of ripening. According to N u k a d a et al., 1986, soluble nitrogen matter content increases from 6.2-12.2 on to 56.0-87.2% during the ripening period of 30-45 days.

Table 6. shows some values of ripening coefficient, Maćej (1989).

Tab. 6. - Ripening coefficient for some cheese varietes, (Maćej, 1989)

Cheese type	Author	Ripening coefficient (%)
Emmental cheese	Dilanjan	31.39
Cheddar	Van Slyke and Price	31.50
Edam cheese	Dilanjan	26.90
Kachkaval cheese (3-4 months)	Djordjević	14.88
Kachkaval cheese (6 – months)	Djordjević	17.53
Somborski cheese	Petrović	22.36
Guculska bryndza	Rudovskaja	33.40
White soft cheese	Živković	9.46
White soft cheese	El Demardash	10.46
White soft cheese	Mišić	20.72
Novosadski cheese	Todorović	13.01
Limburger	Bondžinski	75.87
Romadur	Bondžinski	81.16
Backsteiner	Inihov	27.50
Roquefort	Čebotarev	48.37
Roquefort	Dilanjan	52.50
Brie	Dilanjan	47.10
Camembert	Čebotarev	89.00

It could be seen from data in Table 6. that there are the most intensive proteolytic changes during Camembert cheese ripening, as well as that cheeses with moulds have the biggest ripening coefficient.

Dynamics of titratable acidity and pH

The acidity is one of the most important factors that influences ripening processes, which, on the other hand, influences titratable acidity. However, there both influence product quality. The acidity of Camembert-type cheese influences the moulds growth because they manifest a greater growth in acid environment. It is possible, based on dynamics of cheese acidity, to evaluate the intensity of biochemical processes in cheese as well as of proteolytic processes.

Fig. 1 shows the results for dynamics of titratable acidity and pH of cheese.

Titratable acidity of 1-day old cheese was very high, mean being 237.73°T, while pH was 4.02. The aim of applied technology was to develop high acidity during first ripening day, because it influences faster moulds growth and decreases cheese moisture content. It is well known that co-aggregates based cheese release water faster at the beginning of ripening.

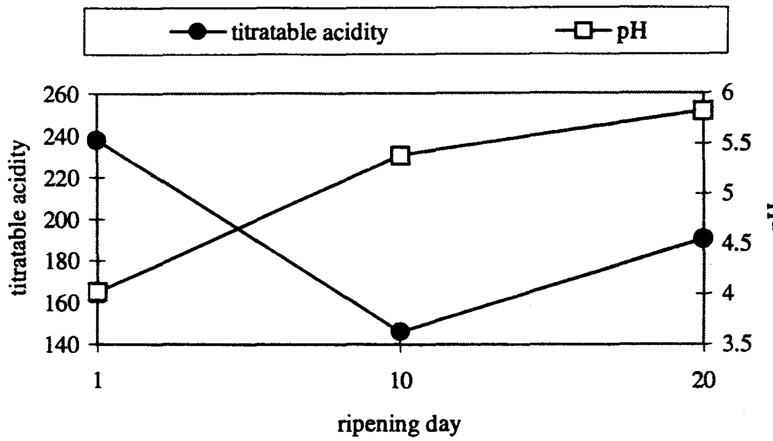

Fig. 1. - Dynamics of titratable acidity and pH during ripening

After 10 ripening days, titratable acidity decreased by 91.55°T, and its mean was 146.18, while pH increased by 1.36 pH-units (mean 5.38).

Further ripening results in increase of both titratable acidity and pH. At the end of investigated ripening period, mean for titratable acidity was 190.13°T, while pH was 5.82.

It could be concluded, based on the gained results, that the great part of lactose was fermented during the first ripening day, as indicated by high value of titratable acidity and low pH value. Further titratable acidity dynamics of cheese primary results from the proteolytic changes and less from lipolytics changes. A large number of primary proteolytic products is formed during the first ten ripening days. These products could react with lactic acid, which influences decrease of titratable acidity. However, between 10th and 20th ripening day, the proteolytic processes were still more intensive. Taking into account dynamics of titratable acidity it could be concluded that a considerable number of other buffer matter, i.e. secondary proteolytic products, is formed in the second ripening stage, which influences increasing of titratable acidity. Nevertheless, titratable acidity was lower at the end than at the beginning of ripening.

The intensive proteolysis during the first ten days resulted in a considerable increase of pH, i.e. pH increased to 5.38, therefore the greatest buffer capacity of cheese (5.1-5.3) is exceeded. Our results agree with the results of other authors.

Noomen, 1978, found positive correlation between pH value at the 5.1-6.0 interval and breakdown of ß-casein, and suggested that plasmin probably contribute to a greater extent of protein degradation in Camembert cheese during ripening. According to Katao-Ka et al., 1987, pH increases to the optimum of 6.0 during ripening. Softness of cheese increased from the surface to the centre, which is a major characteristic of Camembert-type cheese. The results of Nukada et al., 1984, show increase of pH from 4.7-5.2 to 6.9-8.0 during ripening for 30-45 days, as well as a good correlation between ripening coefficient and increase of pH.

Conclusion

According to the above stated, the following conclusions could be drawn:

a) Total nitrogen matter content of cheese shows an increase during ripening period, while total nitrogen content in dry matter slowly decreases during ripening, probably as a consequence of the volatile matter production due to deeper nitrogen matter breakdown;

b) The gained whey contains, on average, 0.0651% nitrogen matter, which is significantly less related to traditional manufacture;

c) The deepest changes occur in milk proteins. As a consequence of protein breakdown, soluble nitrogen matter content shows permanent increase, thus the percent of soluble nitrogen matter in total nitrogen matter is 83.97%, or 8.76-fold greater than on the first day. Sensory characteristics of cheese (aroma, flavour and texture), which result from deep proteolytic changes are very pronounced and characteristic of this cheese type.

d) The titratable acidity on the first ripening day was high, mean being 237.73°T, while pH was 4.02, which was the aim of the applied technology. Titratable acidity decreased during the first ripening stage (1-10 day) from 237.73°T to 146.18°T, due to protein breakdown, while pH increased to 5.38. As a result of such intensive protein breakdown, there was softening of cheese body from the surface to the centre of cheese. Both pH (5.82) and titratable acidity (190.12°T) increased at the end of ripening period.

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KARAKTERISTIKE ZRENJA SIRA TIPA KAMAMBERA PROIZVEDENOG OD MLEKA KOD KOGA JE OBRAZOVAN KOMPLEKS IZMEDJU KAZEINA I SERUM PROTEINA

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Rezime

Sir tipa Kamamber proizveden je od mleka kod kojeg je zagrevanjem na temperaturi od 87°C u toku 10 minuta obrazovan kompleks između kazeina i serum proteina. Na temperaturi od 40°C dodato je 0.35% jogurtne kulture, 400 mg/l $CaCl_2$, suspenzija *Penicilium candidum* i sirilo. Zrenje sira je bilo na temperaturi od 10°C, pri relativnoj vlažnosti od 90-95%, u vremenu od 20 dana.

Sadržaj azota u surutki iznosio je 0.0651% i bio je manji u odnosu na surutku koja se dobija pri tradicionalnoj proizvodnji sireva, što je imalo za posledicu i veći randman.

Najdublje promene za vreme zrenja odvijale su se na proteinima. Nakon 20 dana zrenja sadržaj rastvorljivog azota u ukupnom azotu iznosio je 83.97% i bio 8.76 puta veći u odnosu na prvi dan zrenja. Kao rezultat izraženih proteolitičkih procesa i pH vrednost sira se permanentno povećavala. Na kraju ispitivanog perioda zrenja pH vrednost sira je iznosila 5.82. Senzorne karakteristike sira (ukus, miris, konzistencija) bile su karakteristične za ovu vrstu sira.

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