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EFFECT OF THE RECOMBINANT CHYMOSINS OF DIFFERENT ORIGINS ON PRODUCTION PROCESS OF SOFT CHEESE

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milk-clotting enzymes,
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ABSTRACT

The effect of milk-clotting enzymes (MCE) with different milk-clotting activity (MCA) and proteolytic activity (PA) based on recombinant calf chymosins (Chy-max® Extra: MCA=554 IMCU/g; PA=0.71 UA/g), camel (Chy-max® M: MCA=904 IMCU/g; PA=0.68 PA units/g) and "modified" chymosin (Chy-max® Supreme: MCA=912 IMCU/g; PA=0.26 PA units/g) on the duration of milk coagulation, the composition of whey and fresh cheeses in the production of soft cheese of the Italian type Crescenza at a dose of MCE equal to 1500, 2500 and 3500 IMCU per 100 kg of milk was studied. With an equal introduction dose, the shortest average duration of curd formation is noted for Chy-max Supreme MCE (15.0–27.5 min), long one — for Chy-max M MCE (17.0–31.0 min), and the longest one — for MCE Chy-max Extra (18.0–35.5 min). There was no statistically significant effect ($p < 0.05$) of the type and dose of MCE on the total duration of cheese processing, as well as on the content of fat, protein and dry matter of whey. The type and dose of MCE had an impact on the properties of fresh cheeses: cheese options produced with the maximum dose of Chy-max Supreme had a statistically significant ($p < 0.05$) higher average dry matter content (49.61%) than cheese options made with minimal doses of MCE (47.64–47.91%).

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Научная статья

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ВЛИЯНИЕ РЕКОМБИНАНТНЫХ ХИМОЗИНОВ РАЗНОГО ТИПА НА ПРОЦЕСС ПРОИЗВОДСТВА МЯГКОГО СЫРА

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КЛЮЧЕВЫЕ СЛОВА:

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подсырная сыворотка

АННОТАЦИЯ

Исследовано влияние молокосвертывающих ферментов (МФ), обладающих разной молокосвертывающей активностью (МСА) и протеолитической активностью (ПА), на основе рекомбинантных химозинов теленка (Chy-max® Extra: MCA=554 IMCU/г; ПА=0,71 ед. ПА/г), верблюда (Chy-max® M: MCA=904 IMCU/г; ПА=0,68 ед. ПА/г) и модифицированного химозина (Chy-max® Supreme: MCA=912 IMCU/г; ПА=0,26 ед. ПА/г) на продолжительность свертывания молока, а также на состав сыворотки и свежих сыров при производстве мягкого сыра итальянского типа Кресченца при дозе внесения МФ, равной 1500, 2500 и 3500 IMCU на 100 кг молока. При равной дозе внесения наименьшая средняя продолжительность образования сгустка отмечается у МФ Chy-max Supreme (15,0–27,5 мин), большая — у МФ Chy-max M (17,0–31,0 мин) и наибольшая — у МФ Chy-max Extra (18,0–35,5 мин). Отсутствовало статистически значимое влияние ($p < 0,05$) типа и дозы МФ на общую продолжительность изготовления сыров, а также на содержание жира, белка и сухого вещества сыворотки. Тип и доза МФ оказали влияние на свойства свежих сыров: варианты сыров, произведенные с максимальной дозой Chy-max® Supreme, имели статистически достоверно ($p < 0,05$) большее среднее содержание сухого вещества (49,61%), чем варианты сыров, произведенные с минимальными дозами исследованных МФ (47,64–47,91%).

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1. Introduction

The paper deals with the issues of improving the technology of soft cheeses. Cheeses have a high biological value and are

healthy food products. A significant disadvantage of soft cheeses is their short shelf life, which limits the interest in this product on the part of chain stores and reduces its sales. Another im-

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portant point is the high cost of cheeses, which limits consumer demand for them. This article discusses technological methods aimed at solving these problems.

An increase in the shelf life of soft cheeses can be achieved through the use of milk-clotting enzymes (MCE) with low proteolytic activity [1,2,3,4,5]. Among all types of MCE used in the cheese industry, recombinant chymosins have the lowest level of proteolytic activity [6,7]. The group of recombinant chymosins includes chymosins of various origins, which are arranged in the following sequence in terms of MCA/PA values: calf chymosin < camel chymosin < modified camel chymosin [8,9]. The effect of calf and camel chymosins on proteolysis in cheeses has been extensively studied. The results obtained show a distinct decrease in the level of proteolysis and an increase in the shelf life of cheeses when replacing calf chymosin with camel chymosin [10,11,12]. Chymosin-based MCE was put on the market under the brand name Chy-max® Supreme by Chr. Hansen A/S in 2019. Modified chymosin was obtained by artificial change of the amino acid composition of natural chymosin (presumably camel chymosin), which made it possible to lower its PA level [8].

The use of recombinant chymosins in combination with Direct Vat Starters (DVS) opens up a number of new possibilities for cheesemakers. The improvement in the quality of commercial milk that has taken place to date is associated with its insufficient acidity (at pH 6.6–6.7), which is unfavorable for the activity of “traditional” MCEs (calf rennet, microbial proteases, pepsins). Recombinant chymosins are guaranteed to coagulate milk even with the active acidity of fresh milk, therefore, when using them, the starter activation process, which is carried out to reduce the pH of milk, is not required [12]. On the other hand, activation of the starter is conducted not only for acidification, but also for the accumulation of cells of the starter microflora, which is a necessary condition for the rapid metabolization of milk sugar and lowering the pH of the cheese. To compensate for the insufficient volume of starter microorganisms when using DVS without subsequent activation, it is possible to introduce an increased dose of DVS into the milk. Recently, their cost has significantly decreased, and an economically justified opportunity has appeared to use DVS in an increased dosage [13].

As a result of the combination of the use of recombinant chymosins and a high dose of DVS, many beneficial effects are achieved [12,14]:

- exclusion of starter activation eliminates the risk of activation in milk and subsequent reproduction in cheese of foreign microflora, which has retained its viability after pasteurization;
- rapid coagulation of milk prevents the threat of infection of the starter by a bacteriophage due to the impossibility of circulation of bacteriophages in the milk curd, which ensures the stable development and acid formation of the starter;
- rapid coagulation leads to a reduction in the total duration of the processing of grain in the tank;
- later activation of the DVS-starter and later onset of acid formation (after the formation of the curd) leads to less loss of colloidal calcium phosphate by casein micelles and promotes the formation of a dense curd, which quickly separates moisture during the syneresis process, which also reduces the curd processing time and improves the yield of cheese;
- a high dose of starter leads to an acceleration of the acid formation process and a reduction in the time required to reduce the pH of the cheese.

The new advanced technology ensures consistently high cheese quality and increased production profitability. This effect is achieved by minimizing the loss of milk dry matter in the manufacture of cheese, as well as by reducing labor, energy, time

costs for production, including due to the absence of costs for maintaining a starter department with personnel when using DVS-starter. Reducing the duration of the technological process gives an increase in the mass of milk processed at the enterprise per day, which increases the equipment turnover ratio and reduces the payback period for capital expenditures on equipment and premises.

A practical example of the implementation of an improved technology using recombinant camel chymosin and DVS-starter is the technology for the production of soft cheese of the Italian type Crescenza [2,15]. This technology is of the greatest interest for small cheese factories, since it involves the use of whole milk for the manufacture of cheese. This allows a more cost-effective conversion of milk dry matter and a higher yield of cheese with excellent taste. Another advantage of Crescenza cheese is the use of a culture of thermophilic streptococcus. The use of starter from thermophilic streptococcus guarantees a low level of lactic acid formation [13] and a low level of proteolysis during storage [16], so Crescenza cheese has a long shelf life (more than 21 days), which is of interest to chain stores and expands sales opportunities.

In this paper, the influence of MCE based on different types of recombinant chymosins on the processes occurring during the production and storage of soft cheeses, using the example of Crescenza cheese, was studied. The results obtained are of practical interest in terms of improving the technology for the manufacture of soft cheeses, reducing the cost, increasing the yield of cheeses and increasing their shelf life.

2. Materials and methods

2.1. Materials

In the studies, cow's milk was used from one supplier-manufacturer — AgriVolga LLC (Yaroslavl region, Uglich district, Burmasovo village).

In the production of cheese, lyophilized bacterial concentrates of direct application STI-12 and STI-14 (Chr. Hansen A/S, Denmark) based on the culture of *Streptococcus thermophilus* were used as lactic acid starter. To coagulate milk, milk-clotting enzyme preparations (MCEP) of the following brands were used:

- Chy-max® Extra 600 based on recombinant calf chymosin. Nominal MCA 600 IMCU/cm³ (Chr. Hansen A/S, Denmark);
- 1000 based on recombinant camel chymosin. Nominal MCA 1000 IMCU/cm³ (Chr. Hansen A/S, Denmark);
- Chy-max® Supreme 1000 based on recombinant modified chymosin. Nominal MCA 1000 IMCU/cm³ (Chr. Hansen A/S, Denmark).

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 according to GOST 34430–2018¹, as applied to weakly acidic proteases (at pH 5.3).

2.2.2. Methods for studying the properties of milk, whey and cheeses

The active acidity of milk, whey, and cheeses was determined on a pH-meter pH-150MI (ООО Измерительная Техника, Russia). Potentiometric electrodes of a pH meter immersed in the cheese mass measured the active acidity of cheeses during whey draining. The active acidity of cheese after salting was determined in a suspension of cheese, for the preparation of which 10 g of cheese was ground in a mortar with 10 cm³ of deionized water.

¹ GOST 34430–2018 Enzyme preparations for food industry. Method for the determination of proteolytic activity. M.: Standartinform, 2018. — 12 p.

The mass fraction of moisture in cheeses was determined by drying at a temperature of $102 \pm 2^\circ\text{C}$ according to the Russian state standard GOST 3626–73².

The mass fraction of moisture in whey was determined by the method according to GOST 3626–73 (clause 2.3, in relation to milk).

The mass fraction of total protein in milk and whey was determined by the Kjeldahl method according to the Russian state standard GOST 23327–98³.

The mass fraction of total protein in cheeses was determined by the Kjeldahl method according to the Russian state standard GOST R54662–2011⁴.

The mass fraction of fat in milk and whey was determined by the acid method according to the Russian state standard GOST 5867–90⁵.

The mass fraction of fat in cheeses was determined by the acid method according to the Russian state standard GOST R55063–2012⁶.

2.2.3. Methods for controlling the process of milk curd formation

In order to obtain comparable results, the moment when the curd was ready for cutting was determined online using a measuring system based on the Hot-Wire method [17,18].

2.2.4. Cheese production process

Italian soft cheese of the Crescenza type with a fat content in dry matter of at least 55% was made according to the technological regulations reproduced on the basis of data from Alinovi et al [2] (Table 1).

After acidification to final pH was completed, the fresh cheese was salted by immersion in an 18% salt solution for 1 h at a temperature of $4 \pm 2^\circ\text{C}$. After salting, the cheeses were dried overnight (14–16 h) at a temperature of $4 \pm 2^\circ\text{C}$ and a relative humidity of $85 \pm 5\%$, and then packaged. Packing was carried out in an inert gas atmosphere (a mixture of 70% nitrogen and 30% carbon dioxide) into Amivak CH-B polymer film bags (Atlantis-Pak, Russia) using a Henkelman Boxer 42 packaging machine (Henkelman Vacuum Systems). Packaged cheeses were stored at $3 \pm 1^\circ\text{C}$.

2.2.5. Methods of statistical analysis

The study was conducted based on the design of a full factorial experiment [19], which includes two categorical factors that vary at three levels: the factor “brand of MCEP” and the factor “dose of MCEP”. The introduction doses of MCE in the experiment were selected based on the analysis of scientific literature, based on the technical documentation for MCE, as well as the results of our own research [5,20]. Table 2 shows the experimental plan.

The experiments were carried out in triplicate in a randomized order. Mathematical data processing was carried out using the software packages Microsoft Excel and Statsoft Statistica (v. 5.5).

The influence of the factor “MCEP brand” on the response variables “coagulation duration” and “curd elasticity modulus” was assessed using one-way analysis of variance using Tukey’s

² GOST 3626–73 Milk and milk products. Methods for determination of moisture and dry substances. M.: Standartinform, 2009. — 11 p.

³ GOST 23327–98 Milk and milk products. Determination of mass part of total nitrogen by Kjeldahl method and determination of mass part of protein. M.: Standartinform, 2009. — 8 p.

⁴ GOST R54662–2011 Cheeses and processed cheeses. Determination of protein mass fraction by the Kjeldahl method. M.: Standartinform, 2012–16 p.

⁵ GOST 5867–90 Milk and dairy products. Method of determination of fat. M.: Standartinform, 2009. — 12 p.

⁶ GOST R55063–2012 Kinds of cheese and processed cheese. The rules of tests acceptance, sampling and control methods. M.: Standartinform, 2013. — 28 p.

Table 1

Technological regulations for the production of Crescenza cheese

Process stage		Process parameters
Fat content of standardized milk, %		3.7±0.1
Acidity of the milk mixture before pasteurization, pH units		6.62±0.05
Pasteurization mode of milk mixture		75±1 °C; 30 sec
Dose of introduction of glucono-delta-lactone (GDL), g/kg of milk		0.8±0.2
The duration of ageing of the milk mixture with GDL, min		40±2
Acidity of milk before GDL addition, pH units		6.50±0.02
Introduction dose of milk-starter, U/100 kg of milk *		50
Duration of milk ripening after milk-starter addition, min		10–15
Acidity before the introduction of a milk-clotting enzyme, pH units		6.42±0.02
Introduction dose of MCE, IMCU/100 kg of milk		1 500, 2 500 or 3 500
Temperature of milk coagulation and curd processing, °C		38.5±0.5
Coagulation time, min		15±2
Duration of cutting and formation of curd grain, min		2
Average curd grain size, mm		20±2
Curd holding under whey, min		25±1
Mixing of curd before discharge into molds, min		7.5
Curd grain acidity before molding, pH units		6.30±0.05
Temperature in the fermentation chamber during whey draining, °C		38±1
Whey draining time into the molds, min		220±20
The number of turns of molds during whey draining		3
Indicators of cheese mass before salting	acidity, pH units	5.30±0.05
	temperature, °C	35.5±1.0
Weight of the head before salting, kg		~ 2
Salting duration, min		60
Weight of the head after salting, kg		~ 2

Note:

* U — units of acid-producing activity of DVS-cultures produced by Chr. Hansen. The table shows the nominal durations of the process steps \pm tolerances.

Table 2

Experiment plan

Option	Brand of MCE	Introduction dose of MCE, IMCU/100 kg of milk
1	Chy-max Extra	1 500
2	Chy-max M	1 500
3	Chy-max Supreme	1 500
4	Chy-max Extra	2 500
5	Chy-max M	2 500
6	Chy-max Supreme	2 500
7	Chy-max Extra	3 500
8	Chy-max M	3 500
9	Chy-max Supreme	3 500

pairwise comparison method. The influence of a pair of factors “MCEP brand” and “MCEP dose” on the response variables characterizing the parameters of the technological process at the stage of producing cheese in a cheesemaking tank, indicators of the whey composition and indicators of the composition of cheeses at the beginning of the shelf life was assessed using a two-way analysis of variance by the method of multiple comparisons of Scheffé [19].

3. Results and discussion

3.1. Milk-clotting and proteolytic activity of MCE

One of the goals of the experiment was to evaluate the influence of the level of nonspecific PA on the course of technological processes in the production of cheeses, as well as on the dynamics of proteolysis during the ripening of cheeses. Table 3 shows data on the dose of non-specific PA added to milk when using different doses of different types of MCE for coagulation.

Table 3

Introduction dose of MCE per unit of milk-clotting and total proteolytic activity

Brand of MCE	Milk-clotting activity of MCE (MA), nominal, IMCU/g*	Proteolytic activity of MCE (PA), units PA/g	The introduction of MCE in terms of units PA, at an introduction dose of in terms of units milk-clotting activity, IMCU/100 kg milk		
			1500	2500	3500
Chy-max Extra	554	0,71	1.92	3.21	4.49
Chy-max M	904	0,68	1.13	1.88	2.63
Chy-max Supreme	912	0,26	0.43	0.71	1.00

Note:

* The milk-clotting activity of MCE in the liquid form is recalculated from the nominal activity of 1 cm³ to the nominal activity of 1 g, based on the measured density of these preparations: Chy-max Extra 600 Liquid — 1.083 g/cm³, Chy-max M 1000 — 1.107 g/cm³, Chy-max Supreme — 1.096 g/cm³.

3.2 Milk coagulation process

Krescenza cheese production technology according to Ali-novi et al. [2] provides for the use of direct introduced starters without activation with the MCE introduction 15 minutes after adding the dry starter into milk. A feature of the technology for

the production of soft cheese Crescenza is the use of thermophilic streptococcus starter and a high coagulation temperature (38 °C). Such a coagulation temperature is close to the optimum activity of chymosins (~ 45 °C), which contributes to an increase in MCE activity and makes it possible to use reduced doses of MCE without compromising the duration of milk coagulation and the quality of the resulting cheese.

MCE was introduced into milk after it was kept with GDL until an acidity of 6.42±0.02 pH units was reached and before adding DVS-starter. In order to obtain comparable results, the moment when the curd was ready for cutting was determined online using a measuring system based on the Hot-Wire method. The milk with the introduced MCE solution was stirred for 2 min, after which the sensors of the measuring system were immersed in the product. The moment of completion of mixing is considered the beginning of coagulation. The moment of coagulation completion (readiness of the curd for cutting) was confirmed by a qualified cheesemaker by an organoleptic test for curd fracture.

Figure 1 presents the graphs showing the dependence of the duration of milk coagulation and the modulus of elasticity of the curd at the moment of readiness for cutting on the type and dose of MCE in the manufacture of Crescenza cheese.

Figure 1(A) shows data on the effect of the type and introduction dose of MCEP of different brands on the duration of milk coagulation. At an introduction dose of MCE equal to 1,500 IMCU/100 kg of milk, there are statistically significant differences (Tukey's test, $p < 0.05$) in the average duration of milk coagulation of MCE of different types: Chy-max Supreme (27.5±1.3 min) < Chy-max M (31.0±1.0 min) < Chy-max Extra (35.5±1.5 min). With an increase in the dose of MCE added to milk from 1,500 to 2,500 IMCU/100 kg, the duration of milk coagulation is reduced by ~ 2 times and for different types of MCE is: Chy-max Supreme (15.0±1.0 min) < Chy-max M (19.0±1.0 min) ≈ Chy-max Extra (19.0±1.0 min). There are no statistically significant variances in the duration of milk coagulation between MCE Chy-max M and Chy-max Extra at a dose of 2,500 IMCU/100 kg of milk (Tukey's test, $p < 0.05$). The introduction of MCE into milk at a dose of 3,500 IMCU/100 kg does not lead to a statistically significant (Tukey test, $p < 0.05$) reduction in the duration of coagulation, compared with a dose of 2,500 IMCU/100 kg.

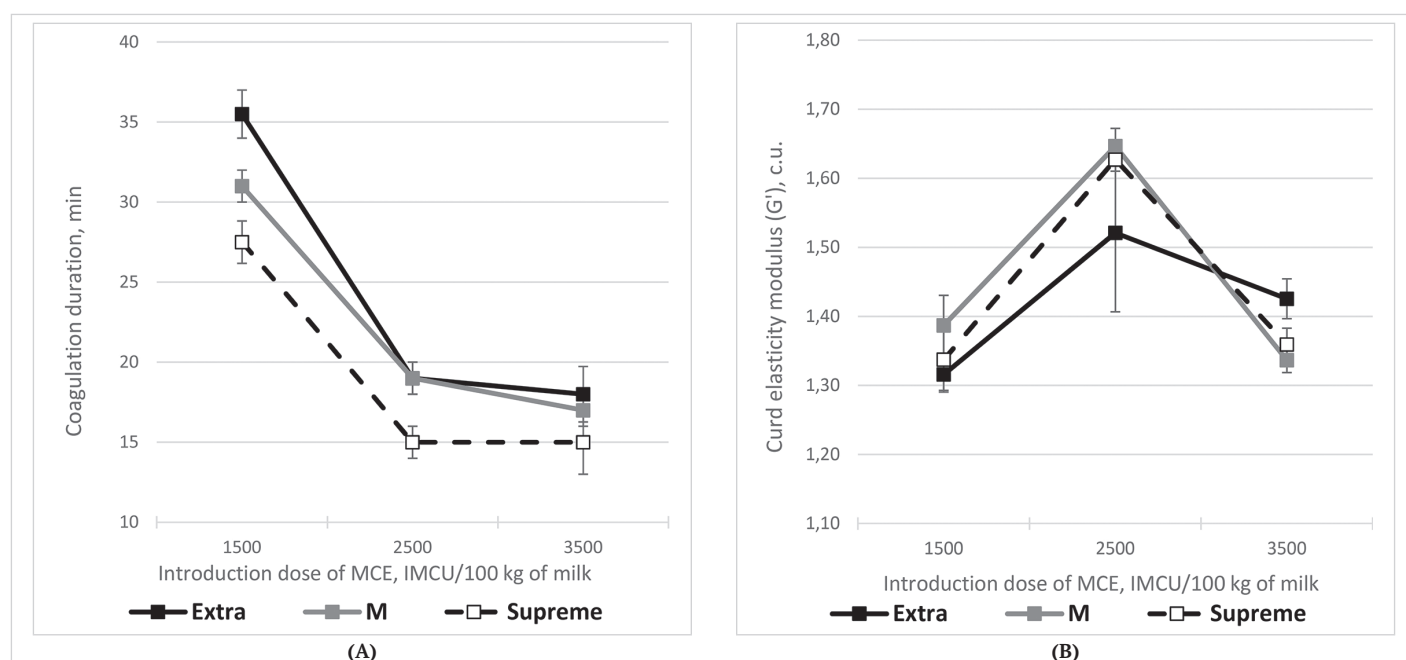


Figure 1. Influence of the type and dose of introduction of MCEP of different brands on: A) the coagulation duration; B) the modulus of elasticity of the curd at the moment of readiness for cutting. Designations for MCEP brands:

Extra — Chy-max Extra; M — Chy-max M; Supreme — Chy-max Supreme. Data are given in the form “mean ± standard deviation” (n = 3)

Thus, the differences in the proteolytic specificity of the studied MCEP, expressed in the level of MCA/PA, led to variances in the dynamics of milk coagulation by the given MCEP. With an equal dose of MCEP in terms of MCA, the shortest duration of milk coagulation was shown by MCEP Chy-max Supreme, which has the highest MCA/PA ratio among the studied MCEP.

There were no statistically significant differences (Tukey's test, $p < 0.05$) in the value of the elasticity modulus of the milk curd at the time of its readiness for cutting, when using different types of MCE at any dose used (Figure 1(B)).

3.3 Curd processing

In the process of making cheeses, the following indicators were studied:

- the duration of the individual stages of the processing procedure in the cheesemaking tank;
- the dynamics of changes in the active acidity of milk and milk curd.

The duration of processing stages of the curd and cheese grain in the cheesemaking tank was determined by the moment of reaching the required density of the curd and cheese grain, estimated by the foreman-cheesemaker.

Table 4 shows the actual values of the process indicators at the stage of making Crescenza cheese in the tank.

The analysis of the data in Table 4, carried out by the Scheffé method of multiple comparisons ($p < 0.05$), shows that at the stage of making cheese in a tank with an equal dose of MCE based on recombinant chymosins of different types, it was possible to identify the following differences:

- at a dose of 1,500 IMCU/100 kg of milk, there are differences in the duration of coagulation between the boiling options with different types of MCEP;
- at a dose of 2,500 and 3,500 IMCU/100 kg of milk, there are differences in the duration of coagulation only in the case of Chy-max Supreme MCEP;
- at a dose of 1,500 IMCU/100 kg of milk — there are differences in the duration of grain processing before molding until the grain acquires a standard density, between the boiling options with MCEP of different types;
- at any dose of the studied MCE, there are no differences in the duration of whey draining and the total duration of cheese production;
- after molding, cheeses made with a minimum dose of Chy-max Extra have the lowest pH.

To establish the effect of the type of MCE used on the amount of milk dry matter lost to whey, indicators of whey composition were evaluated (Table 5).

When analyzing the data obtained (Table 5) using the Scheffé method of multiple comparisons, it was found that there is no statistically significant effect ($p < 0.05$) of the type of MCE in the used dosage on fat, protein and dry matter content of whey. This was explained by the low level of PA of all used MCE, in which there are no negative consequences from the use of MCE in the form of excessive proteolysis of the curd at the stage of processing in the tank, which is associated with an increase in the loss of dry matter of the curd to whey. Therefore, the differences in the PA value between the options of the studied MCE did not lead to differences in the whey composition between the options of cheeses produced with these MCE.

Figure 2 shows a typical micropreparation of a cheese whey sample, which was obtained from one of the cheese samples made during the work.

In the micropreparation of cheese whey, shown in Figure 2, along with fat globules, curd microparticles (so-called cheese dust) are visible. Cheese dust particles are formed as a result of crushing of the cheese curd during its mechanical processing.

Table 4. Technological process indicators at the stage of production of samples of Crescenza cheese in a cheesemaking tank

Process parameter	Dose*	Parameter value for cheeses produced with MCE brand		
		Chy-max® Extra 600	Chy-max® M 1000	Chy-max® Supreme 1000
Active acidity of milk after pasteurization, pH units	1 500	6.57 ± 0.01 ^a	6.55 ± 0.04 ^a	6.52 ± 0.04 ^a
	2 500	6.62 ± 0.02 ^a	6.60 ± 0.04 ^a	6.62 ± 0.03 ^a
	3 500	6.52 ± 0.04 ^a	6.54 ± 0.04 ^a	6.54 ± 0.01 ^a
Active acidity after aging with GDL**, pH units	1 500	6.37 ± 0.01 ^a	6.30 ± 0.02 ^b	6.36 ± 0.03 ^a
	2 500	6.49 ± 0.04 ^a	6.47 ± 0.04 ^a	6.49 ± 0.03 ^a
	3 500	6.37 ± 0.02 ^a	6.37 ± 0.02 ^a	6.38 ± 0.04 ^a
Active acidity before coagulation, pH units	1 500	6.31 ± 0.01 ^a	6.26 ± 0.03 ^a	6.34 ± 0.04 ^a
	2 500	6.42 ± 0.04 ^a	6.42 ± 0.05 ^a	6.44 ± 0.02 ^a
	3 500	6.35 ± 0.04 ^a	6.38 ± 0.01 ^a	6.36 ± 0.06 ^a
Coagulation temperature, °C	1 500	37,7 ± 0.2	38,5 ± 0.4	38,5 ± 0.3
	2 500	37,8 ± 0.3	37,4 ± 0.4	38,6 ± 0.4
	3 500	37,8 ± 0.1	38,7 ± 0.2	38,2 ± 0.3
Coagulation duration, min	1 500	35.5 ± 1.5 ^a	31.0 ± 2.7 ^b	27.5 ± 1.3 ^c
	2 500	19.0 ± 1.0 ^a	19.0 ± 1.0 ^a	15.0 ± 1.0 ^b
	3 500	18.0 ± 1.7 ^a	17.0 ± 1.0 ^a	15.0 ± 2.0 ^b
Duration of cutting and formation of curd grain***, min	1 500	7.0 ± 1.0 ^a	8.0 ± 1.7 ^a	7.0 ± 1.0 ^a
	2 500	8.0 ± 1.0 ^a	8.0 ± 1.0 ^a	9.0 ± 1.0 ^a
	3 500	9.0 ± 1.0 ^a	8.0 ± 1.0 ^a	9.0 ± 1.7 ^a
Curd holding under whey, min	1 500	27.5 ± 1.5 ^a	25.0 ± 2.0 ^a	25.0 ± 2.0 ^a
	2 500	26.0 ± 1.7 ^a	25.0 ± 1.0 ^a	25.0 ± 2.0 ^a
	3 500	25.0 ± 1.0 ^a	25.0 ± 2.0 ^a	25.0 ± 1.7 ^a
Mixing of curd before discharge into molds, min	1 500	8.0 ± 1.0 ^a	9.5 ± 1.3 ^{ab}	11.0 ± 1.0 ^b
	2 500	10.0 ± 1.0 ^a	10.0 ± 2.0 ^a	11.0 ± 1.0 ^a
	3 500	11.0 ± 2.0 ^a	8.0 ± 2.0 ^b	8.0 ± 1.7 ^b
Overall whey draining time into the molds, min	1 500	186.7 ± 10.4 ^a	191.7 ± 12.6 ^a	188.3 ± 7.6 ^a
	2 500	188.3 ± 5.8 ^a	183.3 ± 10.4 ^a	185.0 ± 10.0 ^a
	3 500	183.3 ± 2.9 ^a	183.3 ± 5.8 ^a	181.7 ± 7.6 ^a
Total processing time****, min	1 500	229.2 ± 11.5 ^a	234.2 ± 14.5 ^a	231.3 ± 9.0 ^a
	2 500	232.3 ± 2.1 ^a	226.3 ± 7.5 ^a	230.0 ± 9.8 ^a
	3 500	228.3 ± 3.1 ^a	224.3 ± 9.1 ^a	223.7 ± 11.0 ^a
Active acidity of cheese at the end of whey draining, pH units	1 500	5.34 ± 0.06 ^a	5.28 ± 0.01 ^a	5.21 ± 0.07 ^b
	2 500	5.29 ± 0.03 ^a	5.31 ± 0.04 ^a	5.33 ± 0.04 ^a
	3 500	5.30 ± 0.03 ^a	5.33 ± 0.02 ^a	5.35 ± 0.05 ^a

Note:

* The dose of MCE in the milk mixture was IMCU/100 kg of milk;

** The duration of milk ageing after the introduction of GDL was fixed in time and equaled to 40 min;

*** The duration of cutting and formation of the grain in all options of cheeses was the same time;

**** Time from cutting to reach of final pH.

Data are given in the form "mean ± standard deviation" (n = 3).

The values of the parameters of the same stages of the technological process (separated by thick lines in the table), highlighted by the same superscript symbols, do not have statistically significant differences ($p < 0.05$, multiple comparison using the Scheffé test).

Table 5. Indicators of the whey composition obtained in the production of Crescenza cheese

Serum Composition Index	Dose*	The value of the indicator for whey from cheeses produced with MCE brand		
		Chy-max® Extra 600	Chy-max® M 1000	Chy-max® Supreme 1000
Fat, %	1 500	0.40 ± 0.04	0.30 ± 0.04	0.31 ± 0.04
	2 500	0.30 ± 0.01	0.30 ± 0.03	0.30 ± 0.01
	3 500	0.40 ± 0.02	0.30 ± 0.02	0.30 ± 0.03
Dry matter, %	1 500	7.50 ± 0.05	7.52 ± 0.03	7.41 ± 0.03
	2 500	7.52 ± 0.04	7.53 ± 0.04	7.60 ± 0.04
	3 500	7.52 ± 0.01	7.51 ± 0.02	7.48 ± 0.04
Total protein, %	1 500	0.77 ± 0.02	0.79 ± 0.04	0.78 ± 0.03
	2 500	0.77 ± 0.02	0.79 ± 0.02	0.77 ± 0.02
	3 500	0.79 ± 0.03	0.80 ± 0.03	0.79 ± 0.03

Note: Data are given in the form "mean ± standard deviation" (n = 3).

A high content of cheese dust in the whey means a high loss of dry matter of curd to the whey. A consequence of the use of an increased dose of DVS-starter is a high content of cells of starter microorganisms in the curd already at the initial stage of its processing, which is not typical for cheeses produced by the traditional method using a lower dose of starter culture. Figure 2 shows chains of *Str. thermophilus* trapped in milk curd particles.

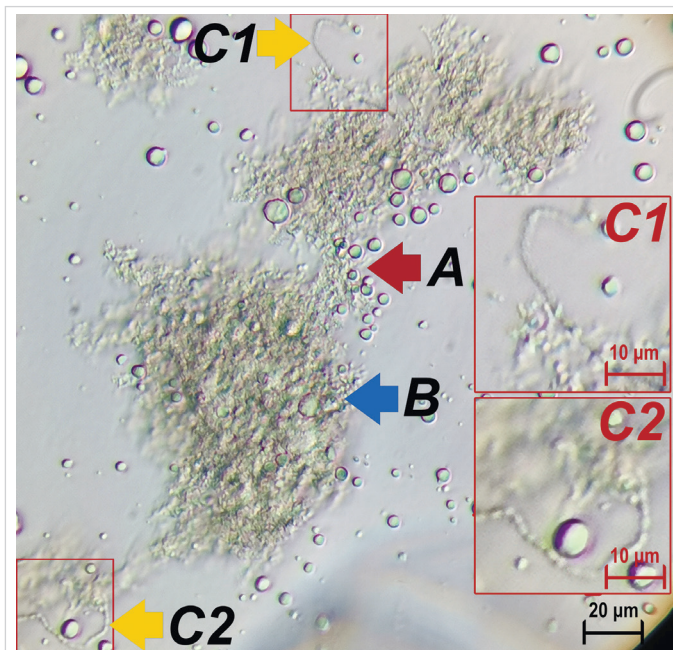


Figure 2. Micropreparation of cheese whey containing microparticles of fat and protein: A) fat globules; B) curd microparticles (cheese dust); C) chains of cells of *Str. thermophilus*

3.4. Physicochemical indicators of cheeses

Table 4 presents the indicators of Crescenza cheese samples produced during the experiment at the beginning of the storage period (7 days).

At the beginning of the storage period, there were no statistically significant differences between the cheese options in terms of fat, protein, salt and pH levels, but there was a tendency to increase the mass fraction of dry matter in cheeses with an increase in the dose of MCE used in cheese production (Table 6). These differences were not statistically significant, except for a single case: cheese options produced with the maximum dose of Chy-max Supreme had a statistically significantly higher dry matter content than cheese options made with the minimum doses of the studied MCE. The obtained data on the composition of cheeses indicate that the type and dose of MCE affect the properties of cheeses already at the stage of their manufacture in a cheesemaking bath.

Table 6. Indicators of Crescenza cheese samples at the beginning of the storage period (7 days)

Dose*	Brand**	Mass fraction, %			
		Dry matter	Fat	Protein	Table salt
1500	Extra	47.64±0.74 ^a	25.67±0.56 ^a	15.77±0.45 ^a	0.77±0.04 ^a
1500	M	47.91±0.37 ^a	25.67±0.64 ^a	15.81±0.29 ^a	0.80±0.04 ^a
1500	Supreme	47.86±0.52 ^a	25.67±0.53 ^a	15.94±0.38 ^a	0.75±0.02 ^a
2500	Extra	48.27±0.67 ^{ab}	26.77±0.18 ^a	15.98±0.36 ^a	0.78±0.03 ^a
2500	M	48.19±0.39 ^{ab}	26.40±0.63 ^a	15.90±0.24 ^a	0.81±0.02 ^a
2500	Supreme	48.95±0.72 ^{ab}	26.77±0.24 ^a	16.30±0.42 ^a	0.77±0.03 ^a
3500	Extra	49.28±0.43 ^{ab}	26.77±0.55 ^a	16.31±0.26 ^a	0.80±0.02 ^a
3500	M	49.22±0.51 ^{ab}	26.40±0.49 ^a	16.24±0.37 ^a	0.77±0.05 ^a
3500	Supreme	49.61±0.67 ^b	26.77±0.74 ^a	16.52±0.33 ^a	0.80±0.04 ^a

Note:

* The dose of MCE in the milk mixture was IMCU/100 kg of milk;

** Designations for MCEP brands: Extra — Chy-max Extra; M — Chy-max M; Supreme — Chy-max Supreme.

Data are given in the form “mean±standard deviation” (n=3).

Data within the same column with the same superscripts are not statistically significant (p<0.05, multiple comparison using Scheffé test).

4. Conclusion

Based on the data obtained, the following conclusions can be drawn:

- ❑ MCE based on recombinant chymosins differ in the level of specific nonspecific proteolytic activity, expressed in terms of the MCA/PA index, according to which recombinant chymosins of different types are arranged in descending order: calf chymosin (MCE Chy-max Extra) > camel chymosin (MCE Chy-max M) > modified camel chymosin (MCE Chy-max Supreme);
- ❑ the level of specific nonspecific PA of recombinant chymosins is significantly lower than that of MCE of animal and microbial origin, therefore, at the stage of making cheese in the tank, even at the maximum dose of MCE based on recombinant chymosins, there is no negative effect in the form of loss of milk dry matter into whey and a decrease in yield cheese;
- ❑ with an equal dose of introduction, the shortest duration of curd formation is observed in Chy-max Supreme MCE (modified chymosin). At the same time, differences in the duration of milk coagulation between recombinant chymosins of different types in one dosage are not significant from a technological point of view. The difference in the duration of milk coagulation by chymosins of different types decreases with an increase in their introduction dose — from ~ 5 min at an introduction dose of 1500 IMCU/100 kg of milk to ~ 2 min at an introduction dose of 3500 IMCU/100 kg of milk;
- ❑ the type and dose of MCE affect the properties of cheese already at the stage of cheese manufacture: cheese options made with the maximum dose of Chy-max Supreme had a statistically significantly higher dry matter content than cheese options produced with the minimum doses of the investigated MCE.

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