

The growing tendency to produce cheese from animal and plant ingredients has led to the diversification of cheese-making technologies and the search for new cheese suitable raw materials. The research object is the technology of cheeses made from combined raw materials: cow's milk and hemp seed proteins. Subjects of research: organoleptic and physical-chemical indicators of cheese product samples; amino acid composition of cheese product samples. The inclusion of vegetable raw materials in the cheese technology affects the technical properties of milk-plant mixtures, in particular, their syrupy properties, and the quality of the finished cheese product. Samples of cheeses were produced according to the traditional technology of Cachotta type cheese. The conducted research established that the proteins of hemp seeds affect the sensory characteristics of cheese. Increasing the content of hemp protein in recipes leads to the appearance of a brown-green color of the cheese product. The taste and smell acquire a characteristic shade of hemp. When vegetable proteins are used in the recipe of cheese, the yield of the finished product increases by 0.3...23.5 %. In experimental samples, the content of fat (by 0.3...2.2 %) and protein (by 1.75...10.4 %) increases. Hemp seed protein balances the amino acid profile in finished cheese. The total content of amino acids in cheese samples was as follows: H10 – 8.25, H15 – 8.35, H20 – 8.44, and H25 – 8.53 mg/g of protein, which is relatively higher than in the control sample H0 – 8.08 mg/g of protein. Taking into account the results of the sensory analysis of the expert commission and the balanced biological profile, the recommended concentration of hemp seed protein in the milk-vegetable mixture is 20 %, which guarantees the acceptable quality of the product. The obtained results are of practical importance, since it can be taken into account that the combination of raw materials of plant and animal origin expands the assortment, increases the raw material base and the output of cheese, and therefore, the profitability of production

Keywords: cheese, plant material, hemp seeds, protein, amino acid, biological value

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DETERMINING THE INFLUENCE OF HEMP SEED PROTEIN ON THE QUALITY INDICATORS OF CHEESE PRODUCT AND THE CONTENT OF NUTRIENTS IN IT

Sergii Odintsov

Postgraduate Student*

Yuliya Nazarenko

PhD, Associate Professor*

Tetiana Synenko

Corresponding author

Doctor of Philosophy (PhD)*

E-mail: t.p.synenko@gmail.com

Svitlana Huba

Senior Lecturer*

*Department of Technology and Food Safety

Sumy National Agrarian University

Herasyma Kondratieva str., 160, Sumy, Ukraine, 40021

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1. Introduction

Shortages of quality cow's milk, growing world population, and concerns about animal welfare have increased the demand for high-quality dairy products made from animal and plant-based ingredients and, in some cases, milk substitutes.

Hemp and industrial hemp products are one of the promising keys to the development of a sustainable and viable food system. In terms of cultivation, hemp has a short harvest period and requires less pesticides and water than other technical crops. In addition, the entire body of the plant – leaves, stems, roots and seeds – can be used without waste [1, 2].

Hemp seeds have a nutritional value comparable to soybeans [3]. Hemp seeds consist of a white kernel and a brown shell. The kernel is rich in protein, unsaturated fatty acids, and dietary fiber. Hemp has a unique high-protein, low-carbohydrate nutritional composition that is markedly different from other plant materials such as rice and wheat [4].

Dairy products such as cheese are obtained using the rennet or acid-rennet process. However, plant proteins have different molecular and functional properties than casein, and as a result, protein clots are not formed under the action of rennet.

This problem can be solved by various methods, such as the selection of a combination of enzymes and strains of microorganisms with different specificities, acids, and heat treatment.

Scientific research on this topic is important in order to understand the potential impact of hemp protein on cheesemaking and the relationship between animal and plant proteins in the curd formation process. The results of such studies are needed in practice because they could make it possible to expand the range of cheese products from combined raw materials.

2. Literature review and problem statement

The production of cheese from vegetable raw materials has started in ancient times. There is the cheese "Sufu", a fermented cheese that is produced by coagulation of soy milk with salts such as calcium sulfate or calcium chloride, pressed, followed by fungal and/or bacterial fermentation [5]. Unlike dairy cheeses, plant-based cheeses have attracted little scientific interest. However, the adaptation of the technology and the choice of other herbal ingredients are relevant.

Works [6, 7] describe the technology of soy cheese obtained by coagulating soy milk with various proteolytic enzymes. It is emphasized that the product had a weak structure and did not resemble hard cheese. The use of acid-thermal method, gluconic-delta-lactone acidifier, and salts (magnesium sulfate or calcium sulfate) for the production of soy curd did not give a satisfactory texture to the final product. The solution to this problem is the combination of raw materials – cow's and soy milk, and the production of a partial analog of milk cheese. This solution is described in [8]. However, the authors showed that with an increase in the proportion of soy milk in the mixture, the characteristics of cheese significantly decrease, and the time of clot formation increases.

Scientists [9] emphasized that the biological value of proteins differs greatly between animal and plant origin. The use of proteins of animal origin leads to the fact that products generally contain a higher proportion of essential amino acids (32...44 %) compared to proteins of vegetable origin (20...35 %).

This is the approach used in [10]. The authors suggest mixing proteins from different sources to increase the nutritional value of the product and balance the amino acid profile. For example, they suggest combining peas and lentils whose proteins are rich in lysine with cereal grains, which are low in lysine but high in sulfur amino acids. However, the combination of proteins of animal and plant origin has not been investigated.

In works [11, 12] the physical-chemical, structural, and functional properties of hemp seed proteins were investigated and compared with soy, pea, whey, and egg proteins. Hemp seed protein has been shown to have a high content of total protein (67.1 g/100 g) and essential amino acids (23.1 g/100 g). The authors determined the potential of using hemp protein in various food products to increase their protein content. However, hemp protein has low water absorption and solubility and high oil absorption, so appropriate processing is required to change its structure and functionality.

Works [13, 14] found that hemp protein has an excellent nutritional amino acid profile with a wide range of essential amino acids and a significant amount of glutamine and arginine. The main protein in hemp seeds is edestine, which makes up about 70 % of hemp protein and has low solubility. The second main protein of hemp is albumin, which has fewer disulfide bonds than edestine, due to which it has a more flexible structure, higher solubility and foaming ability. The authors emphasize that hemp protein has a high degree of digestibility. In addition, most hemp allergens, such as major thaumatin-like protein and lipid transfer protein, have been removed during protein isolation or digestion. Therefore, hemp protein can be used as an ingredient in hypoallergenic products.

Paper [15] examines alternatives to milk based on hemp seeds. It was shown that the shelf life of finished products in the refrigerator was from 15 to 28 days, and a higher hemp content (15 %) led to a longer shelf life. The hemp milk samples showed slight phase separation, but no significant sedimentation or creaming, and all readily recombined after shaking. The authors note that no correlation was observed between the concentration of hemp and the tendency to partition. Hemp milk samples prepared with 3 % and 5 % hemp seeds were the most similar to cow's milk in terms of organoleptic and physicochemical parameters. However, the

functional and technological properties of alternative milk have not been investigated.

In work [16] it was determined that milk from hemp seeds has a high nutritional value. It consists of lipids (1.25...5.00 %), proteins (0.83...4.00 %), carbohydrates (2.5...20.0 %). It also contains vitamin E, minerals (sodium, phosphorus, potassium, magnesium, calcium, sulfur, iron, and zinc), all essential amino acids and polyunsaturated fatty acids. The authors evaluated hemp seed milk in comparison with other types of plant milk, presented its nutritional aspects, and outlined prospects of current research. However, this study does not reveal the technological properties of hemp milk or its suitability for the production of alternative dairy products such as cheese.

In [17], the effect of organic hemp seed powder on the quality indicators of traditional Croatian whey cheese "*Skuta*" obtained by the thermal-acidic curdling method was investigated. It was shown that the addition of hemp seed powder had a significant effect on nutritional value, mineral content, but did not affect microbiological spoilage. However, the work does not consider the use of curd clot formation under the action of enzymes, microbiological starters for the improvement and formation of taste and aroma.

In general, there is insufficient information in the literature on research into the use of hemp protein in cheese technology. All this allows us to state that it is appropriate to conduct a study aimed at the determination of the complex effect of hemp seed protein on cheese production by evaluating sensory indicators, the content of nutrients, and the chemical composition of cheese from combined raw materials.

3. The aim and objectives of the study

The purpose of our study is to determine the effect of hemp seed protein on the quality indicators of cheese product and its content of nutrients. This will make it possible to combine raw materials of vegetable and animal origin for the production of cheese with improved biological value.

To achieve the goal, the following tasks were set:

- to investigate the quality indicators of cheese product samples: organoleptic and physical-chemical;
- to establish the amino acid profile of cheeses made from combined raw materials: cow's milk and hemp seed proteins.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of research is the technology of cheeses made from combined raw materials: cow's milk and hemp seed proteins.

Research subjects: the organoleptic and physicochemical parameters of cheese product samples; amino acid composition of cheese product samples.

Research hypothesis: the inclusion of plant raw materials in cheese technology affects the technical properties of milk-plant mixtures, in particular, their cheese capacity, and the quality of the finished cheese product. The positive functional properties of hemp seed protein determine the expansion of the range of products using them, in particular cheeses with a combined composition of raw materials. It is assumed

that the study of the influence of hemp seed protein on the quality of cheese and the content of nutrients in it would make it possible to expand the assortment of hemp products with a combined composition and increased biological value.

4. 2. Researched raw materials and finished products used in the experiment

The raw milk was obtained from cows at the state enterprise “Experimental farm of the Institute for Agriculture of the Northeast in the National Academy of Agrarian Sciences of Ukraine” (Sumy oblast, Ukraine), which corresponds to the “extra” variety according to DSTU 3662:2018 in terms of quality indicators. The protein content in milk was at the level of (3.2±0.2) %, the fat content was (3.9±0.3) %.

We used hemp seed protein by Nutridem LLC (Kyiv oblast, Ukraine). The protein quality of hemp seeds meets the requirements of TU U 10.8-10.8-3159015717-001-2017. Protein content – 50.0 %, fat – 15.0 %.

10 kg of milk-vegetable mixture was used for the production of cheese. Pasteurization, leavening, fermentation, and subsequent formation of cheese grains were carried out in a laboratory cheese factory. Cheese ripening took place in a laboratory climatic chamber.

Test samples of cheese were made according to the recipe given in Table 1. The cheese samples were assigned code names H0, H10, H15, H20, H25, and H30, according to the protein content of hemp seeds in the milk-vegetable mixture.

Table 1

Formulation of experimental samples of cheeses made from cow’s milk and hemp seed proteins

Title	Ingredient content in the recipe, %				
	control sample	sample 1	sample 2	sample 3	sample 4
	H0	H10	H15	H20	H25
Milk-vegetable mixture, kg:					
Cow’s milk	10.0	9.0	8.5	8.0	7.5
Hemp seed protein powder	–	1.0	1.5	2.0	2.5
Loose components, g/10 kg of milk-vegetable mixture:					
Calcium chloride	2.0	2.0	2.0	2.0	2.0
Rennet enzyme	0.07	0.07	0.07	0.07	0.07
Sourdough from thermophilic cultures of microorganisms – <i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii subsp. bulgaricus</i>	0.15	0.15	0.15	0.15	0.15

The technology of semi-hard cheese of the *Caciotta* type was chosen as the base. The process of manufacturing experimental samples of cheese with hemp seed protein under laboratory conditions consists of the following stages: milk purified from mechanical impurities is heated to a temperature of (60–65) °C. We add hemp seed protein powder according to the recipe (Table 1). The milk-vegetable mixture is pasteurized at a temperature of (90±2) °C with a holding time of 5 minutes. In milk cooled to a temperature of (36±2) °C, dry leaven of direct application is added, in the amount recommended by the manufacturer. Sourdough consists of thermophilic cultures of microorganisms – *Streptococcus*

thermophilus and *Lactobacillus delbrueckii subsp. bulgaricus* (“Dalton”, Italy). Next, a solution of calcium chloride and rennet enzyme “Albamax 600” (100 % chymosin) are added (Caglificio Clerici, Italy). The mixture is fermented at a temperature of (36±2) °C until a dense clot is formed. Next, the clot is cut, the curd grain is processed (kneading, second heating at a temperature of (38±2) °C, drying of the curd grain). The formed cheese heads are self-pressed in a thermal chamber (temperature (50±2) °C, holding time 120 min., number of turns – 3, every 30 min.). Next, the cheese heads are kept for 3–4 hours in brine (salt concentration 18–20 %, temperature 10–14 °C). Then the cheese heads are sent for ripening in a climatic chamber at a temperature of (10±2) °C for 5...30 days. Ripened cheese is stored in a refrigerator at a temperature of (6±2) °C.

4. 3. Methodology for determining quality indicators of samples

Quality assessment of cheese samples was carried out according to generally accepted procedures.

Acidity (pH) of cheese samples was determined by the potentiometric method according to DSTU 8550:2015.

The mass fraction of dry substances in cheese samples was determined by drying to a constant value of the indicator according to DSTU 8552:2015. The mass fraction of protein was determined by the Kjeldahl method according to DSTU 5038:2008. The mass fraction of fat was determined by the acid method (Gerber method) according to DSTU ISO 2446:2019.

Organoleptic indicators of cheese samples were determined according to DSTU 6003:2008, with recommendations described in the international standard ISO 22935-2:2023.

The analysis of amino acids in cheese samples was carried out by the method of ion-exchange liquid column chromatography using the automatic amino acid analyzer “T 339” (Czech Republic, Prague). According to the following procedure: a weighed sample (with a protein content of about 2 mg) is mixed to the bottom of a test tube, 0.5 ml of distilled water and 0.5 ml of concentrated hydrochloric acid are added. The tube is cooled in a mixture of dry ice with acetone or liquid nitrogen. After the contents of the test tube freeze, air is pumped out of it using a vacuum pump to prevent oxidation of amino acids as a result of hydrolysis. Then the test tube is sealed and placed for 24 hours in a thermostat with a constant temperature (106±1) °C. At the end of hydrolysis, the test tube is opened, having previously cooled to room temperature. The contents are quantitatively transferred into a glass beaker and placed in a vacuum desiccator over granulated caustic sodium. Then air is removed from the desiccator using a water pump. After drying the sample, we add 3–4 ml of deionized water to the beaker and repeat the drying procedure. The sample prepared in this way is dissolved in 0.3N lithium citrate buffer (pH 2.2) and applied to the ion exchange column of the amino acid analyzer.

Threefold repeatability of studies was used in our research. The obtained experimental data are represented in units of the international SI system.

The yield of cheese was calculated using equation (1), by weighing the milk-vegetable mixture and the produced cheese:

$$B = \frac{m_{cheese}}{m_{milk}} \cdot 100\%, \tag{1}$$

where *B* is the yield of cheese, %;

m_{cheese} – mass of cheese (freshly produced and 30 days after production), kg;

m_{milk} – mass of milk, kg.

Mathematical and statistical processing of the results was carried out on a computer using Excel software from the Microsoft Office 2016 package (Microsoft Corporation, Washington, USA). The determined value of the reliability of the deviation (p) does not exceed 0.05, which indicates that the value of the accuracy indicator (P) of the results is more than 0.95.

5. Results of research on the influence of hemp seed protein on the quality of cheese and its content of nutrients

5.1. Results of studying the organoleptic and physicochemical parameters of cheese samples made from cow's milk and hemp seed proteins

Fig.1 shows the appearance of cheese samples made from combined raw materials.

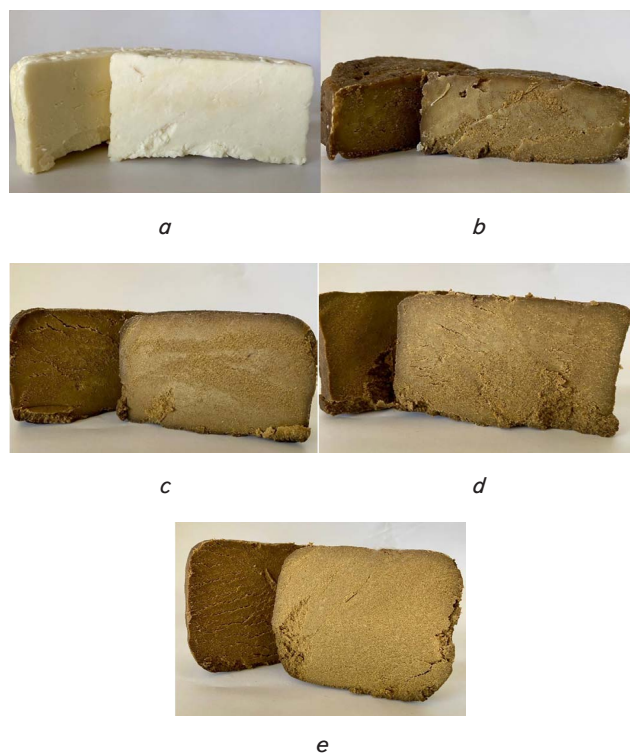


Fig. 1. Appearance of experimental samples of cheese: a – H0; b – H10; c – H15; d – H20; e – H25

Results of the sensory analysis of the general characteristics of the cheese (appearance, taste and smell, consistency, color, cross-section pattern, shape of heads) are represented by the expert group in the form of a profilogram (Fig. 2).

According to the obtained sensory analysis profiles, the samples of cow's milk cheese were given high marks for appearance, taste and aroma, consistency, and color. Add-

ing hemp seed protein and increasing its concentration in the milk-vegetable mixture noticeably changes the appearance, color, and taste. At the same time, the appearance, taste, and smell of experimental cheese samples are maintained at a high level.

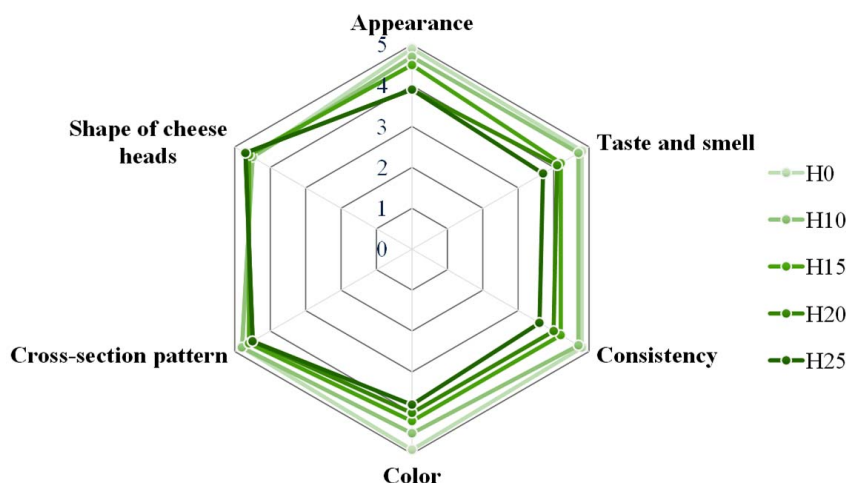


Fig. 2. Sensory profile of cheese samples

Samples of cheese with hemp protein are described by experts as oval-shaped cheeses. The cheese is characterized by a pleasant hemp taste and aroma. The texture is satisfactory, the color is evenly brown-green, and with increasing hemp protein content, the color becomes more intense.

The results of physical and chemical indicators of cheese samples from combined raw materials are given in Table 2.

Table 2

Physicochemical parameters of cheese samples ($n=3, p \leq 0,05$)

Parameter	Sample	Freshly made	In 30 days
Acidity, pH units	H0	5.40±0.01	5.05±0.01
	H10	5.37±0.01	4.93±0.01
	H15	5.32±0.01	4.89±0.01
	H20	5.26±0.01	4.82±0.01
	H25	5.18±0.01	4.79±0.01
Cheese yield, %	H0	13.5±0.2	12.6±0.2
	H10	14.1±0.2	12.9±0.2
	H15	22.7±0.2	21.2±0.2
	H20	28.3±0.2	26.4±0.2
	H25	39.3±0.2	36.1±0.2
Dry matter content, g/100 g	H0	51.85±0.12	57.17±0.12
	H10	52.44±0.25	58.45±0.21
	H15	56.19±0.10	59.35±0.18
	H20	59.90±0.15	60.72±0.15
	H25	64.73±0.31	65.18±0.10
Protein content, g/100 g	H0	22.10±0.12	24.35±0.10
	H10	24.95±0.20	26.10±0.20
	H15	28.73±0.15	30.35±0.15
	H20	31.88±0.14	32.58±0.14
	H25	35.50±0.15	34.75±0.16
Fat content, g/100 g	H0	25.25±0.05	26.80±0.10
	H10	25.90±0.05	27.10±0.05
	H15	26.45±0.10	27.95±0.10
	H20	27.10±0.05	28.50±0.15
	H25	28.25±0.10	29.00±0.05

The addition of hemp seed protein increases the yield of cheese by 0.3...23.5%. The composition of cow's milk cheeses after 30 days of ripening averaged 21.5% protein and 36.1% fat. Increasing the concentration of hemp seed protein in dairy-vegetable mixtures increases the protein content by 1.75...10.4% and the fat content by 0.3...2.2% in cheese products.

5. 2. Results of research on the amino acid profile of cheeses made from cow's milk and hemp seed proteins

The amino acid profile of the studied cheese samples was analyzed chromatographically. The averaged results are represented in the chart below (Fig. 3).

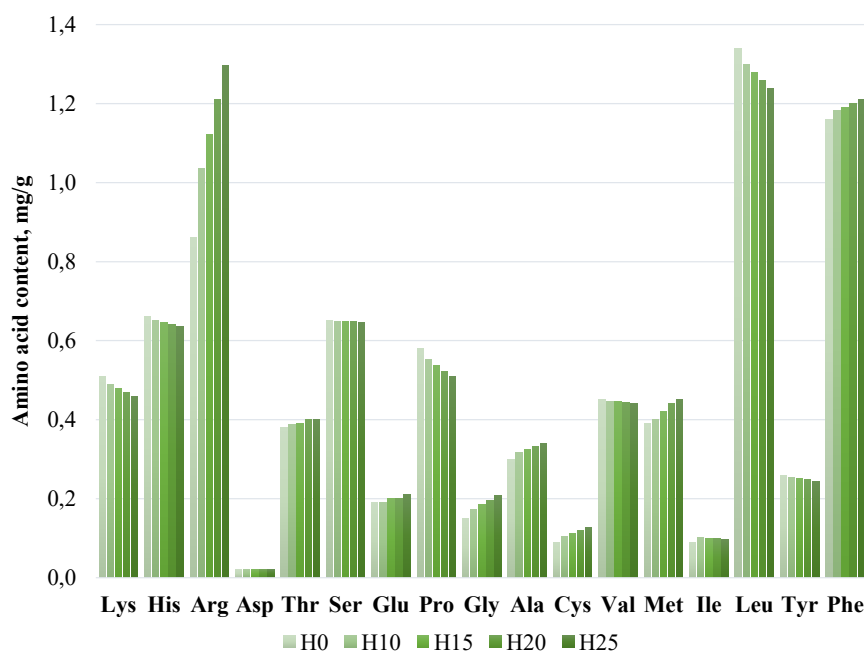


Fig. 3. Averaged amino acid profile of cheese samples

Our study found 17 amino acid residues in the cheese samples. The control sample of cheese (H0) is characterized by a high content of essential amino acids, such as leucine (1.34 mg/g), lysine (0.51 mg/g), phenylalanine (1.16 mg/g), threonine (0.38 mg /g), valine (0.45 mg/g), histidine (0.66 mg/g). And there are also substitute amino acids, in particular, a high content of arginine (0.86 mg/g), proline (0.58 mg/g), serine (0.65 mg/g), and others.

Adding hemp seed protein in cheese samples (H10, H15, H20, H25) increases the content of essential amino acids, such as threonine (0.39...0.4 mg/g), methionine (0.39...0.45 mg/g), phenylalanine (0.18...0.21 mg). And there are also substitute amino acids, in particular, a high content of arginine (1.02...1.3 mg/g), glycine (0.17...0.21 mg/g), alanine (0.32...0.34 mg/g), serine (0.65...0.66 mg/g).

6. Discussion of results of determining the effect of hemp seed protein on the quality of cheese and its content of nutrients

The incorporation of vegetable proteins into cheese technology and establishing the relationship between dairy products and vegetable proteins still require scientific research. Vegetable ingredients in the production of cheese

have been the subject of numerous studies [18, 19]. Plant proteins do not coagulate under the influence of rennet enzymes, so their effective inclusion in cheese technology requires the selection of pretreatment parameters of the milk-vegetable mixture. For example, increased parameters of heat treatment (pasteurization), use of salts, enzymes of a wide spectrum of action, change of pH and leavens with additional properties. In this study, the preheating temperature of the milk-vegetable mixture was (90±2) °C, and the holding time was 5 min. This ensures partial unfolding of protein molecules, and thus free access for enzymes.

Our results can be compared with the conclusions drawn in [6], in which it was shown that with an increase in the proportion of soy in the milk mixture, the characteristics of the curd clot change, and the time of curd formation is prolonged. However, the type of plant material, the content of other nutrients in it (carbohydrates, fats, etc.), fermentation conditions: pH, temperature, sourdough cultures, enzyme preparations, can affect the process of formation of curd and the resulting cheese product.

Results of the organoleptic analysis (Fig. 1) showed that the protein of hemp seeds in the recipe of milk-vegetable mixtures significantly affects the sensory characteristics of cheese. One of the indicators that underwent significant changes was color. An increase in the protein content of hemp seeds in the recipe of the milk-vegetable mixture leads to the formation of a brown-green color of the cheese product. In addition, hemp seed protein affects taste and aroma. During ripening, as a result of enzymatic reactions in the curd mass, the taste and aroma change, acquires an attractive shade, specific

plant and hemp characteristics disappear, in all samples they remain acceptable according to expert evaluation. Results of the organoleptic analysis of the obtained samples of cheeses with hemp seed protein are consistent with the data in [15], which prove that vegetable proteins affect the general sensory characteristics of cheeses. In [20] it was shown that the addition of more than 15% of vegetable proteins negatively affects the taste or consistency of cheese but in some cases the value of 20% of vegetable ingredients in the technology is reached. The use of vegetable proteins in the cheese recipe increased the yield of the finished product (Table 2) by 0.3...23.5%. However, the enrichment of cheese with protein components increases the content of dry substances, the product acquires an unacceptable brittle consistency. In experimental samples, the content of fat (by 0.3...2.2%) and protein (by 1.75...10.4%) increases. To determine the influence of the use of hemp seed protein on the biological value of cheese, the amino acid profile of the experimental samples was investigated. The results showed that hemp seed protein balanced the amino acid profile in the finished cheese. The total content of amino acids in cheese samples was as follows: H10 – 8.25, H15 – 8.35, H20 – 8.44, and H25 – 8.53 mg/g of protein, which is relatively higher than in the control sample H0 – 8.08 mg/g of protein. Such results are explained by

the difference in the amino acid profile of the raw materials: cow's milk and hemp seeds. According to data in [21], hemp seed protein has a balanced amino acid composition and contains all essential amino acids, especially rich in arginine.

Comprehensive studies have shown that the protein of hemp seeds has a significant effect on the quality indicators of cheeses and the content of nutrients in them. Information analysis undoubtedly confirms that the consumption of hemp protein saturates the body with nutrients [13, 14]. Taking into account the results of the sensory analysis of the expert board and the balanced amino acid profile, the recommended concentration of hemp seed protein in the milk-vegetable mixture is 20 %, which guarantees acceptable product quality.

The main limitations of the study are the use of regionally grown hemp seeds. Cultivation, harvesting, and processing methods may differ from those in countries with other climatic and cultural differences. However, the technology of making cheese from combined raw materials can be applied to other countries.

The disadvantage of this study is the study of the influence of hemp seed protein on the quality and content of nutrients in cheese made only by the rennet coagulation method, using the example of the technology of semi-hard cheese of the "Cachotta" type. Further research should investigate the quality indicators of cheese of the combined composition of several different technologies and techniques of protein coagulation.

Our conclusions are of practical importance since it can be taken into account that the combination of raw materials of vegetable and animal origin expands the assortment, increases the raw material base and the yield of cheese, and therefore, the profitability of production. When conducting further research, special attention should be paid to the selection of enzymes, stabilizing additives, establishing the transition of nutrients into the whey.

7. Conclusions

1. A comprehensive study of quality indicators of cheese samples made from combined raw materials showed that hemp seed proteins affect sensory and physical-chemical indicators of cheese. In particular, an increase in the content of hemp protein in the recipes of dairy-vegetable mixtures leads to the appearance of a brown-green color of the cheese prod-

uct. The taste and smell of cheese samples acquires a characteristic hemp shade. However, during ripening, as a result of enzymatic reactions, the taste and aroma change, acquire an attractive shade, and specific hemp characteristics are reduced. It was noted that when vegetable proteins are used in the cheese recipe, the yield of the finished product increases by 0.3...23.5 %. At the same time, the dry matter content increases in the cheese samples, the product acquires an unacceptable brittle consistency. In experimental cheese samples, the content of fat (by 0.3...2.2 %) and protein (by 1.75...10.4 %) increases depending on the amount of hemp seed protein added to the milk-vegetable mixture.

2. Hemp seed protein balances the amino acid profile in the resulting cheese. The total content of amino acids in cheese samples was as follows: H10 – 8.25, H15 – 8.35, H20 – 8.44, and H25 – 8.53 mg/g of protein, which is relatively higher than in the control sample H0 – 8.08 mg/g of protein. In experimental samples of cheese, an increase in the content of arginine, glycine, methionine, phenylalanine is observed due to the increased content of these amino acids in hemp seeds.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study and the results reported in this paper.

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Data availability

All data are available in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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