

THE INFLUENCE OF MILK-CLOTTING ENZYMES ON THE FUNCTIONAL PROPERTIES OF PIZZA-CHEESES

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ABSTRACT

The effect of the type and dose of milk-clotting enzymes (Chy-max® M based on recombinant camel chymosin, Fromase® TL based on Rhizomucor miehei protease) on the physicochemical, functional properties and shelf life of pizza-cheeses was studied. When using a low dose of milk-clotting enzymes (MCE) for milk coagulation (250–1100 IMCU per 100 kg of milk), cheeses were obtained with an increased moisture content (55–57%), excessive acidity (pH 4.8–4.9) and texture defects (incoherent, crumbly, with separation of free moisture). This is due to the formation of a weak curd, which releases moisture poorly during processing. The use of an increased dose of MCE makes it possible to obtain a denser curd, better releasing moisture. Cheese produced with a high dose of milk-clotting enzymes (2000–2800 IMCU per 100 kg of milk) had a lower moisture content (52–53%) and lower acidity (pH 5.0–5.1). The protein matrix is more hydrated in these cheeses, which ensures its better water holding capacity and a more homogeneous and cohesive texture. The use of an increased dose of MCE with a high total proteolytic activity (Fromase) gives undesirable consequences in the form of accelerated proteolysis of cheese mass proteins, rapid loss of functional properties of the cheese, and a decrease in the shelf life of cheese (less than 60 days). Cheese production using an increased dose of MCE with a low level of total proteolytic activity (Chy-max M) allows achieving a low level of proteolysis during cheese ripening and increasing its shelf life.

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1. Introduction

The production of pizza-cheeses in the world is growing every year. Pizza-cheeses are a marginal product for a manufacturer and are in high demand in the market.

Pizza-cheese is a product with its own specific properties that differ from those of other cheeses. Unlike other types of cheese, pizza-cheese is a semi-finished product and is intended for final consumption in a heated state after baking, therefore it must have certain properties in a normal and heated state.

There are a number of mandatory requirements for pizza-cheeses. Cheese should have a clean taste associated with the taste of young cheeses and a coherent, elastic, moderately dense texture, convenient for cutting and grating (this is important for grinding cheese using industrial machines). Once thawed, the cheese should maintain a cohesive texture without separating free moisture, which is required for cheeses used for frozen pizza. When baked on pizza, the cheese should acquire a liquid flowing texture, not form a burn on the surface, not release incoherent fat and moisture, maintain a coherent fibrous structure that allows the formation of long strands when stretched [1].

The cheese should retain its flavor, texture and properties when baked on pizza for a long shelf life. The fibrous structure, capable of stretching when heated, is a feature of pizza-cheeses (“pasta filata”, “string cheese”). To give cheeses a similar structure, limited decalcification of the paracasein-calcium phosphate complex of the cheese is required, followed by its structuring by mechanical stretching in a heated state [2]. In the process of stretching, the cheese mass is heated in hot water at a temperature of 65–80 °C with simultaneous mechanical action (stretching), which allows the curd mass to be stretched into long strands. During the pasta-filata process, the internal structure of the cheese becomes fibrous, since initially shape-

less protein particles are stretched into fibers, between which fat globules are embedded [3].

Fresh pizza-cheese has a distinct fibrous structure and, when heated, can be stretched into strands up to several meters long, but such cheese is not suitable for use in baked goods. Insufficient hydration of proteins in unripened cheese when baked on pizza leads to the separation of free moisture and fat and strong protein burn. To give the cheese mass a sufficient degree of hydration, the cheese is kept for 2–3 weeks (the cheese is “ripened”). During maturation, under the action of milk-clotting enzymes and ferments of the starter culture, limited proteolysis of caseins occurs, as a result of which their hydration increases. When ripe, pizza-cheese acquires resistance to high temperatures during baking of pizza: free moisture and fat are not melted, a desired slight browning of the cheese occurs [4,5].

The problem is that, in view of the sufficiently high amount of moisture (42–52%), proteolysis of proteins proceeds at a high rate and pizza-cheeses overripe quickly. A high degree of proteolysis increases the degree of protein hydration, which, in combination with the high moisture content of the cheese mass, leads to the formation of a viscous, sticky cheese texture, making it impossible to cut and grate it on the industrial devices. During maturation, proteolysis occurs, accompanied by an increase in protein hydration, the formation of cross-linkages between casein submicelles, and a loss of fibrous structure. As a result, after baking on pizza, overripe cheese acquires an excessively fluid (liquid) texture and does not form characteristic strands of the required density and length when lifted [6,7].

A serious requirement from retail chains and pizza producers is a long shelf life of cheeses. In practice, this can be achieved by reducing the rate of proteolysis during storage of cheese. To increase the shelf life, cheese is stored at low temperatures (freezing). This

method has disadvantages in the form of increased energy consumption and labor costs for subsequent defrosting.

Other ways to limit proteolysis can be:

- ❑ the use of MCE with low proteolytic activity [7,8,9,10];
- ❑ the use of a starter culture with low proteolytic activity [11];
- ❑ reducing the dose of the starter culture (with preliminary acidification of milk with organic acids (citric, lactic) or glucono-delta-lactone) [12,13];
- ❑ reducing the dose of MCE incl. by creating conditions that increase its milk-clotting activity (increase in temperature and decrease in milk pH) [14,15];
- ❑ reducing the dose of MCE by increasing the content of casein in milk (normalization of milk by protein by adding milk powder or milk protein concentrate) [16,17];
- ❑ deactivation of MCE and starter by conducting thermomechanical processing of cheese mass at elevated temperatures (62–66 °C) [2,3].

The use of MCE and a starter culture with low proteolytic activity is the simplest method that does not require adjusting the parameters of the technological process. Based on a literature review [9,18,19,20,21], the Chy-max® M preparation based on the recombinant camel chymosin was chosen as the MCE with low proteolytic activity, and the Fromase® TL preparation based on proteases of the microorganism *Rhizomucor miehei* was chosen as the MCE with high proteolytic activity. The culture *Str. thermophilus* was chosen as the starter culture, which has low proteolytic activity [10, 22]. The use of a thermophilic streptococcus starter assumes the use of a higher temperature (36–38 °C) at the stage of making cheese in the vat than using a mesophilic lactococcus starter culture (30–33 °C). This made it possible to test a method for reducing the dose of MCE, based on increasing the activity of milk-clotting enzymes at an elevated coagulation temperature [23].

The aim of the research was to establish the effect of the type and an introduced dose of milk-clotting enzymes on the functional properties of pizza-cheeses and on their shelf life.

2. Materials and methods

2.1. Materials

The studies used cow's milk from one supplier-manufacturer — AgriVolga LLC (Yaroslavl Region, Uglichsky District, Burmasovo village). In the production of cheeses, we used lactic acid

culture of *Streptococcus thermophilus* based on the bacterial concentrate BK-Uglich-TNV (FGBNU Experimental Biofabrika, Russia) with preliminary activation of the culture on sterilized milk at 30 °C for 18 h. For milk coagulation, MCE brands Fromase® 2200 TL (DSM Food Specialties, France) and Chy-max® M 1000 (Chr Hansen A / S, Denmark) were used.

2.2. Methods

2.2.1. Methods for studying the properties of milk-clotting enzymes

The determination of the total proteolytic activity was carried out in accordance with the Russian state standard (GOST) method (GOST «34430–2018»). The method is based on the hydrolysis of a substrate from bovine hemoglobin in a weakly acidic zone (at pH 5.3) by the studied enzyme preparation to low molecular peptides and free amino acids, followed by termination of the enzyme action by precipitation of the unhydrolyzed protein with trichloroacetic acid (TCA) and determination of the resulting peptides and free amino acids.

The one unit (U) of total proteolytic activity (PA) is the amount of enzyme that, in 1 min at a temperature of 30 °C, brings hemoglobin into a non-precipitated TCA state in an amount corresponding to 1 μmol of tyrosine. Activity is expressed in U/g of enzyme preparation.

The amount of protein converted into low molecular peptides and amino acids is determined by the reaction of free amino acids with Folin-Ciocalteu reagent and further determination of the optical density of the resulting blue solutions on a LEKI model SS1207UV photoelectrocolorimeter (MEDIORA OY, Finland) at a light wavelength $\lambda = 670$ nm.

2.2.2. Cheese production process

The technological regulations for the cheese production are shown in Table 1.

The moment of completion of cheddarization was determined by the readiness of the curd mass for thermomechanical treatment by its ability to stretch when heated in hot water.

After cheddarization, thermomechanical treatment of the cheese mass («stretching») was carried out manually until a coherent homogeneous texture was obtained. After stretching, the cheese mass was formed into blocks in the form of a low cylinder weighing 1.0 ± 0.1 kg and a height of 10 ± 2 cm and sent to aging

Table 1

Technological regulations for the cheese production

Stage of the technological process		Process parameters	
Mass fraction of fat in milk mixture	2.4–2.5%		
Milk pasteurization	10 sec at 72 °C		
Calcium Chloride Dose	Based on 35 g of anhydrous salt per 100 kg of milk		
Dose of lactic acid starter culture	1,5% (v/w)		
Milk acidity before coagulation	pH 6.2±0.1		
Milk-clotting enzyme dose, g/100 kg of milk *	Fromase® 2200 TL	Variant “Low” — 0.125	
		Variant “High” — 1.25	
	Chy-max® M 1000	Variant “Low” — 1.15	
		Variant “High” — 2.30	
Coagulation temperature of milk	37.5±0.5 °C		
Size of the grain edges when cutting	1.0±0.2 cm		
Grain processing temperature	37.5±0.5 °C		
Active acidity of the curd mass at the end of processing	pH 5.10±0.5		
Mass fraction of salt in solution during thermomechanical treatment	5%		
Thermomechanical treatment modes	Brine temperature — 80 °C		
	Duration — 10 min		
	Cheese curd temperature after processing — 50–55 °C		

Note: the maximum application rate was taken in accordance with the recommendations given in the documentation for the MCE. The minimum dose was determined based on the rennet test result [23].

for 16–18 hours at a temperature of 8–10 °C to separate free whey and form a closed surface. After aging, the cheeses were packed on a Henkelman Boxer 42 machine (Henkelman Vacuum Systems, The Netherlands) under vacuum (negative pressure 1 Bar; vacuuming time 20 s) into bags made of Amivak CH-B polymer film (Atlantis-Pak, Russia) and sent for storage at a temperature of 3 ± 1 °C.

2.2.3. Methods for studying the properties of cheeses

Determination of active acidity — 10 g of grated cheese was ground in a powder with 10 ml of deionized water; active acidity was determined using a pH-150MI pH-meter (Measuring equipment, Russia).

Determination of the mass fraction of moisture was carried out by drying at a temperature of 102 ± 2 °C according to the Russian state standard GOST 3626–73.

The degree of proteolysis in cheeses was expressed as a percentage of the absolute content of soluble nitrogen from the absolute content of total nitrogen.

Determination of the mass fraction of total and soluble nitrogen was carried out by the Kjeldahl method according to the Russian state standard GOST R54662–2011.

The extraction of water-soluble nitrogen was carried out according to the method [24] in the modification [25]: 20 g of grated cheese was mixed with 40 ml of deionized water, homogenized on a high-speed FSH-2A homogenizer (Jiangsu Jinyi Instrument Technology Company Limited, China) for 1 min. The homogenized mixture was kept at 40 °C for 1 h with continuous stirring for 100 min⁻¹ on a SK-O180-E orbital shaker (DLAB Scientific Co., Ltd, China). The samples were cooled to 4 °C and centrifuged at 3000 g for 30 min. The upper fat layer was removed, and the supernatant was filtered on cellulose acetate filters with a pore size of 0.2 µm (Vladipor, Russia).

The determination of the molecular weight distribution of soluble nitrogenous substances was carried out by high resolution gel filtration using a Superose 12 10/300 GL column (GE Healthcare, Sweden). The eluent was an aqueous solution of 0.05 M Na₂HPO₄ + 0.15 M NaCl, the flow rate of the eluent was 0.5 ml/min; the detector wavelength was 280 nm.

Determination of the ability of cheese to stretch when heated was carried out by a commercial method by the ability of cheese to stretch when lifting the melted cheese with a fork with an estimation of the length of the resulting threads [26].

The microstructure of the cheeses was investigated by the method of light microscopy in transmitted light, on micro-sections of cheese with a thickness of 100 ± 10 µm.

The experiments were repeated three times.

Mathematical processing was performed using Microsoft Excel and Statsoft Statistica software packages.

3. Results and discussion

In the process of cheese production, the following indicators were studied:

- the duration of the individual stages of the processing in the cheese vat;
- dynamics of changes in the active acidity of milk and milk curd.

The introduced dose of different types of MCE according to the rennet test results and according to the recommendation of the MCE manufacturer (the minimum recommended application dose) are shown in Table 2.

Table 2

Application dose and activity of MCE used in the experiment

Indicator	Type and dose of MCE			
	Fromase		Chy-max M	
	Low	High	Low	High
Application dose, g/100 kg	0,125	1,25	1,15	2,3
Milk-clotting activity, IMCU/g	2200	2200	910	910
Introduced dose into milk, IMCU/100 kg	275	2750	1045	2091
Proteolytic activity of MCE, U/g *	118,6	118,6	0,68	0,68
Application dose of proteolytic activity, U/100 kg	14,82	148,25	0,78	1,56
The degree of retention of MCE in the cheese mass, %	3%	3%	30%	30%
Proteolytic activity in cheese mass, U/ kg **	0,44	4,45	0,23	0,47

Note:

* actual value determined by the method according to GOST 34430–2018.

** calculated proteolytic activity based on the degree of MCE retention in the cheese mass according to [21]: for Fromase — $\leq 3\%$, for Chy-max M — $\geq 30\%$.

In the option when the rennet test was used to calculate the dosage of MCE, it was possible to reduce the introduced dose into milk for MCE Fromase by 10 times, for MMCE Chy-max M — by 2 times. This should lead to a significant decrease in the rate of proteolysis in the cheeses of Fromase Low variant. An even greater effect of reducing proteolysis in cheeses with Fromase can be expected due to the low retention of this type of MCE in the cheese mass. The expected level of proteolytic activity in cheeses with a low level of Fromase application has been reduced to the level of cheeses with a high level of Chy-max M.

Differences in MCE dosage resulted in minor differences at the stage of cheese production in the cheese vat. The duration of the processing operations at the stage of cheese production in the vat is shown in Table 3.

Differences in the introduced dose of MCE affected the duration of coagulation. When using a high dose of MCE, the coagulation time was reduced on average: for Fromase — by 3 times, for Chy-max M — by 2.7 times. The differences between the variants

Table 3

Duration of operations at the stage of cheese production in the cheese vat

Stage of technical process	Type and dose of MCE			
	Fromase		Chy-max M	
	Low	High	Low	High
Fermentation	100,00 ± 16,97	100,00 ± 16,97	100,00 ± 16,97	100,00 ± 16,97
Coagulation	35,67 ± 10,06 ^{a)}	11,67 ± 2,99 ^{b)}	35,33 ± 8,84 ^{a)}	13,33 ± 2,99 ^{b)}
Curd processing before cheddarization	22,67 ± 18,21	18,67 ± 4,53	25,67 ± 17,68	23,67 ± 10,06
Cheddarization	45,00 ± 25,93	71,67 ± 5,66	50,00 ± 9,80	66,67 ± 11,32
Total processing time	203,33 ± 58,88	202,00 ± 20,37	211,00 ± 16,75	203,67 ± 10,06
Thermo-mechanical treatment	10,00 ± 0,00	10,00 ± 0,00	10,00 ± 0,00	10,00 ± 0,00

Note: The mean values and confidence intervals of variation are given for the level of statistical significance $p = 0.05$. The values in the table row with the same indices a) and b) have statistically significant differences ($p < 0.05$). The values in the table row that are not marked with indices do not have statistically significant differences ($p < 0.05$)

Table 4

Dynamics of changes in the acidity of cheeses at the stage of production in the cheese vat and during storage

Stage of technical process	Type and dose of MCE			
	Fromase		Chy-max M	
	Low	High	Low	High
Milk before starter introduction	6,53±0,11	6,50±0,12	6,53±0,11	6,50±0,12
Before coagulation	6,22±0,16	6,19±0,06	6,22±0,16	6,19±0,06
After cutting the curd	5,86±0,08 ^{a)}	6,13±0,04 ^{b)}	5,83±0,13 ^{a)}	6,13±0,07 ^{b)}
Before cheddarization	5,65±0,13	5,98±0,02	5,62±0,08	5,95±0,17
After cheddarization	5,07±0,04	5,16±0,09	5,10±0,13	5,12±0,09
After thermo-mechanical treatment	5,08±0,04	5,11±0,08	5,05±0,08	5,06±0,13
1 day	4,80±0,09	5,04±0,11	4,73±0,13	4,98±0,14
8 day	4,89±0,17	5,07±0,08	4,78±0,11	5,03±0,09
30 day	4,90±0,20	5,08±0,13	4,82±0,06	5,05±0,09
60 day	4,87±0,27	5,06±0,09	4,81±0,11	5,03±0,08

Note: The mean values and confidence intervals of variation are given for the level of statistical significance $p = 0.05$. The values in the table row with the same indices a) and b) have statistically significant differences ($p < 0.05$). The values in the table row that are not marked with indices do not have statistically significant differences ($p > 0.05$).

of cheeses with different types and doses of MCE in terms of the duration of other operations and the total duration of processing in the cheese vat were statistically insignificant ($p > 0.05$).

The dynamics of changes in the acidity of cheeses at the stage of production in the cheese vat and during storage is shown in Table 4.

The data in Table 4 shows that the duration of the processing operations affected the dynamics of pH changes in the cheese vat. Differences in pH between the variants of cheeses produced with low and high doses of MCE were noted only at the moment after cutting the curd, which was associated with the different duration of coagulation in these variants. Despite the fact that there were no significant differences in the pH level between the variants of cheeses after the completion of the pasta-filata process, on the 1st day of storage, the pH level in the cheeses acquired different values during storage.

In cheeses produced with different doses of MCE, differences in pH were noted at the beginning of the shelf life, which was maintained until its completion. Despite the fact that the differences between the cheese variants in terms of the average pH level were not statistically significant ($p > 0.05$), there were clear differences in the texture of the cheeses. The

cheeses with a lower pH, produced with a low dose of MCE (Fromase Low and Chy-max M Low variants), had a layered, kind of incoherent, granular texture with the release of free moisture on the cut. Cheese with a higher pH level, produced with a high dose of MCE (Fromase High and Chy-max M High variants) had a homogenic layered texture without separation of free moisture.

Data analysis was carried out to determine the factors influencing the pH of the cheeses. Since the duration of processing of curd in the cheese vat does not differ between the variants of cheeses (table 3), this factor cannot influence the pH of the cheeses. Other factors affecting the pH of the cheeses may be the pH of the curd after the completion of processing in the cheese vat and the moisture content of the curd. The dependence of the pH of the cheese at the beginning of storage on these factors is shown in Figure 1.

Fig. 2 a) it follows that there is a weak relationship between pH at the end of processing and pH of the cheese at the beginning of storage ($R^2 \approx 0.3$). To a greater extent, the pH of cheeses depends on their moisture content. The dependence shown in Fig. 2 b) shows that there is a strong positive relationship between these indicators ($R^2 > 0.9$).

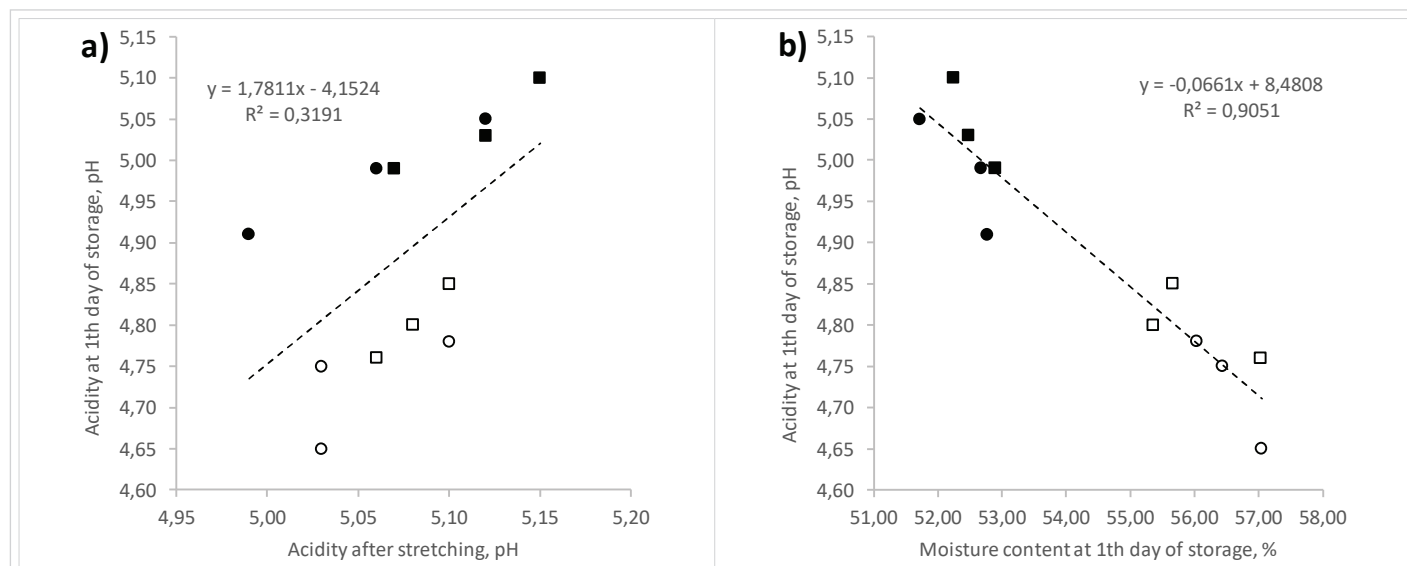


Figure 1. Dependence of the pH of the curd mass on the 1st day of storage on (a) the pH of the curd mass after the completion of processing in the cheese vat, (b) the moisture content in the curd mass on the 1st day of storage for the following cheeses: □ — Fromase Low; ■ — Fromase High; ○ — Chy-max M Low; ● — Chy-max M High; R^2 — coefficient of determination of the regression equation.

In addition, fig. 1 shows that according to the acidity level at the beginning of storage, cheeses are divided into 2 groups: cheeses with a low and high dose of MCE. Cheeses with low dose of MCE have a higher moisture content and lower pH than cheeses with high dose of MCE. This confirms the data of other researchers. In particular, Creamer, et al [27] found that the use of a very low dose of MCE results in a weaker curd, less moisture release and results in a more moist curd.

Consequently, milk coagulation with a low dose of MCE results in a more moist curd mass than using a high dose of MCE. The lactose contained in the aqueous phase of the cheese serves as a source for the formation of lactic acid by microorganisms of the starter culture and a drop in pH. In turn, a low pH level leads to a decrease in the degree of hydration of caseins, a decrease in the water-binding capacity of the cheese mass and the formation of an incoherent texture. When using a high dose of MCE, cheese is obtained with a lower moisture content, with a higher pH and cohesive texture. Since the duration of treatment in the cheese vat does not differ for the variants of cheeses with different doses of MCE, it can be concluded that a high dose of MCE contributes to the production of a denser curd having better syneresis properties.

Figure 2 shows the dynamics of changes in the mass fraction of moisture in cheeses during storage.

The data obtained shows that the pH and mass fraction of moisture in cheeses remains at an approximately constant level from the 1st day until the end of the shelf life. Therefore, the choice of the type and dose of MCE affects the properties of the resulting cheeses already at the stage of processing in the cheese vat.

Another important factor influencing the texture and other functional properties of cheeses is the proteolytic activity of MCE. Studies have shown that a multiple (two or more) increase in the dose of milk-clotting enzyme (rennet) led to an increase in the proteolysis of alpha-S1-casein, the appearance of a more plastic texture and an increase in the severity of the bitter taste in Gouda and Meshanger cheeses [28,29].

Figure 3 shows the dynamics of proteolysis during storage of cheese.

The degree of proteolysis in cheeses during storage depended on the MCE activity in the cheese mass (data in Table 2). The

highest rate and final content of proteolysis products was observed in cheeses produced with a high dose of Fromase. Differences in the dynamics of proteolysis between cheeses made with low dose Fromase and cheeses made with Chy-max M (regardless of dose) were not statistically significant.

Milk-clotting enzymes of different origins (Fromase, based on *R. miehei* protease and Chy-max M based on camel chymosin), apart from differences in the depth of proteolysis, can also give qualitative differences in the composition of proteolysis products. The molecular weight distribution of water-soluble nitrogenous substances (amino acids and peptides) formed as a result of proteolysis by the end of the storage period (60 days) is shown in Figure 4.

From the data obtained on the form of the molecular weight distribution of proteolysis products in cheeses with different doses of MCE (Fig. 4), the following conclusions can be drawn: with a low dose of MCE, there are insignificant differences in the qualitative composition of proteolysis products between MCEs of different origins; with a high dose of MCE, the differences in the nature of proteolysis between MCEs of different origins increase. Fromase with an increased introduced dose forms an increased amount of peptides with a molecular weight >10 kDa. Peptides with such a high molecular weight are tasteless [30]. Proteolysis, accompanied by splitting into large fragments, leads to the rupture of a small number of peptide bonds in a large number of casein molecules that form a protein matrix. This leads to a decrease in the number of spatial bonds at many points of the protein matrix and a decrease in its strength. As a result, the degree of plasticity of the curd is increased. The peptides formed during proteolysis have a higher degree of hydrophilicity than the initial proteins, which leads to an increase in the degree of hydration of the cheese mass.

The cheeses made using Fromase had various degrees of bitter taste: with a low dose –slightly bitter taste, with a high dose – moderately expressed. The cheeses made with Chy-max M did not have a bitter taste at any dose. According to [31], peptides formed during hydrolysis of casein with a molecular weight of less than 6 kDa have a bitter taste. At the same time, with a decrease in molecular weight, the degree of bitter taste of peptides increases. According to [32], peptides with a mass of 2–5 kDa isolated from cheeses have a mild bitter taste, while peptides with

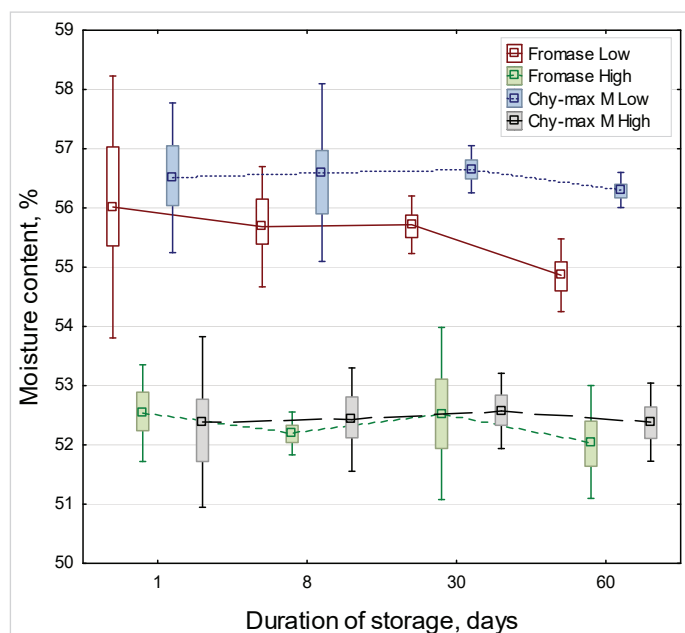


Figure 2. Dynamics of changes in the mass fraction of moisture in cheeses during storage.

Point: Mean; Box: Min-Max; Whisker: Mean \pm 0,95 Conf. Interval

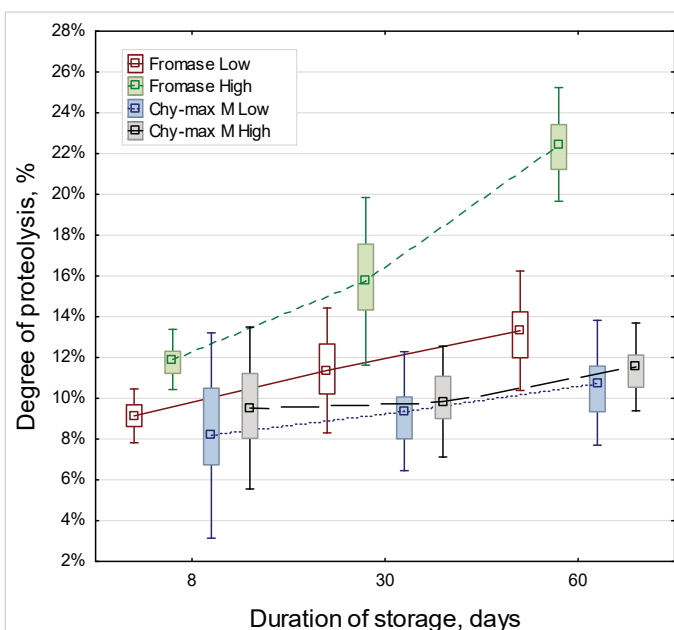


Figure 3. Dynamics of proteolysis (proportion of soluble nitrogen in total nitrogen) in cheeses during storage.

Point: Mean; Box: Min-Max; Whisker: Mean \pm 0,95 Conf. Interval

a mass of less than 1 kDa have the most pronounced bitter taste. The fraction of peptides with a molecular weight of 0.5 kDa to 3.0 kDa had a bitter taste (peptides weighing less than 0.5 kDa did not have a bitter taste) [33]. Peptides with a molecular weight in the range of 3–10 kDa had a less pronounced bitter taste [34].

Cheeses made with Fromase have an increased content of peptides with molecular weights in the range of 0.5–1.0 kDa

and 2.0–5.0 kDa (Figure 4). The presence of these fractions can be the source of the bitter taste in cheeses making with Fromase.

Differences in the degree of proteolysis of cheeses caused differences in the microstructure of cheeses produced with different types and doses of MCE. Photographs of the microstructure of cheeses after 60 days of storage are shown in Figure 5.

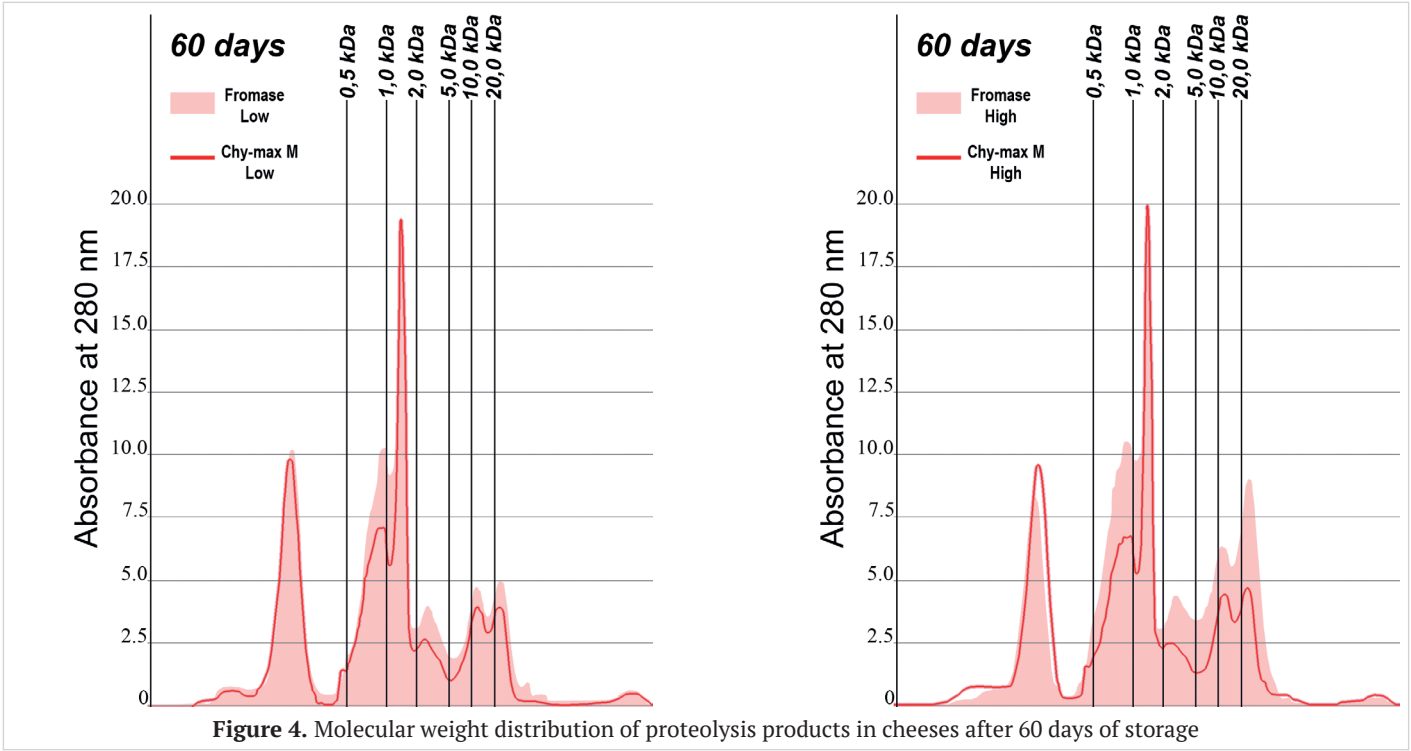


Figure 4. Molecular weight distribution of proteolysis products in cheeses after 60 days of storage

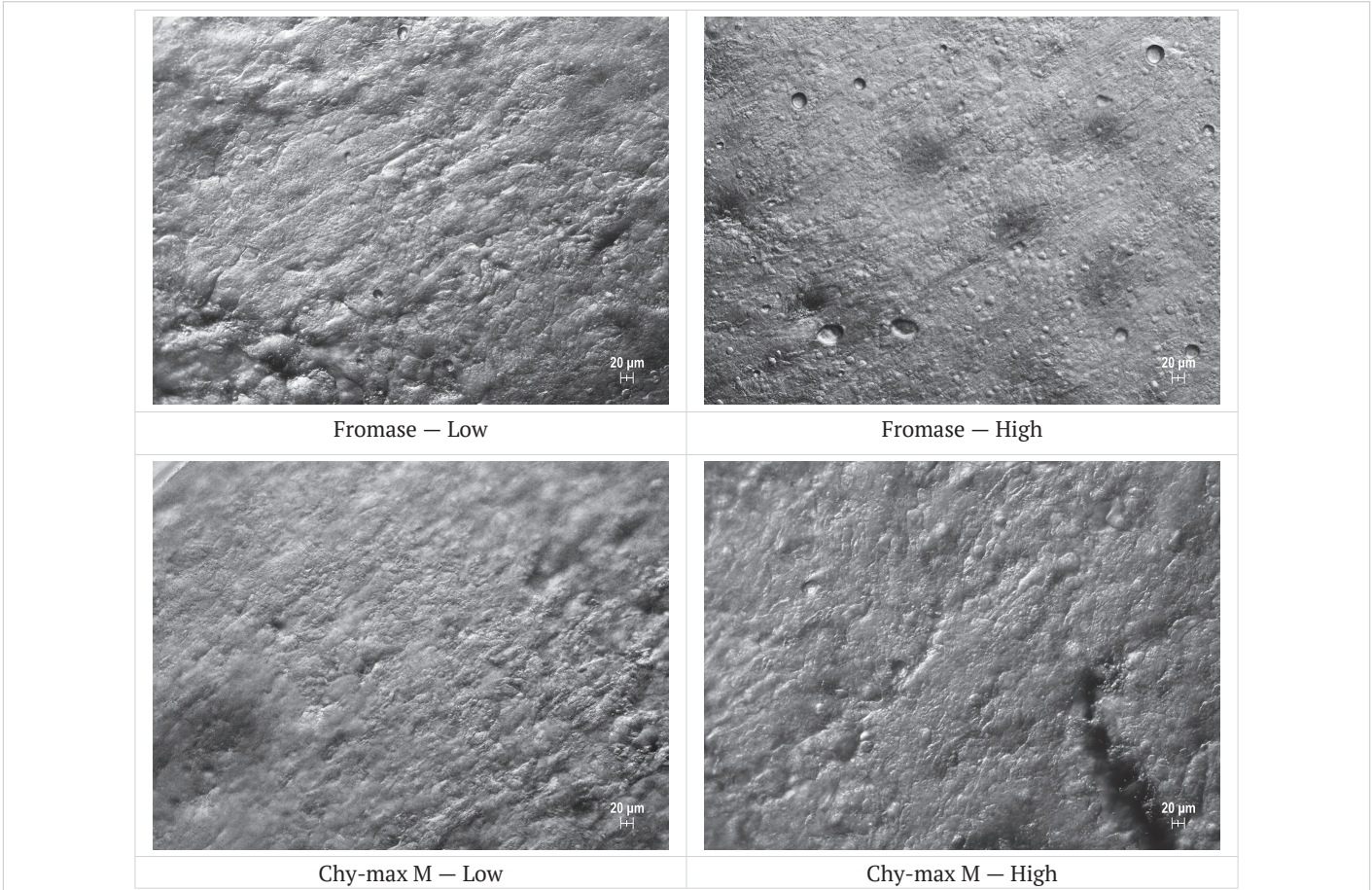


Figure 5. Microstructure of cheeses after 60 days of storage

After stretching processing, the cheese mass has the form of parallel elongated protein fibers with clusters of fat globules and bacteria packed between them [3]. In the process of proteolysis, which occurs in cheese under the action of milk-clotting enzyme and proteolytic enzymes of the starter culture, para-casein decomposes with the formation of water-soluble nitrogenous substances (peptides). As a result of the cleavage of a part of the polypeptide bonds in para-casein molecules, the degree of their hydration increases, and cross-linking between para-casein aggregates increases with the formation of new bonds under the action of the forces of hydrophobic and electrostatic interaction. This leads to the transformation and weakening of the protein matrix of the cheese and a change in the rheological properties of the cheese, which is expressed in an increase in the cohesion and elasticity of the cheese mass. Protein “capsules” surrounding the fat globules are degraded, which leads to the release of fat globules from the casein matrix [3,35].

In the cheese mass, which has undergone strong proteolysis, protein conglomerates, initially in the form of elongated “strands”, swell and the unidirectional fibrous structure of the cheese disappears. This can be seen in the photographs of the microstructure of the cheeses. Cheeses made with Chy-max M and a low dose of Fromase have a low degree of proteolysis and are characterized by a layered structure with pronounced borders between the cheese grains. Cheeses made with a high dose

of Fromase have a high degree of proteolysis and are characterized by a more homogeneous, finely dispersed structure, which is associated with the disappearance of large grains as a result of their hydration.

Differences in the degree of proteolysis in cheeses with different types and dosages of MCE resulted in differences in the structure and texture of these cheeses.

In cheeses with a low dose of MCE, which had a low degree of proteolysis until the end of the storage period, there were no noticeable changes in texture. They retained a pronounced fibrous structure. The disadvantage of the low level of proteolysis in such cheeses was the low degree of protein hydration, due to which the cheeses had an insufficiently coherent texture and contained unbound moisture that was released on the cut.

Cheese with a high level of Chy-max M had a coherent, homogeneous, moderately dense texture. Cheese made with a high level of Fromase, which had the highest degree of proteolysis, acquired a viscous and sticky texture. The use of such cheese for making pizza on a commercial scale is impossible: when sliced, the cheese sticks to the knife, when shredded, cheese chips stick together into lumps.

The appearance of the cheese cut after 60 days of storage is shown in Figure 6.

Tests of the functional properties of cheeses in a heated state were carried out. The results of the fork test of the melted cheese mass are shown in Figure 7.

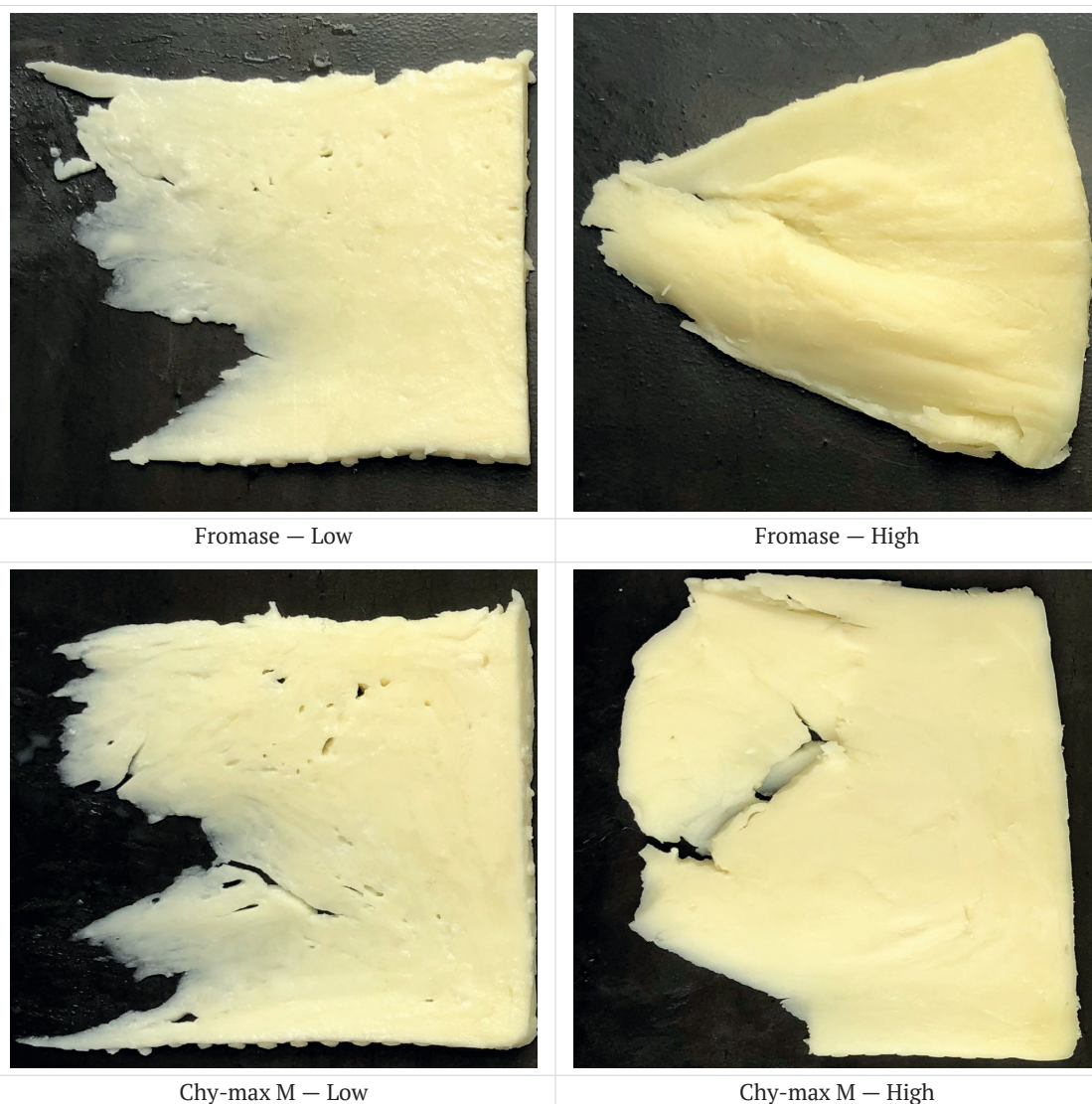


Figure 6. Appearance of the cheese cut after 60 days of storage

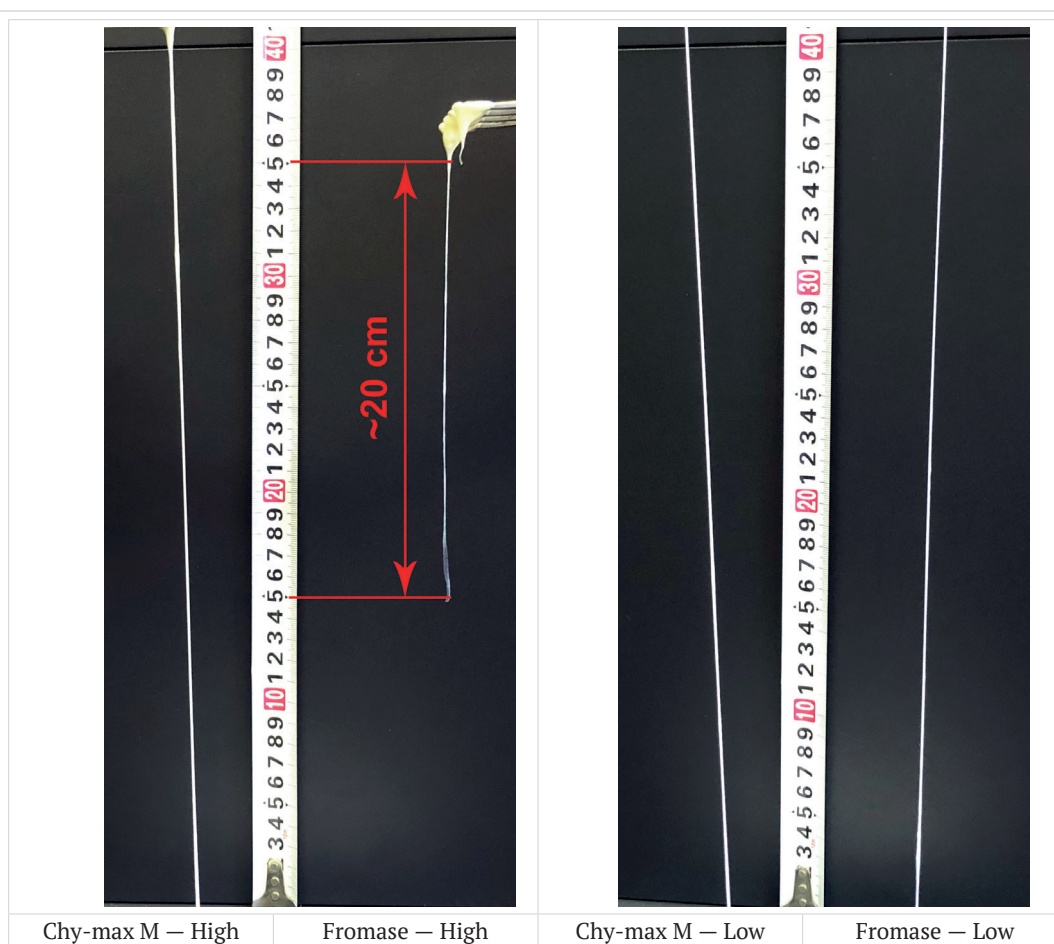


Figure 7. The results of the “fork” test for assessing the ability of the heated curd to stretch

As follows from the data provided in the analytical review, cheese made with a high dose of Fromase and having the highest degree of proteolysis, when heated, acquired an unnecessarily liquid and fluid consistency. The length of the threads formed during the stretching of such cheese did not exceed 20 cm. Cheese that had a low degree of proteolysis (with a low dose of Fromase and with Chy-max M, regardless of the dose), after heating, retained a coherent elastic texture and gave when stretching a thread about 100 cm.

4. Conclusions

When a low dose of milk-clotting enzymes (250–1100 IMCU per 100 kg of milk) was used for milk coagulation, cheeses were obtained with a high moisture content (55–57%), excessive acidity (pH 4.8–4.9) and texture defects (incoherent, crumbly, with separation of free moisture). This is due to the formation of a weak curd, which releases moisture poorly during processing.

The use of an increased dose of MCE makes it possible to obtain a denser curd, better releasing moisture. Cheeses produced with a high dose of milk-clotting enzymes (2000–2800 IMCU per 100 kg of milk) have a lower moisture content (52–53%) and lower acidity (pH 5.0–5.1). The protein matrix is more hydrated in these cheeses, which ensures its better water holding capacity and a more homogeneous and cohesive texture.

The use of an increased dose of a milk-clotting enzyme with a high level of total proteolytic activity (Fromase) gives undesirable consequences in the form of accelerated proteolysis of cheese mass proteins, rapid loss of functional properties of the cheese and a decrease in the shelf life of cheese (less than 60 days). Cheese production using an increased dose of a milk-clotting enzyme with a low level of total proteolytic activity (Chy-max M) allows achieving a low level of proteolysis during cheese ripening and increasing its shelf life.

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