DOI: https://doi.org/10.21323/2618-9771-2023-6-1-103-116

Received 24.02.2023
Accepted in revised 17.03.2023
Accepted for publication 27.03. 2023

Available online at https://www.fsjour.com/jour
Original scientific article
Open access

INFLUENCE OF DIFFERENT MILK-CLOTTING ENZYMES ON THE PROCESS OF PRODUCING SEMIHARD CHEESES

Dmitriy S. Myagkonosov*, Igor T. Smykov, Dmitriy V. Abramov, Irina N. Delitskaya, Elena G. Ovchinnikova

All-Russian Scientific Research Institute of Butter- and Cheesemaking, Uglich, Yaroslavl Region, Russia

KEY WORDS:
Milk-clotting enzymes, enzymatic coagulation, semihard cheese, cheese whey, cheese yield

ABSTRACT
The effect of milk-clotting enzymes (MCEs) of animal origin (Naturen Extra with a mass fraction of chymosins of 95%, "Bovine Pepsin" with a mass fraction of chymosin of 10%), as well as MCEs of microbial origin (Fromase 750 XLG) and recombinant origin (Chy-max Extra and Chy-max Supreme) on the duration of milk coagulation and processing of cheese curd, as well as on the composition of whey and fresh cheeses in the manufacture of semihard cheeses, at an introduction dose of MCE of 1,500 to 6,000 IMCU per 100 kg of milk. Increasing the MCE dose from 1,500 to 2,000–3,000 IMCU/100 kg of milk leads to a decrease in the coagulation duration by an average of 20 min ($p<0.05$). A further increase in the MCE dose to 5,000–6,000 IMCU/100 kg of milk does not lead to a reduction in the coagulation duration. With equal introduction doses, MCE brands of Fromase and "Pepsin" show greater losses of fat (by 0.15–0.60%) and dry matter (by 0.30–0.50%) in whey than MCEs of Naturen brands, Chy-max Extra and Chy-max Supreme. Cheese variants made using different types and doses of MCEs did not have statistically accurate differences in the content of dry matter, fat, protein, salt and pH ($p>0.05$). The use of MCEs of Naturen, Chy-max Extra and Chy-max Supreme brands at a dosage of 5,000–6,000 IMCU/100 kg of milk gives an average of 1.5% higher actual cheese yield compared to MCEs of the Fromase and "Pepsin" brands at an introduction dose of 1 500 IMCU/100 kg of milk.

FUNDING: The article was published as part of the research topic No. FNEN-2019–0010 of the state assignment of the V. M. Gorbatov Federal Research Center for Food Systems of RAS.

Поступила 24.02.2023
Поступила после рецензирования 17.03.2023
Принята в печать 27.03.2023

https://www.fsjour.com/jour
Научная статья
Open access

ВЛИЯНИЕ РАЗЛИЧНЫХ МОЛОКОСВЕРТЫВАЮЩИХ ФЕРМЕНТОВ НА ПРОЦЕСС ИЗГОТОВЛЕНИЯ ПОЛУТВЕРДЫХ СЫРОВ

Мягконосов Д. С.,* Смыков И. Т., Абрамов Д. В., Делицкая И. Н., Овчинникова Е. Г.

Всероссийский научно-исследовательский институт маслоделия и сыроделия, Углич, Ярославская область, Россия

КЛЮЧЕВЫЕ СЛОВА:
молокосвертывающие ферменты, ферментативное свертывание, полутвердые сыры, подсырная сыворотка, выход сыра

АННОТАЦИЯ
Исследовано влияние молокосвертывающих ферментов (МФ) животного происхождения (Naturen Extra с массовой долей химозина 95%, «Говяжий пепсин» с массовой долей химозина 10%), а также МФ микробного происхождения (Fromase 750 XLG) и рекомбинантного происхождения (Chy-max Extra и Chy-max Supreme) на продолжительность свертывания молока и обработки сырного сгустка, а также на состав сыворотки и свежих сыров при изготовлении полутвердых сыров, в дозе внесения МФ, равной от 1 500 до 6 000 IMCU на 100 кг молока. Повышение дозы МФ от 1500 до 2000–3000 IMCU/100 кг молока приводит к уменьшению времени свертывания в среднем на 20 мин ($p < 0,05$). Дальнейшее повышение дозы МФ до 5000–6000 IMCU/100 кг молока не приводит к сокращению продолжительности свертывания. При равных дозах внесения у МФ марок Fromase и «Пепсин» отмечаются большие потери жира (на 0,15–0,60%) и сухих веществ (на 0,30–0,50%) в сыворотку, чем у МФ марок Naturen, Chy-max Extra и Chy-max Supreme. Варианты сыров, изготовленные с использованием разных типов и доз МФ, не имели статистически достоверных отличий по содержанию сухого вещества, жира, белка, соли и по уровню pH ($p > 0,05$). Применение МФ марок Naturen, Chy-max Extra и Chy-max Supreme в дозировке 5000–6000 IMCU/100 кг молока дает в среднем на 1,5% больший фактический выход сыра в сравнении с МФ марок Fromase и «Пепсин» при дозе внесения 1 500 IMCU/100 кг молока.

ФИНАНСИРОВАНИЕ: Статья подготовлена в рамках выполнения исследований по государственному заданию № FNEN-2019–0010 Федерального научного центра пищевых систем им. В. М. Горбатова Российской академии наук.

FOR CITATION: **Myagkonosov, D. S., Smykov, I. T., Abramov, D.V., Delitskaya, I. N., Ovchinnikova, E. G.** (2023). Influence of different milk-clotting enzymes on the process of producing semihard cheeses. *Food Systems*, 6(1), 103-116. https://doi.org/10.21323/2618-9771-2023-6-1-103-116

ДЛЯ ЦИТИРОВАНИЯ: **Мягконосов, Д. С., Смыков, И. Т., Абрамов, Д. В., Делицкая, И. Н., Овчинникова, Е. Г.** (2023). Влияние различных молокосвертывающих ферментов на процесс изготовления полутвердых сыров. *Пищевые системы*, 6(1), 103-116. https://doi.org/10.21323/2618-9771-2023-6-1-103-116

1. Introduction

The composition, quality and cost of cheese depend on the properties of the MCE used in the manufacture.

Enzymatic coagulation of milk begins with the hydrolysis of kappa-casein, which stabilizes micelles, under the MCE action. After the cleavage of the critical fraction of kappa-casein (from 60% or more), the process of aggregation of micelles begins, leading to the formation of a milk curd [1]. The greater the proportion of kappa-casein is split, the higher the density of the resulting curd is and the better the curd releases moisture [2].

The rate of cleavage of kappa-casein and its amount at the beginning of the curd formation process depends on the amount of MCE introduced into the milk. Increasing the MCE amount reduces the coagulation duration [3,4].

Traditionally used MCEs of animal and microbial types have insufficient specificity of proteolytic action and, in addition to kappa-casein, also cleave other proteins, incl. alpha and beta casein, located in the nuclei of micelles. The cleavage of alpha and beta caseins leads to a weakening of the curd strength, which leads to loss of dry matter of the curd in the form of cheese dust due to its crushing during processing. In addition, the dry matter of the curd is lost in the form of water-soluble peptides, which are formed during the hydrolysis of alpha- and beta-caseins under the MCE action [5]. As a result, the cheese yield decreases, the milk consumption for the manufacture of a unit mass of cheese increases [6,7], which leads to an increase in the cheese cost.

The proteolytic activity (PA) of MCE creates the risk of defects in cheese taste (bitterness) and texture (viscous, spreadable consistency) during its ripening and storage, which leads to a limitation of the cheese shelf life. This is especially true for MCEs of animal and microbial origins, which have a high PA [3,8,9]. Therefore, the use of these MCEs in high doses is inappropriate.

Considering the requirements of cheesemakers, MCEs with increased specificity for kappa-casein and a low level of non-specific PA (Chy-max M, Chy-max Supreme, Maxiren XDS) have been created [10,11]. Such MCEs can be used at high introduction doses. By increasing the MCE dose, the coagulation time is reduced, which has a number of positive consequences for improving the quality and reducing the cheese cost:

- duration of free circulation of bacteriophages in liquid milk is reduced, the risk of damage to the bulk starter by bacteriophage is reduced [12];
- time, energy and labor expenditures for the manufacture of a production unit are reduced; the productivity and turnover of technological equipment increases [13].

When using MCE with low PA, there is no cleavage of alpha- and beta-caseins at the stage of curd processing in a cheesemaking tank. The resulting curd has a high density and quickly releases moisture. Due to the high mechanical strength, the curd is resistant to mechanical crushing, which reduces the loss of fat and protein into the whey in the form of cheese dust and makes it possible to increase the cheese yield by 1–2% in comparison with MCEs of animal and microbial origins [11,14].

Part of the MCE introduced into milk transfers into the composition of the cheese mass [15]. MCE preserved in cheese contributes to the proteolysis process in cheese, which depends on the PA value of the enzyme [16,17].

MCEs with low PA are more relevant for non-ripening cheeses — soft cheeses and stretched curd cheeses, for which a minimum level of proteolysis during storage is important to preserve taste and texture for the longest possible time [16,18,19]. To form the taste of ripening cheese varieties, proteolytic modification of cheese mass proteins is necessary. Under the action of proteolysis, the cheese texture is transformed from hard, crumbly to plastic, and cohesive, and a typical cheese taste is formed. An insufficient level of proteolysis leads to an excessively dense texture and an insufficiently pronounced taste of cheeses [17,19,20]. Such a product will not be competitive in a market rich in cheeses from different manufacturers.

A cheese with an attractive taste and texture that is of interest to consumers will bring profit to the manufacturer. As a result, to date, in countries with developed economies and high competition in the marketplace, there has been a tendency to create new cheese varieties with new tastes that can attract the consumer and be in demand. An example of a commercially successful product is "Serra da Estrela" cheese, made from sheep's milk, in the production of which MCE is used based on an extract from artichoke inflorescences "Cynara cardunculus" [21]. Other MCEs are also being sought from other sources to create new cheese varieties. The possibility of using enzymes from plants [22,23], marine organisms [24], and avian pepsins [25] is being studied. The disadvantage of such MCEs is the high PA, which causes the risk of defects in taste and texture in cheeses produced with them and a reduction in their shelf life. This disadvantage can be eliminated by reducing the MCE dose. The resulting negative consequences in the form of obtaining a weaker curd [26] can be compensated in the production of semihard cheeses by increasing the duration and temperature of curd processing [27].

The purpose of this study is to evaluate the quality (taste and texture) and economic indicators of cheese production (yield and process time) obtained by using MCEs of different origins in the manufacture of semihard cheeses.

2. Materials and methods

2.1. Materials

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

In the cheese production, a lactic acid starter based on the BK-Uglich-No. 4 bacterial concentrate (the Federal State Budgetary Scientific Institution "Experimental Biofactory", Russia) was used, consisting of a set of cultures of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, with preliminary activation of the culture in sterilized milk. To coagulate milk, milk-clotting enzyme preparations (MCEs) of the following brands were used:

- Chy-max® Supreme 1000 (recombinant camel chymosin with a modified amino acid sequence; nominal milk-clotting activity — 1,000 IMCU/g; manufacturer — Chr Hansen A/S, Denmark);
- Chy-max® Extra 600 Liquid (recombinant calf chymosin of genetic variant "B"; nominal milk-clotting activity — 600 IMCU/g; manufacturer — Chr Hansen A/S, Denmark);
- Naturen® Extra 220 NB (enzymatic extract from the stomachs of calves with a mass fraction of chymosin of at least 95%; nominal milk-clotting activity — 220 IMCU/g; manufacturer — Chr Hansen A/S, Denmark);
- Fromase® 750 XLG (protease of the fungus *Rhizomucor miehei*; nominal milk-clotting activity — 750 IMCU/g; manufacturer — DSM Food Specialties, France);
- FS10 "Bovine pepsin" (enzyme extract from the stomachs of adult cattle with a mass fraction of chymosin of at least 10%; nominal milk-clotting activity — 1,000 IMCU/g; manufacturer — Zavod endokrinnykh fermentov, LLC, Russia).

2.2 Methods

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

The determination of the total milk-clotting activity was carried out according to GOST ISO 11815–2015[1].

The determination of the total proteolytic activity was carried out according to GOST 34430–2018[2], 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-150MI pH meter (Izmeritelnaya Tekhnika, LLC, Russia) equipped with a FC200B combined pH electrode (Hanna Instruments Inc., USA). The active acidity of cheeses during self-pressing was measured with a potentiometric electrode of a pH meter immersed in cheese mass. 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 cm^3 of deionized water.

The mass fraction of moisture in cheeses was determined by drying at a temperature of 102 ± 2 °C according to the Russian state standard GOST 3626–73[3].

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 23327–98[4].

The mass fraction of total protein in cheeses was determined by the Kjeldahl method according to the Russian state standard GOST R54662–2011[5].

The mass fraction of fat in milk and whey was determined by the acid method according to the Russian state standard GOST 5867–90[6].

The mass fraction of fat in cheeses was determined by the acid method according to the Russian state standard GOST R55063–2012[7].

The optical density of whey was measured after dilution of whey with water (1:10) in a cuvette with an optical path length of 10 mm at a wavelength of 650 nm on a LEKI spectrophotometer of model SS1207UV (MEDIORA OY, Finland).

The molecular weight distribution of soluble proteins in whey was determined by high resolution gel filtration using an AKTA Pure 25 chromatographic system (Cytiva, Sweden) equipped with a Superose 12 10/300 GL column (GE Healthcare, Sweden). Eluent — aqueous solution of 0.05 M Na_2HPO_4 + 0.15 M NaCl (pH 6.50), eluent flow rate — 0.5 ml/min; detector wavelength — 280 nm. The column was calibrated according to the release time of protein substances with a known molecular weight: IgG (180 kDa), aldolase (158 kDa), BSA (69 kDa), ovoalbumin (43 kDa), β-Lg (36.0 kDa), α-La (14.4 kDa), cytochrome C (12.3 kDa), tryptophan (0.204 kDa). The calibration curve was based on a logarithmic regression model [28]. To determine the molecular weight distribution of soluble protein substances, whey was filtered on filter paper "type 102" (China standard GB / T 1914–2007), and then on a cellulose acetate filter with a pore size of 0.45 μm (Vladipor, Russia).

The cheese yield was calculated using the equation given in [29].

The actual cheese yield was calculated using equation (1):

$$Y_a = \frac{M_{cheese}}{M_{milk} + M_{starter}} \times 100\%, \quad (1)$$

where

Y_a — actual cheese yield, %;
M_{cheese} — mass of cheese, kg;
M_{milk} — mass of milk used for cheese production, kg;
$M_{starter}$ — mass of the starter used for cheese production, kg.

Milk fat yield (MFY), expressed as kg milk fat transferred from milk into cheese, was calculated using equation (2)

$$MFY = \frac{M_{cheese} \cdot \%F_c}{M_{milk} \cdot \%F_{milk}} \times 100\%, \quad (2)$$

where

MFY — proportion of milk fat that has transferred into cheese, %;
$\%F_c$ — mass fraction of fat in cheese, %;
$\%F_{milk}$ — mass fraction of fat in milk used for cheese production, %.

Milk protein yield (MPY), expressed as kg milk protein transferred from milk into cheese, was calculated using equation (3)

$$MFY = \frac{M_{cheese} \cdot \%P_c}{M_{milk} \cdot \%P_{milk}} \times 100\%, \quad (3)$$

where

MPY — proportion of milk protein transferred into cheese, %;
$\%P_c$ — mass fraction of protein in cheese, %;
$\%P_{milk}$ — mass fraction of protein in milk used for cheese production, %.

2.2.3. Methods for controlling the process of milk curd formation

To obtain comparable results, the moment of the curd readiness for cutting was determined online using a measuring system based on the Hot-Wire method [30,31].

2.2.4. Microscopic studies

Microscopic studies of whey were carried out on "wet mount" type microscope preparations by light microscopy in transmitted light, using a dark field condenser. Photographs were taken with a Canon EOS600D digital camera. The photographs were corrected using the Digital Photo Professional software v.4.5 (Canon Inc., Japan).

2.2.5. Cheese production process

A semi-hard cheese was produced with a mass fraction of dry matter of 59% and a mass fraction of fat in dry matter of 55%. Cheese was made in laboratory-scale 12 l-capacity cheese vats with water jacket, equipped with electric paddle mixers. Milk was normalized:

- by fat, to obtain a mass fraction of milk fat of 5.0±0.1%, by adding fresh cream with a fat content of 34–38%;
- by protein, to obtain a mass fraction of total milk protein of 3.5 ± 0.05%, by adding dry milk protein concentrate of Ledor MPC85 LH with a protein content of 85% (Hochdorf Swiss Nutrition AG, Switzerland).

Table 1 gives the technological regulations for the cheese production.

[1] GOST ISO 11815–2015 Milk. Determination of total milk-clotting activity of bovine rennet. M.: Standartinform, 2015. — 10 p.
[2] GOST 34430–2018 Enzyme preparations for food industry. Method for the determination of proteolitic activity. M.: Standartinform, 2018. — 12 p.
[3] GOST 3626–73 Milk and milk products. Methods for determination of moisture and dry substance. M.: Standartinform, 2009. — 11 p.
[4] 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.
[5] GOST P 54662–2011 Cheeses and processed cheeses. Determination of protein mass fraction by the Kjeldahl method. M.: Standartinform, 2012. — 16 p.
[6] GOST 5867–90 Milk and dairy products. Method of determination of fat. M.: Standartinform, 2009. — 12 p.
[7] GOST P 55063–2012 Kinds of cheese and processed cheese. The rules of test acceptance, sampling and control methods. M.: Standartinform, 2013. — 28 p.

Table 1. **Technological regulations for the production of semi-hard cheese**

Таблица 1. **Технологический регламент производства полутвердого сыра**

Indicator	Meaning
Acidity of the milk mixture before pasteurization, pH	6,62±0,05
Pasteurization mode of milk mixture	72,0±0,5 °C; 30 sec
Introduction dose of calcium chloride, g/100 kg of milk	Based on 35 g of anhydrous salt per 100 kg of milk
Acidity of milk before milk-starter addition, pH	6,50±0,02
Introduction dose of milk-starter *, kg/100 kg of milk	0,7
Duration of milk ripening after milk-starter addition, min	10–15
Acidity before the introduction of a milk-clotting enzyme, pH	6,42±0,02
Introduction dose of MCE **, IMCU/100 kg of milk	1 500–6 000
Coagulation temperature, °C	33,5±0,5
Coagulation time, min	15–50
Duration of cutting and formation of curd grain, min	15–20
Duration of stirring before cooking, min	25±1
Temperature of the cooking, °C	40–41
Duration of temperature increasing to cooking temperature, min	15
Duration of the stirring at cooking temperature, min	40–50
Whey draining time into the molds, min	30
The number of turns of molds during whey draining	3
Pressing duration, min	120–180
Head weight after pressing, kg	~1,5
Acidity of cheese before salting, pH	5,30±0,05
Salting duration, h	2

Note:
* activated starter culture in sterile milk is used
** the introduction dose of MCE is based on the design of the experiment.
The table shows the nominal durations of the process steps±deviations.

After pressing, fresh cheeses were salted by immersion in a 22% NaCl solution for 8 h at a temperature of 4±2 °C. After salting, the cheeses were dried for 14–16 hours at a temperature of 4±2 °C and a relative humidity of 85±5%, and then packaged. After salting and drying, fresh cheeses were packed. Packing was carried out under vacuum (pressure $0.96\pm0.01\cdot10^{-5}$ Pa; duration of vacuum treatment — 17 s) into bags made of Amivak CH-B polymer film (Atlantis-Pak, Russia) using a Henkelman Boxer 42 packaging machine (Henkelman Vacuum Systems). Packed cheeses were ripened at a temperature of 11±1 °C.

2.2.6. Methods of statistical analysis

The study was based on the design of the experiment that included two categorical factors: "MCE brand" and "MCE dose".

Table 2 shows the design of the experiment.

Table 2. **Design of experiment**

Таблица 2. **План эксперимента**

MCE brand	Introduction dose of MCE	
	Factor level	IMCU/100 kg of milk
Fromase 750 XLG	-1	1,500
Pepsin FS-10	-1	1,500
Naturen Extra	-1	1,500
Fromase 750 XLG	0	3,000
Pepsin FS-10	0	3,000
Naturen Extra	0	3,000
Chy-max Extra	0	3,000
Chy-max Supreme	0	2,000
Naturen Extra	+1	6,000
Chy-max Extra	+1	6,000
Chy-max Supreme	+1	5,000

Two replications of the experiments were carried out in a randomized order.

Mathematical data processing was carried out using the software packages of Microsoft Excel and Statsoft Statistica (v. 5.5). The effect of the "MCE brand" and "MCE dose" factors on the response variables was assessed using two-way analysis of variance (ANOVA) using the Tukey pairwise comparison method [32].

3. Results and discussion

3.1. Milk-clotting and proteolytic activity of MCE

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

Table 3. **Introduction dose of MCE in units of milk-clotting and total proteolytic activity**

Таблица 3. **Доза внесения МФ в единицу молокосвертывающей и общей протеолитической активности**

MCE brand	Milk-clotting activity of MCE (MA), nominal, IMCU/g*	Proteolytic activity of VAG (PA), PA/g units	Introduction of MCE in terms of units of PA, at the level of the factor "introduction dose of MCE"***		
			–1	0	+1
Fromase® 750 XLG	776**	56.91	110.01	220.01	—
FS10 "Bovine pepsin" (15/85)	1087**	14.36	19.82	39.63	—
Naturen® Extra 220 (95/5)	193*	0.95	7.38	14.77	29.53
Chy-max® Extra 600 Liquid	554*	0.48	—	2.60	5.20
Chy-max® Supreme 1000	912*	0.28	—	0.61	1.54

Note:
* Nominal (declared by the manufacturer) milk-clotting activity of MCE.
** Actual (measured) milk-clotting activity of MCE.
*** The level of the "MCE dose" factor in IMCU units is given in Table 2.

The milk-clotting activity of MCE in liquid dosage form, recalculated from the nominal activity of 1 cm^3 to the nominal activity of 1 g, based on the measured density of these preparations: Chy-max Supreme — 1.096 g/cm^3; Chy-max Extra 600 Liquid — 1.083 g/cm^3, Naturen® Extra 220–1.134 g/cm^3, Fromase® 750 XLG — 1.180 g/cm^3. The specimen FS10 "Bovine Pepsin" has a dry dosage form.

Their dosage was based on the proteolytic activity of MCE in the cheese production.

Chymosin-based MCEs have been used in the cheese production at doses of 3,000 and 6,000 IMCU/100 kg milk, respectively, for Naturen Extra and Chy-max Extra MCE, and 2,000 and 5,000 IMCU/100 kg milk for Chy-max Supreme, which corresponds to the minimum and maximum used doses of these MCEs recommended by the manufacturer. Naturen Extra MCE has also been studied at a dose of 1,500 IMCU/100 kg of milk, which is ½ of the minimum introduction dose recommended by the MCE manufacturer. Introduction doses of MCEs from 1,200 to 1,600 IMCU/100 kg of milk are obtained using the "VNIIMS rennet-probe" method to calculate the minimum amount of MCE required for milk coagulation within ~30 min [33,34].

For MCE based on microbial protease (Fromase 750 XLG) and bovine pepsin ("Bovine Pepsin" FS-10), which have higher levels of PA than MCEs based on chymosins, introduction doses of 1,500 and 3,000 IMCU/100 kg of milk were chosen, due to the high probability of obtaining a bitter taste in cheese at a higher dose of these MCEs, as proved by the results of studies of microbial proteases [8, 35] and pepsins [6, 36] in production of cheeses.

3.2 Milk coagulation and curd processing

To coagulate milk, working solutions of MCE were prepared with a concentration of 5.0 IMCU/см³. The introduction dose of MCE working solutions was calculated to achieve a concentration of MCE from 1,500 to 6,000 IMCU per 100 kg of processed milk, depending on the factor levels in the design of the experiment.

MCE was added into milk after the introduction of the starter of lactic acid microorganisms and holding the milk until an acidity of 6.45±0.05 pH was reached. To obtain comparable results, the moment of the curd readiness 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 into the product. The moment when mixing is completed is considered the moment of the beginning of coagulation.

The moment of coagulation completion (curd readiness for cutting) was confirmed by a qualified cheesemaker by an organoleptic test for curd fracture. The curd, ready for cutting, when lifted with a spatula, gave a fracture with sharp edges with the release of a transparent yellowish-green whey.

The duration of the curd processing stages was not a fixed value, but was determined by its readiness for the next processing stage in accordance with the regulations for the production of semihard cheeses. Process formation and stirring of curd grain was continued until a sufficient degree of compaction was obtained and a dense layer was formed on the surface of the grains, protecting the grains from destruction during kneading thoroughly. Processing during and after cooking continued until the cheese grains acquired the necessary density and stickiness, making it possible to form a coherent cheese mass from individual grains, which can be pressed.

The processing of cheese grains in the cheese vat was considered completed after the cheese grains acquired the required density (which indicated a sufficient degree of dehydration) while maintaining sufficient stickiness of the grains. The readiness of the grain was determined by the method generally accepted in the cheesemaking practice — a "grinding test" for the grain.

To establish the influence of experimental factors (type and dose of MCE) on the duration of coagulation and the total duration of grain processing in the cheesemaking tank, analysis of variance of the data was carried out. Table 4 shows the results of the analysis of variance.

Table 4. **Mean sum of squared deviations, level of statistical significance and coefficients of determination of the ANOVA model for response variables**

Таблица 4. **Средняя сумма квадратов отклонений, уровень статистической достоверности и коэффициенты детерминации модели ANOVA для переменных отклика**

Factor	df	Coagulation duration, min	Processing time (without coagulation), min
Type	4	10.32 (–)	14.83 (–)
Dose	2	805.36 (***)	172.97 (**)
Error	17	4.58	19.04
R^2		0.96	0.61

Note:
df — number of degrees of freedom
Factor keys: Type — MCE type; Dose — MCE dose.
Error — part of the response variable variation related to an error;
R^2 — coefficient of determination for the ANOVA model.
The level of statistical significance of the factor effect evaluation (in parentheses): "–" — statistically inaccurately (p>0.05); "*" — p<0.05; "**" — p<0.01; "***" — p<0.001.

The results of the analysis of variance show that the MCE dose (p<0.001) affects the coagulation duration and the total duration of grain processing in the cheesemaking tank, and there is no influence of the MCE type (p>0.05). The analysis of variance model containing the selected set of factors provides a reliable description of the variation of the response variables "coagulation duration" at the level of R^2 = 0.96 and "processing duration" at the level of R^2 = 0.61.

Figure 1 shows the graphs reflecting the dependence of the duration of milk coagulation and the stages of the milk curd processing on the introduction dose of various types of MCEs in the manufacture of experimental variants of semihard cheeses.

The graphs visualizing the influence of experimental factors on the response variables (Figure 1) show that with an equal dose of MCE, there are no statistically accurate differences between MCEs of different types (Tukey's test, p>0.05) in the coagulation duration and in the total duration of curd processing in the cheesemaking tank. Increasing the MCE dose from the minimum level (1,500 IMCU/100 kg) to the average level (2,000 for Chy-max Supreme MCE and 3,000 IMCU/100 kg for other MCEs) results in a statistically accurate (Tukey's test, p<0.05) reduction in the coagulation duration by an average of 20 minutes. Further increase in the MCE dose to the maximum level (5,000 for Chy-max Supreme MCE and 6,000 IMCU/100 kg for other MCEs) does not provide a significant result in terms of reduction in the coagulation duration (Tukey's test, p>0.05).

At the same time, with an increase in the MCE dose from the average to the maximum one, it leads to a statistically significant (Tukey test, p<0.05) reduction in the duration of curd processing in the tank by an average of 10 minutes.

Based on the obtained results on the effect of the type and dose of MCE on the duration of the stages of processing the cheese curd in the tank, it can be concluded that the use of high doses of MCE (5,000–6,000 IMCU/100 kg of milk) for milk coagulation can be considered an appropriate technological method, leading to a reduction in the time spent on cheese production and to an increase in the turnover of technological equipment.

3.2 Whey composition

To establish the influence of experimental factors on the indicators of the composition of whey obtained in the manufacture of cheeses using different types and doses of MCEs, an analysis of variance of the data was carried out. Table 5 shows the results of the analysis of variance.

Table 5. **Mean sum of squared deviations, level of statistical significance and coefficients of determination of the ANOVA model for response variables**

Таблица 5. **Средняя сумма квадратов отклонений, уровень статистической достоверности и коэффициенты детерминации модели ANOVA для переменных отклика**

Factor	df	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of dry matter, %	Optical density *, units
Type	4	0.08625 (**)	0.00076 (–)	0.102 (*)	0.02594 (*)
Dose	2	0.001583 (–)	0.00743 (–)	0.049 (–)	0.00259 (–)
Error	17	0.01527	0.00363	0.031	0.00923
R^2		0.69	0.36	0.68	0.56

Note:
df — number of degrees of freedom
Factor keys: Type — MCE type; Dose — MCE dose.
Error — part of the response variable variation related to an error;
R^2 — coefficient of determination for the ANOVA model.
The level of statistical significance of the factor effect evaluation (in parentheses): "–" — statistically inaccurately (p>0.05); "*" — p<0.05; "**" — p<0.01; "***" — p<0.001.

Based on the results of the analysis of variance shown in Table 5, the following conclusions can be drawn. The factor significantly (p<0.05) influencing the composition of cheese whey is "MCE type", but not "MCE dose". At the same time, the MCE type has a statistically accurate effect on the total content of dry matter and fat in whey, but not on the content of whey protein.

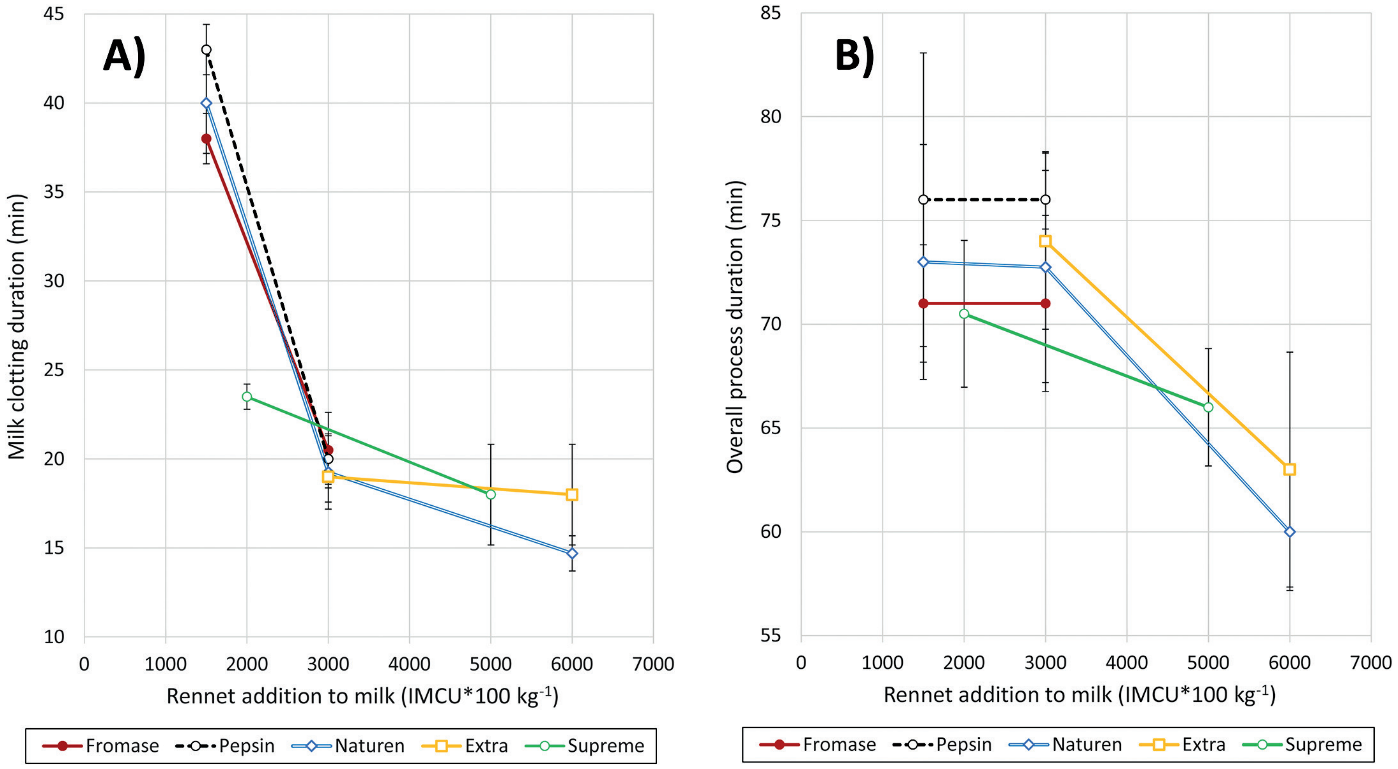

Figure 1. **Influence of the type and introduction dose of MCE of different brands on the duration of milk coagulation (A) and the processing duration of the curd in the cheesemaking tank without considering the coagulation time (B). Keys of MCE brands on the graph: Fromase — Fromase 750 XLG; Pepsin — "Bovine Pepsin" FS-10; Naturen — Naturen Extra 220; Extra — Chy-max Extra; Supreme — Chy-max Supreme. Data are given in the form "mean value ± standard deviation"**

Рисунок 1. **Влияние типа и дозы внесения МФП разных марок на продолжительность свертывания молока (А) и на продолжительность обработки сгустка (B) в сыродельной ванне (без учета времени свертывания). Обозначения марок МФ на графике: Fromase — Fromase 750 XLG; Pepsin — «Пепсин говяжий» ФС-10; Naturen — Naturen Extra 220; Extra — Chy-max Extra; Supreme — Chy-max Supreme. Данные приведены в форме «среднее значение ± стандартное отклонение»**

The effect of factors (type and dose of MCE) established by the method of variance analysis on indicators of the composition of cheese whey is visualized in the form of graphs presented in Figure 2.

The difference between various types of studied MCEs is the level of non-specific PA (Table 3). The data given in Figures 2 (A) and 2 (B) can show that at equal introduction doses, MCEs with high PA (Fromase, Pepsin) have statistically significant ($p < 0.05$, Tukey's test) large losses of fat and dry matter of the curd into whey than MCEs with lower PA (Naturen, Chy-max Extra and Chy-max Supreme). In addition, there are similar differences between the MCE variants in terms of protein losses in whey and in whey optical density (Figures 2 (C) and 2 (D)), but these differences are not statistically accurate ($p > 0.05$, Tukey's test).

According to data [8], MCEs with high PA cause proteolytic cleavage of alpha- and beta-caseins in the cheese curd at the stage of processing in the cheesemaking tank. Under the action of proteolysis, the strength of the curd and its resistance to mechanical stress decrease. Such a curd is subjected to crushing, followed by the loss of part of the dry matter into whey in the form of "cheese dust". In addition, as a result of proteolytic cleavage, water-soluble peptides are formed from caseins, which transfer into whey.

The results of the analysis of variance (Table 5) and graphs visualizing them (Figure 2) allow us to conclude that the type of used MCE affects the loss of milk dry matter in the form of "cheese dust", and not in the form of water-soluble proteolysis products (peptides) formed during caseins cleavage. This conclusion is confirmed by the results of microscopic and chromatographic studies of cheese whey. Figure 3 shows a photo of a micropreparation of cheese whey taken at the end of the curd processing. Figure 3 (1) demonstrates the appearance of fat globules; Figure 3 (2) gives the appearance of a particle of cheese dust.

On the fracture of the "cheese dust" particle, it can be seen (Figure 3 (2)) that fat globules occupy the main volume of the particle. Therefore, the loss of dry matter in the form of "cheese dust" leads mainly to the loss of fat from the curd being processed.

Figures 4 and 5 show the chromatograms of whey obtained from the manufacture of cheeses with different types and doses of MCEs, reflecting the content of water-soluble protein substances in whey.

The peak in the free volume of the chromatogram (V0) is formed by the smallest particles ("cheese dust" and fat globules) passing through a filter with a pore size of 0.45 µm used for filtration before chromatography. Figure 4 shows that whey samples from cheeses produced with MCEs of Fromase and "Pepsin" at the minimum introduction doses (1,500 IMCU/100 kg of milk) show a higher peak free volume (V0). This means the presence of a greater amount of cheese dust in the composition of whey obtained from cheeses made with minimal MCEs doses of Fromase and "Pepsin".

Water-soluble products of proteolysis potentially formed from curd caseins under the action of MCE have a mass of less than 5 kDa [37]. On the chromatograms of cheese whey obtained from cheeses produced with different combinations of the type and dose of MCEs, there are no significant differences in the area of the peaks of substances with a molecular weight of less than 5 kDa. This means that during the grain processing in the tank, there is no proteolytic effect of MCE on alpha- and beta-caseins, to the extent that leads to the formation of water-soluble proteolysis products and their loss from the curd to the whey.

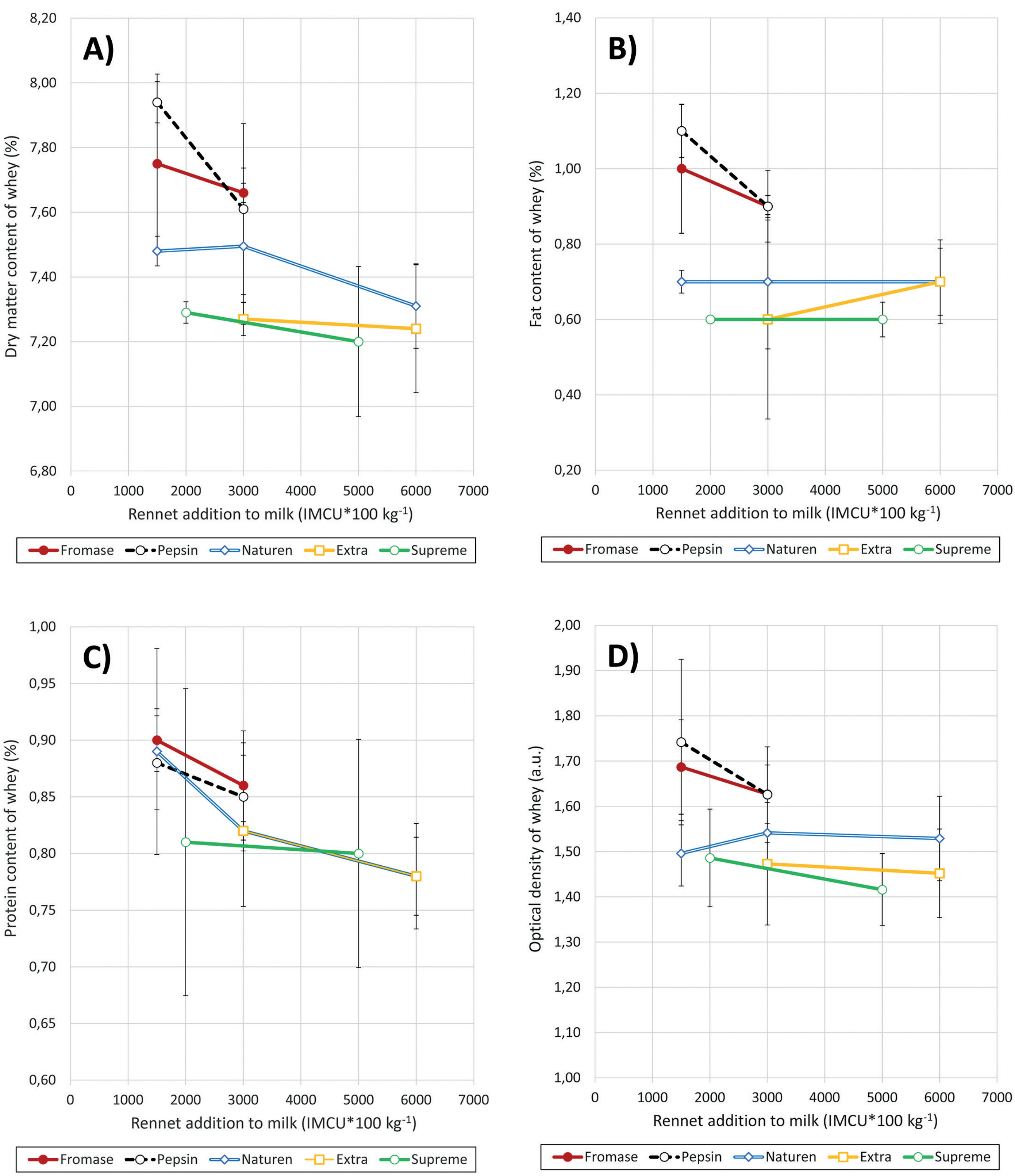

Figure 2. **The influence of the type and introduction dose of MCE of different brands on the mass fraction of dry matter of whey (A), the mass fraction of whey fat (B), the mass fraction of whey protein (C) and the optical density of whey (D). The keys of the MCE brands on the graph are similar to Figure 1. The data are given in the form of "mean value ± standard deviation"**

Рисунок 2. **Влияние типа и дозы внесения МФП разных марок на: массовую долю сухого вещества сыворотки(A), массовую долю жира сыворотки (B), массовую долю белка сыворотки(C), оптическую плотность сыворотки (D). Обозначения марок МФ на графике аналогичны с Рисунком 1. Данные приведены в форме «среднее значение ± стандартное отклонение»**

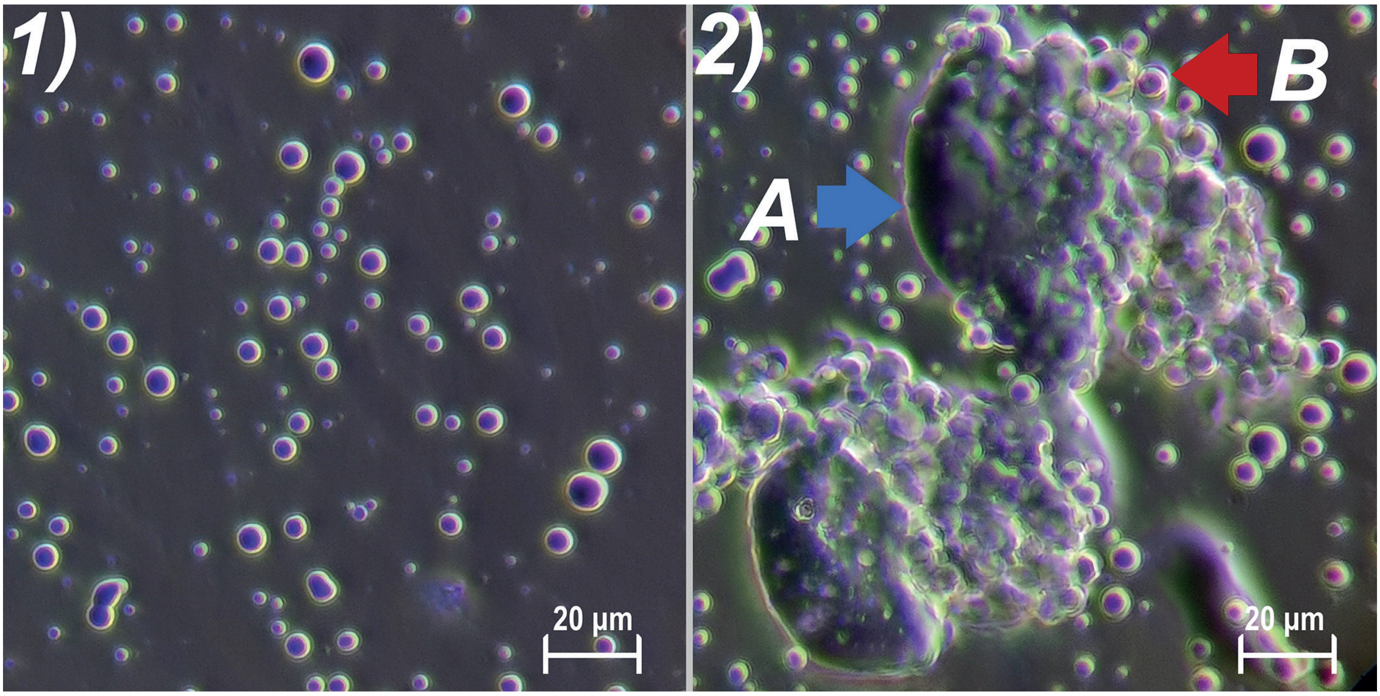

Figure 3. **Whey micropreparation 1) fat globules; 2) cheese dust particles (A) containing a large number of fat globules (B)**
Рисунок 3. **Микропрепарат сыворотки: 1) жировые шарики; 2) частицы сырной пыли (А), содержащие большое количество жировых шариков (B)**

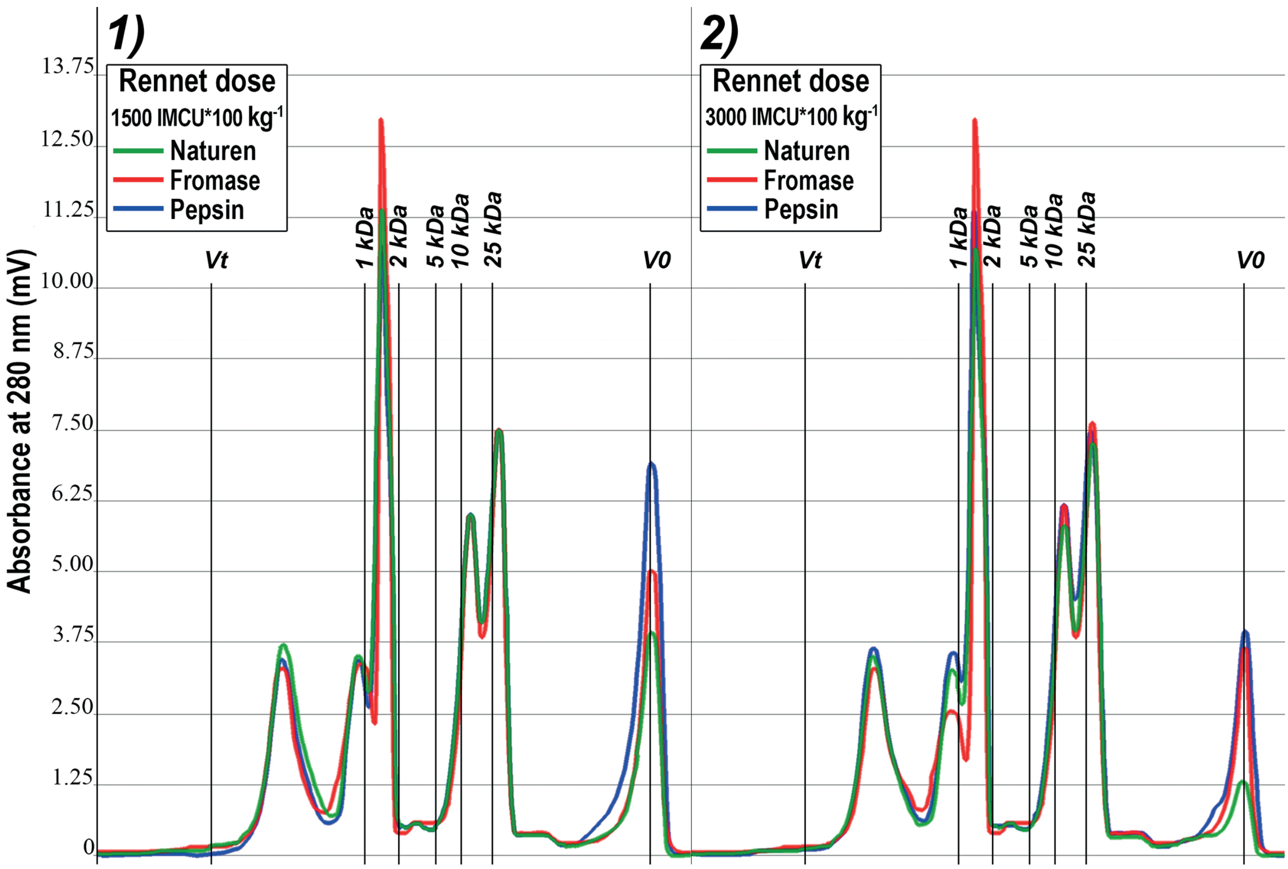

Figure 4. **Chromatograms of cheese whey samples obtained from cheese variants produced with rennet enzyme (Naturen), microbial MCE (Fromase), and MCE based on bovine pepsin (Pepsin)**
Рисунок 4. **Хроматограммы образцов подсырной сыворотки, полученной от вариантов сыров, произведенных с сычужным ферментом (Naturen), с МФ микробного происхождения (Fromase) и с МФ на основе говяжьего пепсина (Пепсин)**

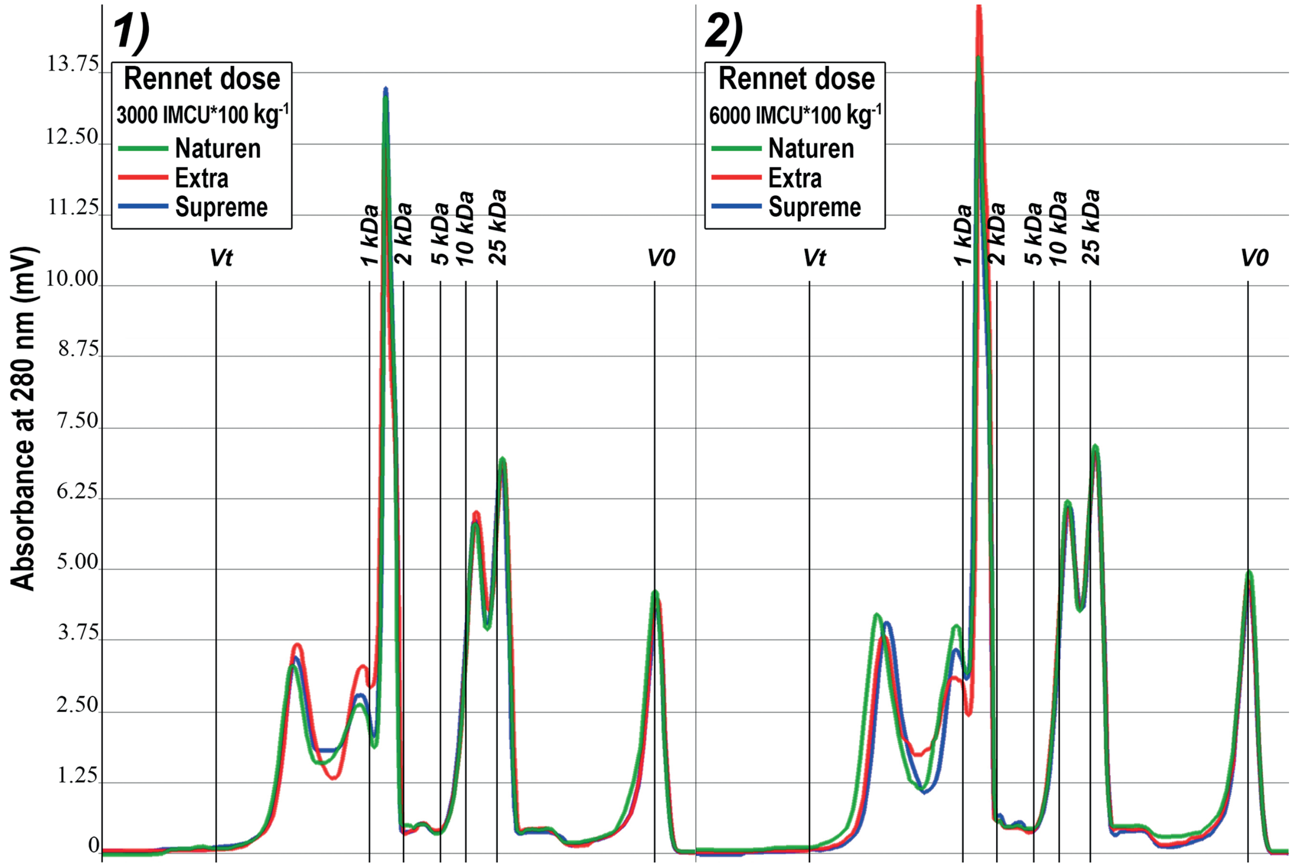

Figure 5. **Chromatograms of cheese whey samples obtained from cheese variants produced with rennet enzyme (Naturen), MCE of Chy-max Extra (Extra) and MCE of Chy-max Supreme (Supreme)**

Рисунок 5. Хроматограммы образцов подсырной сыворотки, полученной от вариантов сыров, произведенных с сычужным ферментом (Naturen), с МФ Chy-max Extra (Extra) и с МФ Chy-max Supreme (Supreme)

The influence of the MCE type on the optical density of the whey (Table 5, Figure 2 (D)), is also confirmed by the fact that the dry matter of the curd is lost in the form of optically opaque particles, i.e. "cheese dust".

Based on the obtained results on the effect of the type and dose of MCE on the amount of dry matter loss of the curd in cheese whey, it can be concluded that the use of higher doses of MCE with a high MCA/PA ratio, such as Chy-max Extra and Naturen (6000 IMCU/100 kg of milk) leads to a decrease in the loss of milk dry matter to whey by ~0.6% (from 7.8% to 7.2%). At the same time, the use of Chy-max Supreme MCE makes it possible to achieve equally low losses already at an introduction dose of 2,000 IMCU/100 kg of milk.

3.4. Physicochemical indicators of cheeses

The aim of the experiment was to obtain cheeses of the same composition. To achieve this goal, the duration of mechanical processing of the curd in the tank was adjusted (see section 3.2).

Table 6 shows the physicochemical indicators of test cheeses at the beginning of the shelf life (in 7 days).

Cheese variants made using different types and doses of MCEs did not have statistically accurate differences in the content of dry matter, fat, protein, salt and pH level (Tukey's test, $p > 0.05$).

Table 6. **Physicochemical parameters of test cheeses at the beginning of storage**

Таблица 6. **Физико-химические показатели экспериментальных сыров в начале хранения**

MCE brand	Dose*	Mass fraction of dry matter, %	Mass fraction of fat, %	Mass fraction ofprotein, %	Mass fraction of salt, %	pH
Fromase 750XLG	-1	58.28±0.27	30.80±0.61	20.18±0.56	1.24±0.03	5.11±0.06
	0	58.23±0.40	32.27±0.11	20.15±0.28	1.15±0.08	5.08±0.05
Pepsin FS-10	-1	56.85±0.44	30.80±1.10	20.01±0.54	1.10±0.06	5.10±0.07
	0	58.70±1.28	31.53±0.53	20.55±0.82	1.41±0.02	5.12±0.06
Naturen Extra	-1	58.04±0.44	31.10±0.55	20.67±0.15	1.30±0.03	5.11±0.04
	0	58.95±1.34	31.53±1.12	20.52±0.64	0.95±0.05	5.15±0.03
	+1	57.08±0.96	31.12±0.57	19.25±0.79	1.36±0.04	5.11±0.03
Chy-max Extra	0	58.92±0.01	33.00±0.01	19.56±0.23	1.36±0.03	5.13±0.02
	+1	56.97±0.17	31.02±1.00	19.13±0.69	1.29±0.01	5.09±0.03
Chy-max Supreme	0	57.58±0.87	33.00±0.28	19.31±0.64	1.36±0.04	5.13±0.02
	+1	56.92±1.07	31.77±0.59	19.31±0.62	1.33±0.05	5.09±0.01

Note:
* Introduction dose of MCE into the milk mixture, IMCU/100 kg of milk: "-1" — 1,500; "0" — 2,000 for Chy-max Supreme MCE and 3,000 for other MCEs; "+1" — 5,000 for the Chy-max Supreme MCE and 6,000 for other MCEs. Data are presented in the format "mean value±standard deviation".

3.5. Conversion efficiency of milk dry matter

To establish the influence of experimental factors on the efficiency of converting milk dry matter into cheese, an analysis of variance of the data was carried out. Table 7 shows the results of the analysis of variance, reflecting the degree of influence of the type and dose of MCEs used in the cheese production, on the indicators characterizing the efficiency of the conversion of milk dry matter.

The results of the analysis of variance, given in Table 7, show that the MCE dose has a significant effect on the actual cheese yield and on the weight of the cheese head ($p < 0.001$). In addition, the MCE dose affects, although less significantly, the degree of transition of fat and protein into the composition of the cheese mass ($p < 0.05$).

The influence of the experimental factors (type and dose of MCE) on the indicators characterizing the efficiency of the conversion of milk dry matter is visualized in the form of graphs in Figure 6.

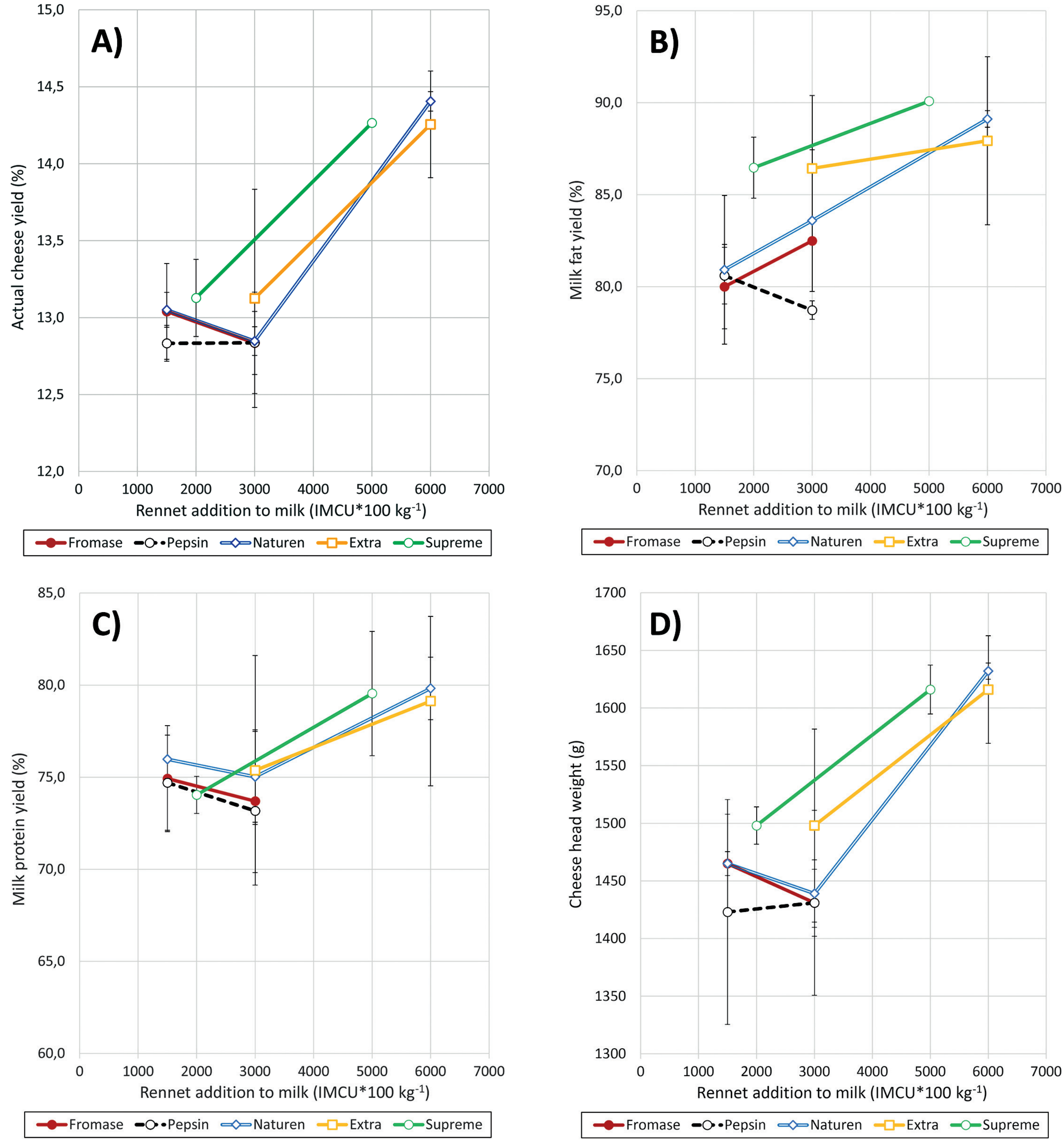

Figure 6. **Influence of the type and introduction dose of MCE of different brands on the actual cheese yield (A), the degree of transition of fat into the cheese composition (B), the degree of transition of protein into the cheese composition (C), the weight of the cheese head (D). The keys of the MCE brands on the graph are similar to Figure 1. The data are given in the form of “mean value ± standard deviation”**

Рисунок **6. Влияние типа и дозы внесения МФП разных марок на: фактический выход сыра (A), степень перехода жира в состав сыра (B), степень перехода белка в состав сыра (C), массу сырной головки (D). Обозначения марок МФ на графике аналогичны с Рисунком 1. Данные приведены в форме «среднее значение ± стандартное отклонение»**

Table 7. **Mean sum of squared deviations, level of statistical significance and coefficients of determination of the ANOVA model for response variables**

Таблица 7. **Средняя сумма квадратов отклонений, уровень статистической достоверности и коэффициенты детерминации модели ANOVA для переменных отклика**

	df	Actual cheese yeild, %	Percentage transferred to cheese		Weight of the cheese head, g
			Milk fat, %	Milk protein, %	
Type	4	0.035 (–)	15.5 (–)	2.1 (–)	2005 (–)
Dose	2	2.917 (***)	35.1 (*)	38.9 (*)	37776 (***)
Error	17	0.068	7.9	7.4	2157
R^2		0.88	0.68	0.48	0.78

Note:
df — number of degrees of freedom
Factor keys: Type — MCE type; Dose — MCE dose.
Error — part of the response variable variation related to an error;
R^2 — coefficient of determination for the ANOVA model.
The level of statistical significance of the factor effect evaluation (in parentheses): "–" — statistically inaccurately ($p>0.05$); "*" — $p<0.05$; "**" — $p<0.01$; "***" — $p<0.001$.

The reason for the influence of the "MCE dose" factor on the actual cheese yield and the weight of the cheese head is the reduction in the total duration of processing of the cheese curd with an increase in the MCE dose. Other conditions being equal, reducing the processing duration leads to the preservation of more moisture in the curd. Cheeses with high doses of MCE had a shorter duration of curd processing in the tank (Figure 1), and as a result, a higher moisture content, compared to cheeses produced with lower doses of MCE and subjected to longer mechanical processing. Figure 7 (A) visualizes the relationship between the MCE dose used to coagulate the milk, the duration of curd processing, and the mass fraction of moisture in the cheese.

In addition, a factor that implicitly affects the cheese yield and the weight of the cheese head is the "MCE type". The use of MCE in the cheese production, which have a low level of PA, helps to reduce fat loss to whey (Figure 2 (B)) and increase the degree of transfer of milk fat to cheese (Figure 6 (B)). Emmons [6] showed that loss of whey fat leads to a proportional decrease in fat content in cheese and a decrease in cheese yield. On the contrary, a decrease in fat loss leads to an increase in the cheese yield and the weight of the cheese head. Figure 7(B) shows the relationship between moisture and fat content of cheeses and actual cheese yield.

The data obtained indicate the effect of the use of types and doses of MCEs in the study on the duration of the stages of processing the cheese curd, on the loss of dry matter of the curd in the cheese whey, the composition of fresh cheeses (mass fraction of moisture and fat) and on the cheese yield. The regularities discovered in this work coincide with the results of studies by other authors discussed in the introduction to this article.

Conclusions

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

- the studied MCEs of different origins (animal, microbial, and recombinant) differ significantly in the level of specific nonspecific proteolytic activity, expressed in terms of the MCA/PA index, according to which the studied MCEs of different types are arranged in descending order: *R. miehei* protease (Fromase) > bovine pepsin (PS-10) > calf rennet (Naturen) > recombinant calf chymosin (Chy-max Extra MCE) > modified camel chymosin (Chy-max Supreme MCE);
- despite significant differences in the level of specific nonspecific proteolytic activity, the duration of milk coagulation by the studied MCEs depends only on the dose of units of milk-clotting activity, but not on the proteolytic activity of MCEs. The exception is Chy-max Supreme, which has the highest MCA/PA. When adding Chy-max Supreme MCE to milk at a dose of 2,000 IMCU/100 kg of milk, the duration of milk coagulation is provided at a level that requires the introduction of other studied MCEs at a dose of

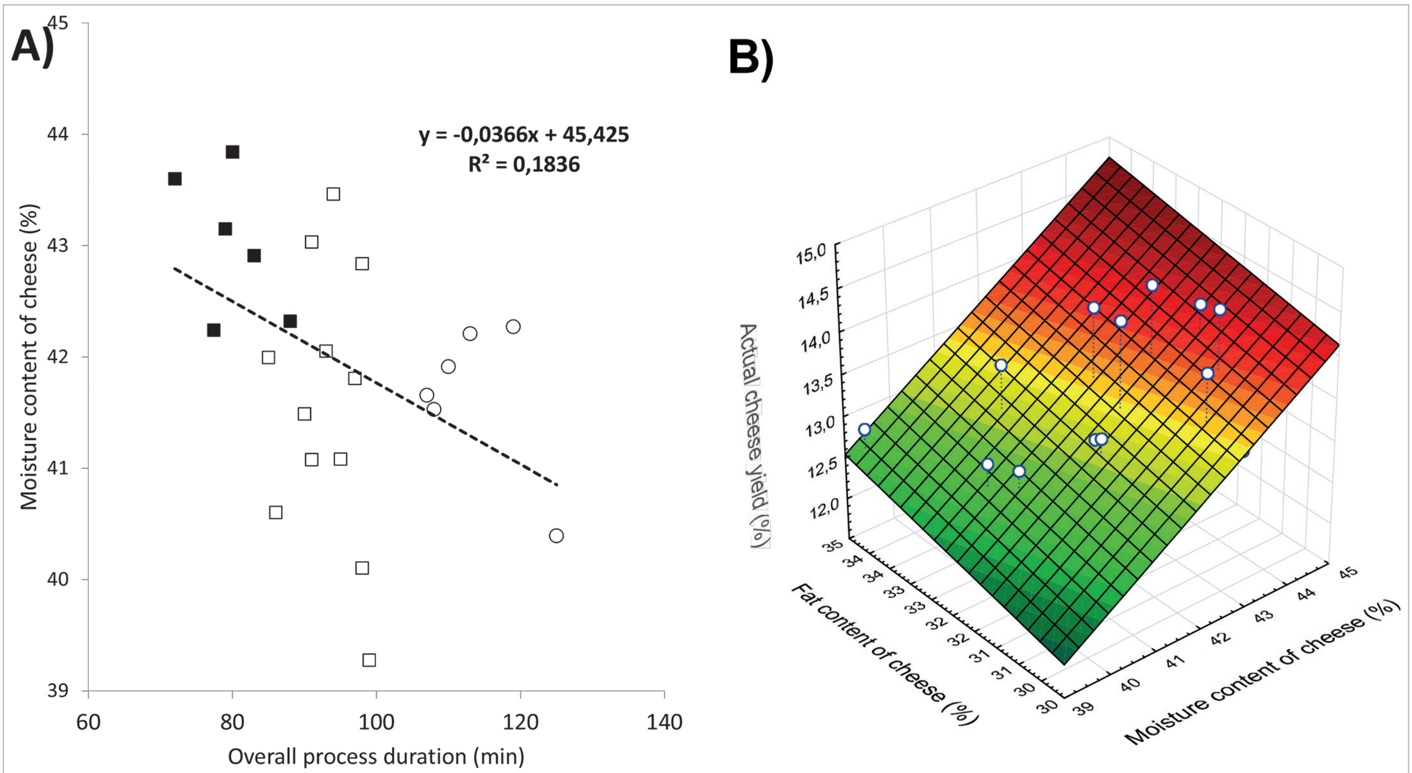

Figure 7. **A) Effect of the total curd processing time, include the coagulation time, on the moisture content of the cheese. Introduction dose of MCE (see Table 2): ○ — level "-1"; □ — level "0"; ■ — level "+1". B) The dependence of the actual cheese yield on the indicators of the cheese composition**

Рисунок 7. **А) Влияние общей продолжительности обработки сырного сгустка на содержание влаги в сыре. Доза внесения МФ (см. Таблицу 2): ○ — уровень «-1»; □ — уровень «0»; ■ — уровень «+1»; В) Зависимость фактического выхода сыра от показателей состава**

3,000 IMCU/100 kg of milk. In addition, the dose of Chy-max Supreme MCE at 5,000 IMCU/100 kg of milk provides the same duration of milk coagulation as other tested MCEs at a dose of 6,000 IMCU/100 kg of milk;

- the level of specific non-specific proteolytic activity of MCEs does not affect the total duration of grain processing in the cheesemaking tank. There are no significant ($p<0.05$) differences between MCEs with different levels of MCA/PA in terms of the total duration of grain processing in the tank, which is significantly ($p<0.05$) affected by the MCE dose introduced for milk coagulation. With an increase in the MCE dose, the duration of grain processing in the tank is reduced;
- the level of specific non-specific proteolytic activity of MCEs affects the proportion of milk dry matter lost in the whey composition during the processing of the cheese curd. In variants of cheeses made with the use of MCEs with a low level of MCA/PA (Fromase, "Bovine Pepsin"), the highest waste of milk dry matter into whey is noted. When using natural calf rennet (Naturen Extra), there is less release of milk dry matter into whey. In the variants with the use of MCEs with the highest level of MCA/PA (Chy-max Extra and Chy-max Supreme), the lowest release of milk dry matter into whey among the studied types of MCEs is noted;
- the use of MCEs with a low level of PA at an increased dosage (6,000 IMCU/100 kg of milk for Naturen and Chy-max Extra or 5,000 IMCU/100 kg of milk for Chy-max Supreme) can increase the actual cheese yield by an average of 1.5% (from 12.7% to 14.2%).

REFERENSES

1. Fox, P. F., Cogan, T. M., Guinee, T. P. (2017). Rennet-Induced Coagulation of Milk. Chapter in a book: Cheese: Chemistry, Physics and Microbiology. Elsevier: Academic Press, 2017. https://doi.org/10.1016/B978–0–12–417012–4.00005–3
2. Fox, P. F., Guinee, T. P., Cogan, T. M., McSweeney, P. L. H. (2017). Cheese: Enzymatic Coagulation of Milk. Chapter in a book: Fundamentals of Cheese Science, 2nd Ed. New York: Springer. 2017. https://doi.org/10.1007/978–1–4899–7681–9_7
3. Chitpinityol, S., Crabbe, M. D. C., (1998). Review. Chymosin and aspartic proteinases. *Food Chemistry*, 61(4), 395–418.
4. Harboe, M., Broe, M. L. Qvist, K. B. (2010). The Production, action and application of rennet and coagulants. Chapter in a book: Technology of cheesemaking. 2nd Ed. Chichester: Blackwell Publishing Ltd., 2010. https://doi.org/10.1002/9781444323740.ch3
5. Jaros, D., Rohm, H. (2017). Rennets: Applied Aspects. Chapter in a book: Cheese: Chemistry, Physics and Microbiology. Elsevier: Academic Press. 2017. https://doi.org/10.1016/B978–0–12–417012–4.00003-X
6. Emmons, D. B., Reiser, B., Giroux, R. N., Stanley, D. W. (1976). Cheddar cheese made with bovine pepsin. I. Yield and quality of cheese. *Canadian Institute of Food Science and Technology Journal*, 9(4), 189–200. https://doi.org/10.1016/S0315–5463(76)73674–5
7. Meinardi, C. A., Alonso, A. A, Hynes, E. R., Zalazar, C. A. (2002). Influence of milk-clotting enzymes on acidification rate of natural whey starter culture. *International Journal of Dairy Technology*, 55(3), 139–144. https://doi.org/10.1046/j.1471–0307.2002.00052.x
8. Jacob, M., Jaros, D., Rohm, H. (2010). The effect of coagulant type on yield and sensory properties of semihard cheese from laboratory-, pilot- and commercial-scale productions. *International Journal of Dairy Technology*, 63(3), 370–380. https://doi.org/10.1111/j.1471–0307.2010.00598.x
9. Yasar, K., Guzeler, N. (2011). Effects of coagulant type on the physicochemical and organoleptic properties of Kashar cheese. *International Journal of Dairy Technology*, 64(3), 372–379. https://doi.org/10.1111/j.1471–0307.2011.00679.x
10. Dekker, P. (2019). Dairy Enzymes. Chapter in a book: Industrial Enzyme Applications. 1st Ed. Weinheim: Wiley-VCH Verlag GmbH & Co., 2019. https://doi.org/10.1002/9783527813780.ch2_3
11. Ha-La biotec. Chy-max® Supreme-cheese production at a new level. Retrieved from https://halabiotec.com.br/wp-content/uploads/2019/06/Ha-La_Biotec_147.pdf Accessed April 12, 2022. (In Portuguese)
12. Fox, P. F., Cogan, T. M., Guinee, T. P. (2017). Factors that Affect the Quality of Cheese. Chapter in a book: Cheese: Chemistry, Physics and Microbiology. Elsevier: Academic Press, 2017. https://doi.org/10.1016/S1874–558X(04)80084–8
13. Mozzarella is the biggest category in the commercial cheese market. Retrieved from https://mejeriteknikselskab.dk/sites/default/files/dms/Seminarprogrammer/ulf_mortensen_new_syrning_og_koagulering_i_mozzarella_apr2018_final_version.pdf Accessed April 12, 2022
14. Chr Hansen. Working together to produce more cheese from milk. Retrieved from https://sdtstatic.s3.amazonaws.com/media/uploads/2019/05/13/1CHR%20HANSEN190508%20SDT%20Presentation%20CHR%20Hansen.pdf Accessed March 01, 2023.
15. Wilkinson, M. G., Kilcawley, K. N. (2005). Mechanisms of incorporation and release of enzymes into cheese during ripening. *International Dairy Journal*, 15(6–9), 817–830. https://doi.org/10.1016/j.idairyj.2004.08.021
16. Moynihan, A. C., Govindasamy-Lucey, S., Jaeggi, J. J., Johnson, M. E., Lucey, J. A., McSweeney, P. L. H. (2014). Effect of camel chymosin on the texture, functionality, and sensory properties of low-moisture, part-skim Mozzarella cheese. *Journal of Dairy Science*, 97(1), 85–96. https://dx.doi.org/10.3168/jds.2013–7081
17. Soodam, K., Ong, L., Powell, I. B., Kentish, S. E., Gras, S. L. (2015). Effect of rennet on the composition, proteolysis and microstructure of reduced-fat Cheddar cheese during ripening. *Dairy Science and Technology*, 95, 665–686. https://doi.org/10.1007/s13594–015–0250–5
18. Alinovi, M., Cordioli, M., Francolino, S., Locci, F., Ghiglietti, R., Monti, L. et al. (2018). Effect of fermentation-produced camel chymosin on quality of crescenza cheese. *International Dairy Journal*, 84, 72–78. https://doi.org/10.1016/j.idairyj.2018.04.001
19. Myagkonosov, D. S., Abramov, D. V., Delitskaya, I. N., Bukcharina, G. B. (2022). Effect of the recombinant chymosins of different origins on the quality and shelf life of soft cheeses. *Food Systems*, 5(3), 239–248. https://doi.org/10.21323/2618–9771–2022–5–3–239–248
20. McCarthy, C. M., Wilkinson, M. G., Guinee, T. P. (2017). Effect of coagulant type and level on the properties of half-salt, half-fat Cheddar cheese made with or without adjunct starter: improving texture and functionality. *International Dairy Journal*, 75, 30–40. https://doi.org/10.1016/j.idairyj.2017.07.006
21. Bansal, N., Drake, M. A., Piraino, P., Broe, M. L., Harboe, M., Fox, P. F. et al. (2009). Suitability of recombinant camel (Camelus dromedarius) chymosin as a coagulant for Cheddar cheese. *International Dairy Journal*, 19(9), 510–517. https://doi.org/10.1016/j.idairyj.2009.03.010
22. Sousa, M. J., Malcata, F. X. (1996). Effects of processing conditions on the caseinolytic activity of crude extracts of Cynara cardunculus L. *Food Science and Technology International*, 2(4), 255–263. https://doi.org/10.1177/108201329600200408
23. Arbita, A., Kristijarti A., Ardelia I. (2015). The effects of the types of milk (cow, goat, soya) and enzymes (rennet, papain, bromelain) toward Cheddar cheese production. *Makara Journal of Technology*, 19(1), Article 7. https://doi.org/10.7454/mst.v19i1.3028
24. Ben Amira, A., Besbes, S., Attia, H., Blecker, C. (2017). Milk-clotting properties of plant rennets and their enzymatic, rheological, and sensory role in cheese making: A review. *International Journal of Food Properties*, 20(sup1), S76–S93. https://doi.org/10.1080/10942912.2017.1289959
25. Osuna-Ruíz, I., Tiznado-Garzón, R., Salazar-Leyva, J. A., García-Magaña, M. de L., Benítez-García, I., Moreno-Hernández, J. M. et al. (2023). Milk-clotting and proteolytic properties of a partially purified pepsin from Yellowfin Tuna (*Thunnus albacares*) and its potential for cheesemaking. *Food and Bioprocess Technology*, 2023. https://doi.org/10.1007/s11947–023–03030–3
26. Eskander, M. (2017). Utilization chicken gizzard lining immobilized pepsin for soft white cheese manufacture. *Syrian Journal of Agricultural Research*, 4(4), 49–58. (In Arabic)
27. Creamer, L. K., Iyer, M., Lelievre, J. (1987). Effect of various levels of rennet addition on characteristics of Cheddar cheese made from ultrafiltered milk. *New Zealand Journal of Dairy Science and Technology*, 22(3), 205–214.
28. McSweeney, P. L. H. (2007). Cheese problems solved. (2007). Woodhead Publishing, 2007
29. Visser, S., Slangen, C. J., Robben, A. J. P. M. (1992). Determination of molecular mass distributions of whey protein hydrolysates by high-performance size-exclusion chromatography. *Journal of Chromatography A*, 599(1–2), 205–209. https://doi.org/10.1016/0021–9673(92)85474–8
30. Fox P. F., Guinee T. P., Cogan T. M., McSweeney P. L. H. (2017). Cheese Yield. Chapter in a book: Fundamentals of Cheese Science. Springer, Boston, MA, 2017. https://doi.org/10.1007/978–1–4899–7681–9_10
31. Smykov, I. T. (2018). Milk curd cutting time determination in cheesemaking. *Food Systems*, 1(2), 12–20. https://doi.org/10.21323/2618–9771–2018–1–2–12–20 (In Russian)
32. Smykov, I. T. (2019). Self segmenting of rennet induced milk gel in cheesemaking tank. *International Journal of Dairy Technology*, 72(4), 591–600. https://doi.org/10.1111/1471–0307.12650
33. Montgomery, D. C. (2013). Design and analysis of experiments. Wiley, 2013
34. Myagkonosov, D. S., Mordvinova, V. A., Abramov, D. V., Ovchinnikova, E. G., Municheva, T. E. (2020). Rennet test is an important tool for pro-

ducing high quality cheese. *Cheesemaking and Buttermaking*, 2, 30–33. https://doi.org/10.31515/2073–4018–2020–2–28–31 (In Russian)

35. Myagkonosov, D. S., Mordvinova, V. A., Abramov, D. V., Ovchinnikova, E. G., Municheva, T. E. (2020). Technological properties of milk clotting enzymes of different origin. Part II. Influence of the type of milk clotting enzyme used on proteolysis processes during cheese ripening. *Cheesemaking and Buttermaking*, 5, 10–13. https://doi.org/10.31515/2073–4018–2020–5–10–13 (In Russian)
36. Myagkonosov, D. S., Mordvinova, V. A., Delitskaya, I. N., Abramov, D. V., Ovchinnikova, E. G. (2020). The influence of milk-clotting enzymes on the functional properties of pizza-cheeses. *Food Systems*, 3(3), 42–50. https://doi.org/10/21323/2618–9771–2020–3–3–42–50
37. Garg, S. K., Johri, B. N. (1994). Rennet: Current trends and future research. *Food Reviews International*, 10(3), 313–355. https://doi.org/10.1080/87559129409541005
38. Jacob, M. (2011). Milk coagulation enzymes of various origins and their influence on cheese yield and cheese quality. Dissertation. Dresden: Technical University of Dresden, 2011. Retrieved from https://d-nb.info/1067190643/34 Accessed March 01, 2023. (In German)

БИБЛИОГРАФИЧЕСКИЙ СПИСОК

1. Fox, P. F., Cogan, T. M., Guinee, T. P. (2017). Rennet-Induced Coagulation of Milk. Chapter in a book: Cheese: Chemistry, Physics and Microbiology. Elsevier: Academic Press, 2017. https://doi.org/10.1016/B978–0–12–417012–4.00005–3
2. Fox, P. F., Guinee, T. P., Cogan, T. M., McSweeney, P. L. H. (2017). Cheese: Enzymatic Coagulation of Milk. Chapter in a book: Fundamentals of Cheese Science, 2nd Ed. New York: Springer. 2017. https://doi.org/10.1007/978–1–4899–7681–9_7
3. Chitpinityol, S., Crabbe, M. D. C., (1998). Review. Chymosin and aspartic proteinases. *Food Chemistry*, 61(4), 395–418.
4. Harboe, M., Broe, M. L. Qvist, K. B. (2010). The Production, action and application of rennet and coagulants. Chapter in a book: Technology of cheesemaking. 2nd Ed. Chichester: Blackwell Publishing Ltd., 2010. https://doi.org/10.1002/9781444323740.ch3
5. Jaros, D., Rohm, H. (2017). Rennets: Applied Aspects. Chapter in a book: Cheese: Chemistry, Physics and Microbiology. Elsevier: Academic Press. 2017. https://doi.org/10.1016/B978–0–12–417012–4.00003-X
6. Emmons, D. B., Reiser, B., Giroux, R. N., Stanley, D. W. (1976). Cheddar cheese made with bovine pepsin. I. Yield and quality of cheese. *Canadian Institute of Food Science and Technology Journal*, 9(4), 189–200. https://doi.org/10.1016/S0315–5463(76)73674–5
7. Meinardi, C. A., Alonso, A. A, Hynes, E. R., Zalazar, C. A. (2002). Influence of milk-clotting enzymes on acidification rate of natural whey starter culture. *International Journal of Dairy Technology*, 55(3), 139–144. https://doi.org/10.1046/j.1471–0307.2002.00052.x
8. Jacob, M., Jaros, D., Rohm, H. (2010). The effect of coagulant type on yield and sensory properties of semihard cheese from laboratory-, pilot- and commercial-scale productions. *International Journal of Dairy Technology*, 63(3), 370–380. https://doi.org/10.1111/j.1471–0307.2010.00598.x
9. Yasar, K., Guzeler, N. (2011). Effects of coagulant type on the physicochemical and organoleptic properties of Kashar cheese. *International Journal of Dairy Technology*, 64(3), 372–379. https://doi.org/10.1111/j.1471–0307.2011.00679.x
10. Dekker, P. (2019). Dairy Enzymes. Chapter in a book: Industrial Enzyme Applications. 1st Ed. Weinheim: Wiley-VCH Verlag GmbH & Co., 2019. https://doi.org/10.1002/9783527813780.ch2_3
11. Ha-La biotec. Chy-max® Supreme-cheese production at a new level. Retrieved from https://halabiotec.com.br/wp-content/uploads/2019/06/Ha-La_Biotec_147.pdf Accessed April 12, 2022. (In Portuguese)
12. Fox, P. F., Cogan, T. M., Guinee, T. P. (2017). Factors that Affect the Quality of Cheese. Chapter in a book: Cheese: Chemistry, Physics and Microbiology. Elsevier: Academic Press, 2017. https://doi.org/10.1016/S1874–558X(04)80084–8
13. Mozzarella is the biggest category in the commercial cheese market. Retrieved from https://mejeritekniskselskab.dk/sites/default/files/dms/Seminarprogrammer/ulf_mortensen_new_syrning_og_koagulering_i_mozzarella_apr2018_final_version.pdf Accessed April 12, 2022.
14. Chr Hansen. Working together to produce more cheese from milk. Retrieved from https://sdtstatic.s3.amazonaws.com/media/uploads/2019/05/13/1CHR%20HANSEN190508%20SDT%20Presentation%20CHR%20Hansen.pdf Accessed March 01, 2023
15. Wilkinson, M. G., Kilcawley, K. N. (2005). Mechanisms of incorporation and release of enzymes into cheese during ripening. *International Dairy Journal*, 15(6–9), 817–830. https://doi.org/10.1016/j.idairyj.2004.08.021
16. Moynihan, A. C., Govindasamy-Lucey, S., Jaeggi, J. J., Johnson, M. E., Lucey, J. A., McSweeney, P. L. H. (2014). Effect of camel chymosin on the texture, functionality, and sensory properties of low-moisture, part-skim Mozzarella cheese. *Journal of Dairy Science*, 97(1), 85–96. https://dx.doi.org/10.3168/jds.2013–7081
17. Soodam, K., Ong, L., Powell, I. B., Kentish, S. E., Gras, S. L. (2015). Effect of rennet on the composition, proteolysis and microstructure of reduced-fat Cheddar cheese during ripening. *Dairy Science and Technology*, 95, 665–686. https://doi.org/10.1007/s13594–015–0250–5
18. Alinovi, M., Cordioli, M., Francolino, S., Locci, F., Ghiglietti, R., Monti, L. et al. (2018). Effect of fermentation-produced camel chymosin on quality of crescenza cheese. *International Dairy Journal*, 84, 72–78. https://doi.org/10.1016/j.idairyj.2018.04.001
19. Myagkonosov, D. S., Abramov, D. V., Delitskaya, I. N., Bukcharina, G. B. (2022). Effect of the recombinant chymosins of different origins on the quality and shelf life of soft cheeses. *Food Systems*, 5(3), 239–248. https://doi.org/10.21323/2618–9771–2022–5–3–239–248
20. McCarthy, C. M., Wilkinson, M. G., Guinee, T. P. (2017). Effect of coagulant type and level on the properties of half-salt, half-fat Cheddar cheese made with or without adjunct starter: improving texture and functionality. *International Dairy Journal*, 75, 30–40. https://doi.org/10.1016/j.idairyj.2017.07.006
21. Bansal, N., Drake, M. A., Piraino, P., Broe, M. L., Harboe, M., Fox, P. F. et al. (2009). Suitability of recombinant camel (Camelus dromedarius) chymosin as a coagulant for Cheddar cheese. *International Dairy Journal*, 19(9), 510–517. https://doi.org/10.1016/j.idairyj.2009.03.010
22. Sousa, M. J., Malcata, F.X. (1996). Effects of processing conditions on the caseinolytic activity of crude extracts of Cynara cardunculus L. *Food Science and Technology International*, 2(4), 255–263. https://doi.org/10.1177/108201329600200408
23. Arbita, A., Kristijarti A., Ardelia I. (2015). The effects of the types of milk (cow, goat, soya) and enzymes (rennet, papain, bromelain) toward Cheddar cheese production. *Makara Journal of Technology*, 19(1), Article 7. https://doi.org/10.7454/mst.v19i1.3028
24. Ben Amira, A., Besbes, S., Attia, H., Blecker, C. (2017). Milk-clotting properties of plant rennets and their enzymatic, rheological, and sensory role in cheese making: A review. *International Journal of Food Properties,* 20(sup1), S76–S93. https://doi.org/10.1080/10942912.2017.1289959
25. Osuna-Ruíz, I., Tiznado-Garzón, R., Salazar-Leyva, J.A., García-Magaña, M. de L., Benítez-García, I., Moreno-Hernández, J.M. et al. (2023). Milk-clotting and proteolytic properties of a partially purified pepsin from Yellowfin Tuna (*Thunnus albacares*) and its potential for cheesemaking. *Food and Bioprocess Technology*, 2023. https://doi.org/10.1007/s11947–023–03030–3
26. Eskander, M. (2017). Utilization chicken gizzard lining immobilized pepsin for soft white cheese manufacture. *Syrian Journal of Agricultural Research*, 4(4), 49–58. (In Arabic)
27. Creamer, L. K., Iyer, M., Lelievre, J. (1987). Effect of various levels of rennet addition on characteristics of Cheddar cheese made from ultrafiltered milk. *New Zealand Journal of Dairy Science and Technology*, 22(3), 205–214.
28. McSweeney, P. L. H. (2007). Cheese problems solved. (2007). Woodhead Publishing, 2007
29. Visser, S., Slangen, C. J., Robben, A. J. P. M. (1992). Determination of molecular mass distributions of whey protein hydrolysates by high-pergomance size-exclusion chromatography. *Journal of Chromatography A*, 599(1–2), 205–209. https://doi.org/10.1016/0021–9673(92)85474–8
30. Fox P. F., Guinee T. P., Cogan T. M., McSweeney P. L. H. (2017). Cheese Yield. Chapter in a book: Fundamentals of Cheese Science. Springer, Boston, MA, 2017. https://doi.org/10.1007/978–1–4899–7681–9_10
31. Смыков И. Т. (2018). Определение момента готовности молочного сгустка к разрезке при производстве сыров. Пищевые системы, 1(2), 12–20. https://doi.org/10.21323/2618–9771–2018–1–2–12–20
32. Smykov, I. T. (2019). Self segmenting of rennet induced milk gel in cheesemaking tank. *International Journal of Dairy Technology*, 72(4), 591–600. https://doi.org/10.1111/1471–0307.12650
33. Montgomery, D. C. (2013). Design and analysis of experiments. Wiley, 2013
34. Мягконосов, Д. С., Мордвинова, В. А., Абрамов, Д. В., Овчинникова, Е. Г., Муничева, Т. Э. (2020). Сычужная проба — важный инструмент для получения сыра высокого качества. *Сыроделие и маслоделие*, 2, 30–33. https://doi.org/10.31515/2073–4018–2020–2–28–31
35. Мягконосов, Д. С., Мордвинова, В. А., Абрамов, Д. В., Овчинникова, Е. Г., Муничева, Т. Э. (2020). Технологические свойства молокосвертывающих ферментов разного происхождения. Часть II. Влияние вида используемого МФП на процессы протеолиза при созревании сыров. *Сыроделие и маслоделие*, 5, 10–13. https://doi.org/10.31515/2073–4018–2020–5–10–13
36. Myagkonosov, D. S., Mordvinova, V. A., Delitskaya, I. N., Abramov, D. V., Ovchinnikova, E. G. (2020). The influence of milk-clotting enzymes on the functional properties of pizza-cheeses. *Food Systems*, 3(3), 42–50. https://doi.org/10/21323/2618–9771–2020–3–3–42–50
37. Garg, S. K., Johri, B. N. (1994). Rennet: Current trends and future research. *Food Reviews International*, 10(3), 313–355. https://doi.org/10.1080/87559129409541005
38. Jacob, M. (2011). Milk coagulation enzymes of various origins and their influence on cheese yield and cheese quality. Dissertation. Dresden: Technical University of Dresden, 2011. Retrieved from https://d-nb.info/1067190643/34 Accessed March 01, 2023. (In German)

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Dmitry S. Myagkonosov, Candidate of Technical Sciences, Senior Researcher, Head of Research Department in Applied Biochemistry and Enzymology, All-Russian Scientific Research Institute of Butter- and Cheesemaking 19, Krasnoarmeysky Boulevard, Uglich, 152613, Yaroslavl Region, Russia Tel.: +7–915–973–63–13 E-mail: d.myagkonosov@fncps.ru ORCID: https://orcid.org/0000–0003–4443–7573 * corresponding author	**Мягконосов Дмитрий Сергеевич** — кандидат технических наук, старший научный сотрудник, руководитель направления исследований по прикладной биохимии и энзимологии, Всероссийский научно-исследовательский институт маслоделия и сыроделия 152613, Ярославская область, Углич, Красноармейский бульвар, 19 Тел.: +7–915–973–63–13 E-mail: d.myagkonosov@fncps.ru ORCID: https://orcid.org/0000–0003–4443–7573 * автор для контактов
Igor T. Smykov, Doctor of Technical Sciences, Chief Researcher, Department of Physical Chemisry, All-Russian Scientific Research Institute of Butter- and Cheesemaking 19, Krasnoarmeysky Boulevard, Uglich, 152613, Yaroslavl Region, Russia Tel.: +7–48532–9–81–21 E-mail: i_smykov@mail.ru ORCID: https://orcid.org/0000–0002–5663–3662	**Смыков Игорь Тимофеевич** — доктор технических наук, главный научный сотрудник, отдел физической химии, Всероссийский научно-исследовательский институт маслоделия и сыроделия 152613, Ярославская область, Углич, Красноармейский бульвар, 19 Тел.: +7–48532–9–81–21 E-mail: i_smykov@mail.ru ORCID: https://orcid.org/0000–0002–5663–3662
Dmitry V. Abramov, Candidate of Biological Sciences, Senior Researcher, Head of Biochemical Research in Cheesemaking and Buttermaking, All-Russian Scientific Research Institute of Butter- and Cheesemaking 19, Krasnoarmeysky Boulevard, Uglich, 152613, Yaroslavl Region, Russia Tel.:+ 7–910–970–42–97 E-mail: d.abramov@fncps.ru ORCID: https://orcid.org/0000–0001–8326–1932	**Абрамов Дмитрий Васильевич** — кандидат биологических наук, старший научный сотрудник, руководитель направления биохимических исследований по сыроделию и маслоделию, Всероссийский научно-исследовательский институт маслоделия и сыроделия 152613, Ярославская область, Углич, Красноармейский бульвар, 19 Тел.: +7–910–970–42–97 E-mail: d.abramov@fncps.ru ORCID: https://orcid.org/0000–0001–8326–1932
Irina N. Delitskaya, Candidate of Technical Sciences, Senior Researcher, Department of Cheesemaking, All-Russian Scientific Research Institute of Butter- and Cheesemaking 19, Krasnoarmeysky Boulevard, Uglich, 152613, Yaroslavl Region, Russia Tel.: +7–48532–98–1–28 E-mail: i.delitskaya@fncps.ru ORCID: https://orcid.org/0000–0003–3587–4050	**Делицкая Ирина Николаевна** — кандидат технических наук, старший научный сотрудник, отдел сыроделия, Всероссийский научно-исследовательский институт маслоделия и сыроделия 152613, Ярославская область, Углич, Красноармейский бульвар, 19 Tel.: +7–48532–98–1–28 E-mail: i.delitskaya@fncps.ru ORCID: https://orcid.org/0000–0003–3587–4050
Elena G. Ovchinnikova, Researcher, Department of Biochemistry, All-Russian Scientific Research Institute of Butter- and Cheesemaking 152613, Yaroslavl Region, Uglich, Krasnoarmeysky Boulevard, 19. Tel.: + 7–48532–98–1–94 E-mail: e.ovchinnikova@fncps.ru ORCID: https://orcid.org/0000–0003–4891–4330	**Овчинникова Елена Григорьевна** — научный сотрудник, отдел биохимии, Всероссийский научно-исследовательский институт маслоделия и сыроделия 152613, Ярославская область, Углич, Красноармейский бульвар, 19 Tel.: + 7–48532–98–1–94 E-mail: e.ovchinnikova@fncps.ru ORCID: https://orcid.org/0000–0003–4891–4330

Contribution	**Критерии авторства**
Authors equally relevant to the writing of the manuscript, and equally responsible for plagiarism.	Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.
Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.