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The influence of microparticulated whey proteins with polyphenols and micellar casein concentrate on quality indicators and biological value of semi-hard cheese

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Abstract. The aim of the research is to study the combined effect of micellar casein concentrate and whey protein microparticulate with polyphenols on the process of rennet coagulation of milk, organoleptic, physico-chemical parameters and biological value of experimental cheese samples. Cow's milk obtained from the supplier “Chapaevskoye” farm with a mass concentration of fat 3.9%, micellar casein concentrate with a ratio of casein: whey proteins 96:4, microparticulate of whey proteins with the inclusion (encapsulation) of dihydroquercetin in their structure, produced at JSC “Dairy Plant Stavropolsky” were used as research objects. The mechanism of reducing the astringency of polyphenols by whey proteins is considered. The relevance of using milk protein concentrates in cheese production is substantiated. The study of the combined effect of micellar casein concentrate and microparticulated whey proteins with the inclusion (encapsulation) of dihydroquercetin on rennet coagulation of milk is carried out. A comparative analysis of quality attributes and amino-acid score of semi-hard cheese enriched with dairy protein concentrates with "Rossijsky" cheese is given. The research results showed that the standardized dairy mixture with micellar casein concentrate and microparticulate with polyphenols is suitable for the production of rennet cheese. According to all physico-chemical parameters, the experimental sample is close to the control one. The mass fraction of protein is 0.5% higher, the mass fraction of moisture is 1.2% and the mass fraction of fat is 2.7% lower compared to the control sample. Since the control sample of “Russian” cheese has a lack of essential amino acids such as methionine and cysteine, it is concluded that the experimental cheese shows higher performance due to the presence of a microparticulate of whey proteins rich in sulfur-containing amino acids. The research results allow to conclude that cheese enriched with micellar casein concentrate and a microparticulate of whey proteins with the inclusion (encapsulation) of dihydroquercetin in their structure is characterized by stable organoleptic and physico-chemical parameters and a higher biological value compared to its counterpart, "Russian" cheese.

Keywords: micellar casein concentrate, microparticulated whey proteins, dihydroquercetin, semi-hard cheese

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Научная статья
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Влияние микропартикулята сывороточных белков с полифенолами и концентрата мицеллярного казеина на показатели качества и биологическую ценность полутвердого сыра

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Аннотация. *Целью работы является исследование совместного влияния концентрата мицеллярного казеина и микропартикулята сывороточных белков с полифенолами на процесс сычужного свёртывания молока, органолептические, физико-химичесике показатели и биологическую ценность опытных образцов сыра. В качестве объектов исследований использовали молоко коровье-сырье, полученное от поставщика ООО СП «Чапаевское» с массовой долей жира 3,9%, концентрат мицеллярного казеина с соотношением казеин: сывороточные белки 96:4, микропартикулят сывороточных белков с включением (инкапсулированием) в их структуру дигидрокверцетина, произведенные на АО «Молочный комбинат «Ставропольский». Рассмотрен механизм снижения терпкости полифенолов сывороточными белками. Обоснована актуальность использования концентратов молочных белков в сыроделии. Проведено исследование совместного влияния на сычужное свёртывание молока концентрата мицеллярного казеина и микропартикулята сывороточных белков с включением (инкапсулированием) в их структуру дигидрокверцетина. Дан сравнительный анализ показателей качества и аминокислотного скора сыра, обогащенного концентратами молочных белков с «Российским» сыром. Результаты исследований показали, что нормализованная смесь с концентратом мицеллярного казеина и микропартикулятом с полифенолами пригодна для производства сычужного сыра. По всем физико-химическим показателям экспериментальный образец близок к контрольному. Массовая доля белка на 0,5% выше, массовая доля влаги на 1,2 % и массовая доля жира на 2,7 % ниже по сравнению с контрольным образцом. Поскольку контрольный образец сыра «Российский» имеет недостаток незаменимых аминокислот, таких как метионин и цистеин, сделан вывод, что опытный сыр демонстрирует более высокие показатели благодаря наличию микропартикулята сывороточных белков, богатого серосодержащими аминокислотами. Результаты исследований позволяют сделать вывод о том, что сыр, обогащенный концентратом мицеллярного казеина и микропартикулятом сывороточных белков с включением (инкапсулированием) в их структуру дигидрокверцетина характеризуется стабильными органолептическими и физико-химическими показателями и более высокой биологической ценностью по сравнению с аналогом – «Российским» сыром.*

Ключевые слова: концентрат мицеллярного казеина, микропартикулят сывороточных белков, дигидрокверцетин, полутвердый сыр

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Introduction. Efforts to enrich conventional food products with biologically active substances (BAS) are necessary to meet the modern need for products that provide not only basic nutritional properties but also beneficial properties. One such ingredient is polyphenols (PP) [4].

Plant-based polyphenolic compounds are known as some of the main antioxidants in the human diet [8]. For example, dihydroquercetin, an extract of giant larch, has high antioxidant activity. Dihydroquercetin prevents autooxidation of food products and increases their shelf life. Even small amounts of dihydroquercetin help protect the body from the harmful effects of free radicals. To mask the tart and astringent taste characteristic of this compound, it must be bound or encapsulated with other components of the food system [2, 10]. In this case, PF passes "unnoticed" into the taste buds and is then released in the gastrointestinal tract (Figure 1).

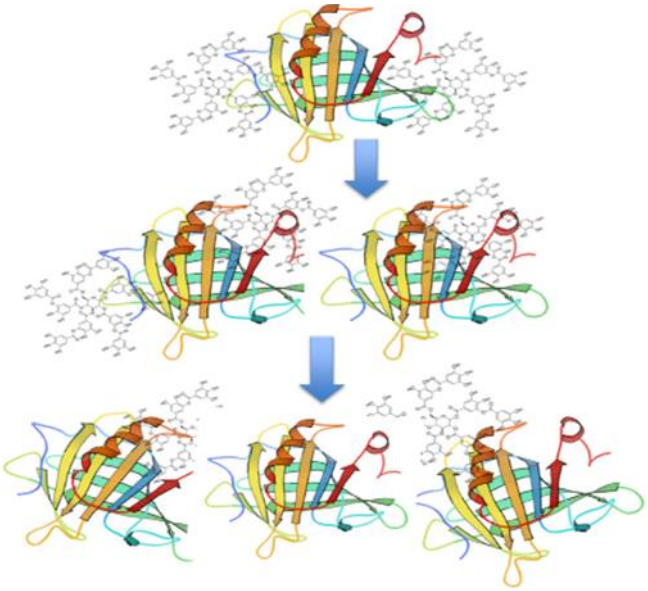

Figure 1 – Mechanism of astringency reduction by whey proteins. Hydrophobic binding, polyphenols with protein, protein crosslinking, aggregation of protein molecules

The functional properties and biological activity of protein-polyphenol complexes or conjugates depend on the nature of the proteins and polyphenols used, as well as on the type of cross-linking method used.

In the future, it will be important to be able to design complexes and conjugates with specific functional properties more rationally. This requires a better understanding of the structure-function relationships of whey protein-polyphenol complexes and conjugates so that they can be tailored for specific applications. In addition, there is growing interest in the use of innovative processing technologies such as ultrasound and high-pressure treatment to modify the structural and functional properties of these systems. Ultrasound treatment can be used to increase the solubility and thermal stability of a soy protein-cyanidin-3-galactoside conjugate, and high-pressure treatment can be used to improve the functional properties of an α-LA-pelargonidin-3-glucoside conjugate [3].

The combination of whey protein microparticles and polyphenolic compounds may lead to the creation of new products in the field of healthy nutrition. For example, for the production of functional cheeses enriched with polyphenols. Such cheeses may have improved organoleptic properties, an increased shelf life and increased biological value [5].

In cheesemaking, rennet caseins were previously used to standardize protein in normalized mixtures, and, less commonly, dry milk; currently, milk protein concentrates (MPCs) are most often used. The structure of casein in micellar casein concentrate (MCC) is comparable to that in

MPC, but the main difference between them is the presence of a larger amount of whey protein in the latter.

The aim of the work is to study the combined effect of micellar casein concentrate and microparticulate whey proteins with polyphenols on the process of rennet coagulation of milk, organoleptic, physicochemical indicators and biological value of experimental cheese samples.

Materials and research methods. The objects of the research were raw cow's milk obtained from the supplier OOO SP Chapaevskoye with a fat mass fraction of 3.9%, micellar casein concentrate with a casein: whey protein ratio of 96:4, microparticulate whey proteins with the inclusion (encapsulation) of dihydroquercetin in their structure, produced at JSC Dairy Plant Stavropolsky.

The introduction of whey protein microparticles with the inclusion (encapsulation) of dihydroquercetin into their structure into cheese-ready milk was carried out on the basis of regulatory documents - MR 2.3.1.1915-04 "Recommended levels of consumption of food and biologically active substances", MR 2.3.1.0253-21 "Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation". The dose of introducing microparticles with PF into milk for cheese production was selected at 0.3% by weight, which will provide 20% of the adequate intake level (AIL) when consuming 100 g of the product, since the AIL of flavonols, which include dihydroquercetin, for adults is 30 mg / day [6, 7]. Thus, per 1 liter of milk, 3 g of whey protein microparticulates with the inclusion (encapsulation) of dihydroquercetin in their structure were added to 1 experimental sample.

Milk was normalized for protein by adding micellar casein concentrate with a casein: whey protein ratio of 96:4. The calculation of the introduction of micellar casein concentrates was carried out based on the material balance equation, in this case, the protein balance [1]. Thus, 7 g of micellar casein concentrate were introduced per 1 liter of milk. To obtain a cheese curd, the normalized mixture was weighed, then heated to (32 ± 1) °C and 1 g of a 2% rennet solution was introduced. After introducing the enzyme, the time was noted. The readiness of the curd was determined, a cut was made with a spatula, then the curd was lifted along the cut with its flat part.

Research results and their discussion. The research involved milk with a fat content of 3.9%, micellar casein concentrate with a casein:whey protein ratio of 96:4, and whey protein microparticles with dihydroquercetin included (encapsulated) in their structure.

The results are presented in tables 1,2.

Addition of micellar casein concentrate with a casein:whey protein ratio of 96:4 to cheese-ready milk % and KSB-UF-55 "Microparticulate" with inclusion in the structure of proteins (encapsulation) of biologically active substances, for which dihydroquercetin was selected, allowed to evaluate the physicochemical parameters of the normalized mixture. The control sample was normalized by ultrafiltration. The results are given in Table 3.

The mass fraction of fat in the control sample is slightly higher, since normalization was carried out by ultrafiltration.

Technological indicators of rennet and whey are presented in Table 4.

Table 1 – Physicochemical parameters of the raw milk used

Indicators	Raw milk
Titratable acidity, °T	16.0 ± 0.5
Active acidity, pH units	6.85 ± 0.02
Mass fraction of protein, %	3.42 ± 0.5
Mass fraction of fat, %	3.94 ± 0.1
Mass fraction of dry matter, %	12.94±0.2
Lactose	4.87 ± 0.01
Freezing point	0.53 ± 0.02

Table 2 – Physicochemical parameters of micellar casein concentrate and whey protein microparticles

Name of the indicator	Samples	
	KMK 96 :4	MPC-DHA
Titratable acidity, °T	11.5 ± 1.0	15 ± 1.0

Active acidity, pH units	6.99 ± 0.02	6.52 ± 0.02
Mass fraction of protein, %	75 ± 0.22	55 ± 0.2
Mass fraction of fat, %	1 ± 0.5	2.5 ± 0.5
Mass fraction of moisture, %	4.7 ± 0.20	2.4 ± 0.2
Ash content, %	7.10 ± 0.05	3.0 ± 0.04
Mass fraction of lactose, %	10.06 ± 0.1	4.68 ± 0.23
Solubility index, cm3 ^{of} wet sediment	0.05 ± 0.5	0.5 ± 0.5
Purity group	1	1
Calculated DHA content, mg%	-	175±0.5

Table 3 – Physicochemical parameters of the normalized mixture

Name of the indicator	Normalized mixture	
	Experimental sample	Control
Titratable acidity, °T	16.5 ± 0.5	16.0 ± 0.5
Active acidity, pH units	6.82 ± 0.02	6.85 ± 0.02
Mass fraction of protein, %	4.25 ± 0.05	4.20 ± 0.05
Mass fraction of fat, %	4.10±0.1	4.20 ± 0.1
Mass fraction of dry matter, %	13.58 ± 0.2	12.94 ± 0.2
Lactose	4, 95 ± 0.01	4.87 ± 0.01

Table 4 – Technological indicators of rennet and whey

Samples	Coagulation time, min	Clot density, units	Condition of casein clot	Appearance of the serum
Control	30	Dense, 8	A clot with a smooth surface, dense	Transparent, greenish-yellow, without visible particles of casein dust
Experimental sample	32	Dense, 7	A clot with a smooth surface, dense	Whitish, without visible casein dust particles

The experimental sample took a little longer to form a clot, the whitish whitish whitish whitish whitish whitish whitish whey, the clot less dense than the control whey. The physicochemical parameters of the whey are presented in Table 5.

Table 5 – Physicochemical parameters of serum

Name of the indicator	Experimental sample	Control
Titratable acidity, °T	12.5 ± 0.5	12.5 ± 0.5
Active acidity, pH units	6.57 ± 0.05	6.55 ± 0.05
Mass fraction of dry matter, %	6.4 ± 0.2	6.2 ± 0.2
Density at 20 ºC, kg/m³	1023±1	1021±1
Mass fraction of fat, %	0.4 ± 0.1	0.3 ± 0.1
Mass fraction of protein, %	1.11 ± 0.05	1.0 ± 0.5

In all respects, the experimental sample is close to the control one.

Studies have shown that a normalized mixture with micellar casein concentrate and microparticulate matter with polyphenols is suitable for the production of rennet cheese.

The studies of the physicochemical characteristics of the finished product were carried out using standard, generally accepted methods. A comparison of the organoleptic indicators of cheese enriched with micellar casein concentrate and whey protein microparticulate with the inclusion (encapsulation) of dihydroquercetin in their structure was carried out with "Rossiysky" cheese (Table 6).

Table 6 – Organoleptic characteristics of experimental and control cheese samples

Name of the indicator	Prototype	Control sample
Appearance	The crust is clean, smooth, without damage.	The crust is clean, smooth, without a thick subcortical layer, without damage
Taste and smell	Cheesy, creamy, without any foreign tastes or odors.	Cheesy, slightly sour, without any foreign tastes or smells.
Consistency	The dough is tender, flexible, and homogeneous throughout.	The dough is plastic and homogeneous throughout.
Drawing	The cross-section shows a uniformly distributed pattern consisting of irregular, angular and slit-shaped eyes.	The cross-section shows a uniformly distributed pattern consisting of irregular, angular and slit-shaped eyes.
Dough color	Yellow with a slight green tint, uniform throughout the mass	Yellow, uniform throughout the mass

The experimental sample had a creamier taste and a more delicate dough consistency compared to the control sample.

The results of the study of the physicochemical quality indicators of the control and experimental cheese samples are presented in Table 7.

Table 7 – Results of the study of physicochemical quality indicators of control and experimental cheese samples

Physicochemical parameters	The value of the indicator for ND	Test results	
		Prototype	Control sample
Mass fraction of fat, %	-	30.4 ± 0.8	33.1 ± 0.8
Mass fraction of fat in terms of dry matter, %	not less than 1	47.2	50.5
Mass fraction of protein, %	-	29.8 ± 0.5	28.3 ± 0.5
Mass fraction of moisture, %	no more than 43	35.6 ± 0.2	34.4 ± 0.2
Mass fraction of moisture in defatted substance, %	-	51.1	51.4

The physicochemical parameters of the experimental sample are close to the control sample. The protein mass fraction is 0.5% higher, the moisture mass fraction is 1.2% lower, and the fat mass fraction is 2.7% lower compared to the control sample.

The biological value of a product is determined by the amino acid composition of the protein and its digestibility and is characterized by such an indicator as the amino acid score, which indicates the significance of each essential amino acid separately in the object [3] (Figure 2).

The data indicate an excess of amino acids phenylalanine + tyrosine. Since the control sample of cheese "Rossiyskiy" has a deficiency of essential amino acids, such as methionine and cysteine, it can be concluded that the experimental cheese has higher indicators due to the microparticulate whey proteins, as it is rich in sulfur-containing amino acids. Amino acids methionine and cysteine play important physiological roles. Thus, cysteine has the ability to affect the oxidation-reduction potential of the system and has a destructive effect on lipids and proteins.

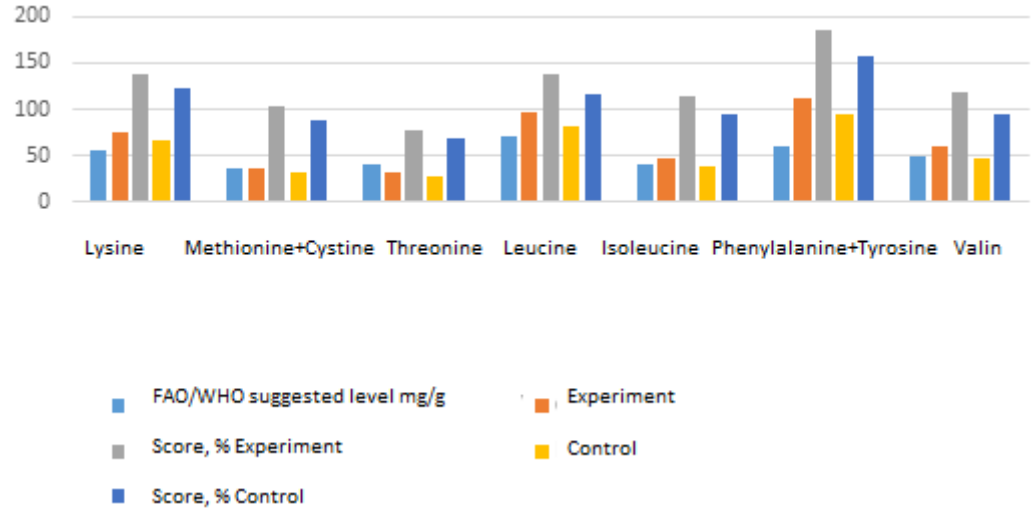

Figure 2 – Amino acid score of experimental and control cheese compared to FAO/WHO protein (mg/g protein)

In the presence of cysteine, the body's resistance to ionizing radiation increases. Methionine is involved in the synthesis of glycerophospholipids, which are part of biological cell membranes, and it is also necessary for the regeneration of liver cells. The limiting amino acid is threonine.

Conclusion. The results of the research allow us to conclude that cheese enriched with micellar casein concentrate and whey protein microparticulate with the inclusion (encapsulation) of dihydroquercetin in their structure is characterized by stable organoleptic and physicochemical indicators and a higher biological value compared to its analogue, “Rossiysky” cheese.

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