Вивчено комплексний вплив процесів неферментативного каталізу – кріомеханолізу і заморожування сичугових сирів при підготовці до плавлення, який призводить до кріодеструкції важкорозчинних паразказеїнаткальційфосфатний нанокомплексів в розчинну форму. Установлено, що відбувається їх кріодеструкція і трансформація в наноформу (на 55…60 %). Розроблено технологію оздоровчих плавлених сирних виробів. Розкрито механізми процесів заморожування та неферментативного каталізу

Ключові слова: неферментативний каталіз, механоліз, заморожування, низькотемпературне подрібнення, твердий сичуговий сир, нанокомплекси, плавлені сирні вироби

Изучено комплексное влияние процессов неферментативного катализа – криомеханолиза и замораживания сычужных сыров при подготовке к плавлению, которое приводит к деструкции труднорастворимых паракaзеинаткальцийфосфатных нанокомплексов в растворимую форму. Установлено, что происходит их криодеструкция и трансформация в наноформу (на 55…60 %). Разработана нанотехнология оздоровительных плавленых сырных изделий. Раскрыты механизмы процессов замораживания и неферментативного катализа

Ключевые слова: неферментативный катализ, механолиз, замораживание, низкотемпературное измельчение, твердый сычужный сыр, нанокомплексы, плавленые сырные изделия

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TECHNOLOGY OF HEALTHY PROCESSED CHEESE PRODUCTS WITHOUT MELTING SALTS WITH THE USE OF FREEZING AND NON-FERMENTATIVE CATALYSIS

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1. Introduction

A global problem in the international practice in many countries of the world today is the imbalance in the food diets. There is a shortage of milk, fish, meat, fruits and berries, that is, the basic food products that affect health and immunity of the population. The need for vitamins, proteins, mineral substances, carotene and other biologically active substances in the food diet is satisfied by 50 % [1–3]. According to statistical data, almost 50 % of the Earth's population is starving [1, 3]. The problem of imbalance and deficit is complicated by the deterioration in ecological situation on the entire Earth, which in turn leads to additional reduction in the immunity of population and the necessity for an increase in basic products and the amount of BAS in the food diet.

In addition, today in the world there is another important global problem, which leads to the deterioration of health of the population, which is worth paying attention to. It is linked to the decrease in quality of food products and the use of synthetic components in their production. An analysis of data of the periodic literature revealed that over the last 10–15 years, in international practice, to significantly reduce the cost of production and to increase their own profits, most of the enterprises in food industry have widely used various types of artificially created food additives (colorings, taste intensifiers, sweeteners, thickeners, preservatives, etc.)

[1, 3]. There is also another way to reduce the deficit of food, which is currently applied in international markets. It is the design and use of artificially produced food products in the food diet, the range of which is growing rapidly every year. At present, there are synthetic analogues of milk, meat, flour, cereals, vegetables and other food products, which are no different from the natural ones in physical appearance and taste [1, 2]. Their application allows obtaining a wide range of, at first glance, traditional products, in the production of which one can apply non-standard, or in smaller amount of raw materials of vegetable, animal origin or their substitutes. Over the last 10–15 years another method has become widely spread. There appeared so-called powder industry; at present, producing meat, dairy, bread and bakery, confectionery products, juices, beverages and other foods is already impossible to imagine without the use of food additives, mixtures and substitutes. By the calculations of scientists from the USA, annual consumption of different food additives by the country's population is on average about 2,5 kg [1, 2].

The disadvantages of the use of man-made products and the products obtained with the use of artificial additives are low digestibility, and the negative impact on the health of people. It was found that daily consumption of finished products of industrial production, obtained with the use of certain kinds of food additives or artificial ingredients may cause allergy [1, 2]. However, the powder industry and production of the artificially created products at the expense of low cost of such products is getting more and more spread in the world. Working engineers, practical workers and researchers are working at the development of new and the improvement of traditional technologies of production with the use of food additives and artificial components of working engineers – practice and researchers. In different countries there are certain segments of the population who prefer such artificially created products because their prices are significantly lower than the cost of natural products.

In the food industry of the most developed countries such as Japan, the United States, England, Germany, France and others, in parallel with the production of cheap products manufactured in this way, the direction of producing healthy products appeared. They differ from the traditional ones by the content of natural BAS that promote immunity [3–5].

One of the ways of development of technologies of these products is the search for innovative technological methods that make it possible to completely exclude the need for the use of food additives and synthetic components in their production and to obtain high quality products.

The healthy products include combined dairy-vegetable products, including processed cheeses, enriched with natural herbal supplements. The traditional technologies of producing processed cheeses include the need for the use of food additives – melting salts in the process of preparing the ground mass of rennet cheese for melting. In this regard, it is urgent to find the technological methods that will make it possible to completely exclude the need for application of melting salts in the technology of getting processed cheeses with the use of solid rennet cheeses as main raw material.

It is known that processed cheeses are very popular with the population of all countries of the world. This is due to their high taste properties, nutritional value and simple production technology. For the human organism they are a valuable source of important functional nutrients, fully fledged proteins, essential amino acids, lipids, mineral substances, vitamins and others [3, 5, 6, 9]. However, they are characterized by the low content of biologically active substances and reduced terms of shelf life. In Ukraine, the range of processed cheeses which are high in BAS is limited. Difficulties in making them with the use of rennet cheeses as raw materials are related to the fact that their composition includes complicated low solubility lipidproteincalciumphosphate complexes (LPCPC). In them, lipids and polypeptide chains are intertwined, stitched together using calcium bridges, disulphide and phosphoramide bonds, hydrogen bonds, and intermolecular interactions. It prevents their peptization and solving in the process of melting cheeses and getting a homogeneous fluid mass [3, 6, 7]. So, one of the main technological operations in the technology of processed cheeses is softening of cheeses and getting a homogeneous fluid mass in the course of heating in presence of melting salts. In this case, peptization and pasteurization of LPCPC take place simultaneously. In this case, peetization and pasteurization are going simultaneously. Traditionally, in the manufacture of processed cheeses for increasing the peptization of LPCPC, softening and giving the cheese mass fluidity, different melting salts are used, such as salts of citric acid, pyrophosphates, phosphates and the other in the quantity from 30 to 100 kg per 1 t of product, which are harmful substances for human organism [5–8].

In this regard, it is relevant to search for technological methods that would significantly reduce the amount of melting salts in the manufacture of processed cheeses [5, 10]. Scientific research, which is reflected in this article is aimed at finding these technological methods. The authors proposed as an innovation to use the integrated influence on the raw materials (solid rennet cheeses) of freezing and finely dispersed grinding. The latter are accompanied by processes of disaggregation, cryodestruction, mechanoactivation, cryomechanochemistry, and destruction of low-solubilitylipid-protein nanocomplexes and nanoassociates and polypeptide chains. It is necessary for peptization – increasing their dissolution, obtaining fluid cheese mass with gel texture. The use of these technological methods is aimed at the reduction or exclusion of melting salts in the course of obtaining processed cheese products with the use of rennet cheeses as the main component.

2. Literature review and problem statement

By cryomechanodestruction the authors of the work imply the new technological method, which includes the influence on the raw material of freezing and finely dispersed grinding which leads to disaggregation, destruction, cryodestruction of nanocomplexes and nanoassociates of difficult-to-solve substances that are found in them. The result is a more complete extraction of these valuable components form raw materials. The specified technological method, according to the authors, is an alternative to the fermentative processing of food raw materials. It should be noted, that the use of processes of mechanic destruction and cryomechanodestruction has already found its implementation in such industries as chemical, metallurgical, textile, aviation, etc. in such countries as Japan, Russia, Kazakhstan and others [3, 13]. Thus, for example, the use of processes of cryo- and mechanochemistry allowed developing technologies of powder metallurgy, technologies of plastics, which do not have scratches, technologies of textile products with water and dirt resistant properties, and others. In food industry, both in Ukraine and in international practice these processes have almost not been studied [13–16].

At present, in the world in various industries there is literally a "boom" of creation of nanotechnologies, including food industry. It is explained by the fact that there appeared a possibility of the focused obtaining of dispersed systems with particles in the nano-range (1...100 nm) and of controlling their structure and fractional composition, which gives the opportunity to conduct research and development at the molecular, nuclear, and micromolecular level. This allows getting the materials, systems, structures, etc. with fundamentally new properties. This is connected with the fact that new properties are obtained in the range of nano-dimensional particles of different materials, which are not typical for a large sample. The evident example is gelatin, which is dissolved after swelling in hot water after a few hours, while finely dispersed ground gelatin (in nano-meter range) is dissolved in cold water (at a temperature of +18...+20 °C) within a minute. Similar examples are numerous. The essence of the processes that occur in the nano – dimensional form is impossible to describe using the patterns which are known to modern science. Here, there is a necessity of in-depth fundamental research.

Authors have been examining for 30 years the application in food industry of cryogenic grinding and processes of mechanic activation, cryomechanodestruction (without using cold) and freezing. In particular, the authors obtained finely dispersed nanopowders and homogeneous pastes from fruit, berries, vegetables, medicinal and spicy-aromatic vegetable raw materials, and products of beekeeping [17, 18]. For the first time in the world practice in processing of various raw materials, the new phenomena and effects were revealed and their mechanisms were disclosed. It was shown that the processes of cryomechanodestruction, mechanic activation, freezing and cryomechanochemistry lead to substantial destruction and disaggregation of biological nanocomplexes – biopolymer-BAS and the release of BAS from the hidden and inactive forms [19]. We also revealed the cryodestruction of biopolymers (proteins, polysaccharides, cellulose, and pectin substances), their destruction to the individual monomers, leading to the effect of "enrichment", more complete extraction from raw materials, and better assimilation by the human organism [3, 17].

An analysis of periodical literature over the past 10 years showed that scientists had discovered a significant impact of cryogenic grinding of gelatin which led to a substantial change in its original properties and dissolution at room temperature [17]. It was also found that the use of the processes of finely dispersed mechanical grinding in obtaining polymer plastic, latex, etc. led to a decrease in their molecular weight, the appearance of new functional groups and links, and to a change in their solubility [3, 13]. In this regard, it could be assumed that the use of the processes of freezing and cryomechanodestruction would lead to destruction, disaggregation, mechanolysis and peptization of complicated complex of LPCPC of solid rennet cheeses. This will help their better melting with reduced amounts of melting salts or without them. In the dairy industry, there is a continuous search for technological methods aimed at reducing the amount of melting salts in the production of processed cheese. Leading scientists of Ukraine and Russia managed to decrease them only by 20 % [3, 5, 10–12].

Thus, the analysis of the literature showed that the works considering processed cheeses are mainly dedicated to their enrichment with various food supplements [17–19]. But the range of processed cheese products, which today exists in the market and food additives that are used in their manufacture are characterized by the low content of BAS [5].

In this regard, it is relevant to study the impact of methods of deep processing, such as freezing and finely dispersed grinding in the preparation of rennet cheeses for melting. The aim is to get processed cheese products of high quality without the use of melting salts. In particular, the studies of the influence of the integrated action of freezing and finely dispersed grinding on cryodestruction of lipid-protein nanocomplexes and nanoassociates present the interest for researching. It is expedient to study protein destruction, transformation of bound α-amino acids into a free form, conformational changes of protein molecules, etc. It is urgent to develop nanotechnologies of processed cheese products without the use of melting salts and their enrichment with various vegetable biologically active substances, which have an immunomodulatory, detoxicating and antioxidation influence.

The obtained semi-finished processed cheeses for healthy eating that were received without melting salts, were used by the authors in manufacturing cheese fillings for "PanCakes", croissants, snacks, sauces, dressing sauces, dipping sauces, original snacks – falafels, etc.

3. The aim and tasks of the studies

The purpose of the work is the study of the impact of such processes as freezing, mechanic activation and non-fermentative catalysis on the destruction of low-soluble lipid-protein nanocomplexes of solid rennet cheeses without melting in developing the nanotechnology of healthy processed cheese products.

To accomplish the set goal, it was necessary to solve the following tasks:

– to explore the patterns and mechanisms of the influence of freezing and non-fermentative catalysis – mechanolysis on more complete extraction of hidden protein forms from low-solubilitylipid-protein nanocomplexes of hard rennet cheeses;

– to study the integrative influence on raw materials of non-fermentative catalysis and freezing on mechanolysis (destruction) of protein and conformational changes of protein molecules of solid rennet cheese;

– to develop nanotechnologies of healthy processed cheese products without melting salts based on solid rennet cheeses with the use of the processes of freezing, non-fermentative catalysis and natural vegetable enriching additives as innovation;

– to study the chemical composition of the new cheese products – fillings for confectionery products "PanCakes" obtained by this technology and to compare their quality with the analogues and original raw materials.

4. Materials and methods of studying

4. 1. Materials and equipment used in the experimental research

The study was carried out with the use of solid rennet cheese nanopowders from natural spices (black pepper, sweet pepper peas, coriander), spicy (ginger, garlic) and carotenoid (carrots, peppers) vegetables (Fig. 1). In addition, the authors studied the processed cheese mass after cryoprocessing (or cheesy semi-finished products) and new healthy processed cheese products in the form of fillings for confectionery "PanCake", obtained by nanotechnology and cheese snacks – falafels (Fig. 2).

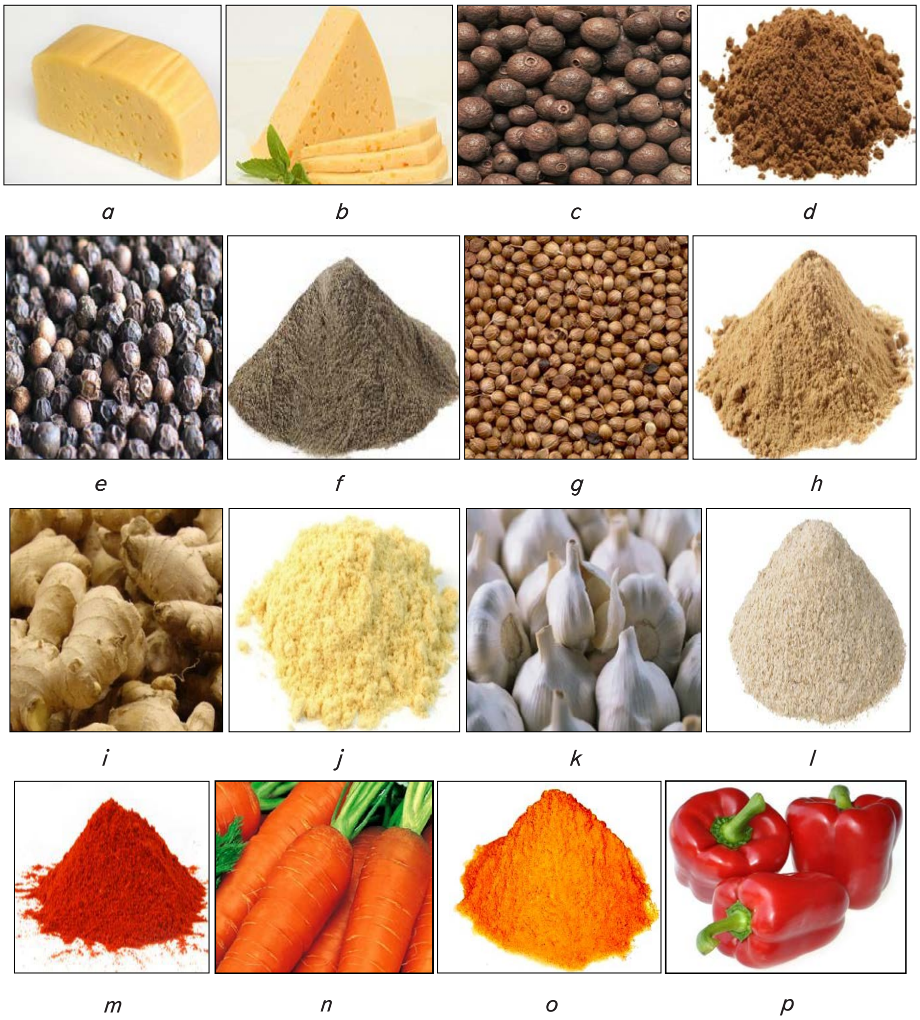


Fig. 1. Objects of research: *a, b* – samples of solid rennet cheese "Rosiisky"; *c–p* – nanopowders, spices, spicy and carotenoid vegetables (*c* – sweet pepper; *d* – nanopowder from sweet pepper; *e* – black pepper peas; *f* – nanopowder from black pepper peas; *g* – coriander; *h* – nanopowder from coriander; *i* – ginger; *j* – nanopowder from ginger; *k* – garlic; *l* – nanopowder form garlic; *m* – nanopowder from carrot; *n* – carrot; *o* – nanopowder from paprika; *p* – paprika)

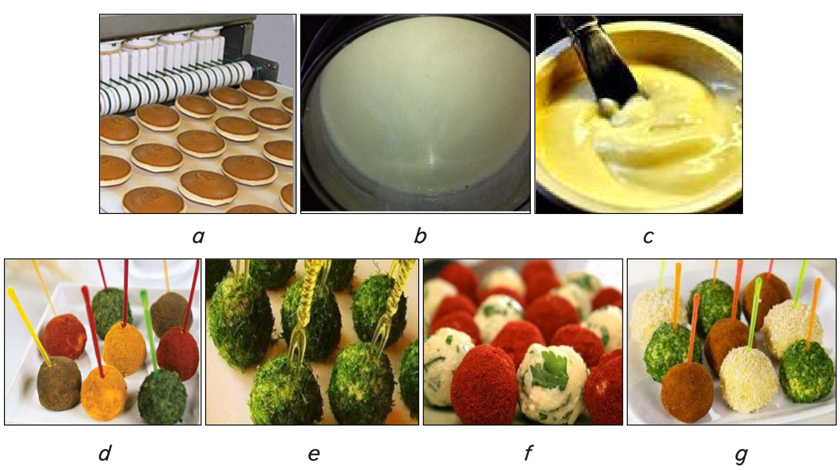


Fig. 2. Products from processed cheese: *a* – confectionery products "Pancake" with new cheese filling, developed by the authors; *b, c* – paste – like processed cream (semi-finished products for fillings, falafels, dressing sauces, dipping sauces, etc.); *d–g* – cheese snacks falafels (*d* –"Assorted"; *e* –"Emerald"; *f* –"Piquant"; *g* – "Exotic")

One can get acquainted with the procedure of samples preparation in more detail in paper [20].

4. 2. Methods for determining indices of the studied samples

The following indices were used as criteria for assessing the quality of rennet cheeses (original and ground): protein (total nitrogen), free and bound amino acids, hydrophilic and hydrophobic residues of amino acids, fat, dry substances, humidity, organic acids, and minerals. In more detail these procedures are described in the article [21]. You can get acquainted with the procedure of defining the indices of the studied samples in work [20].

One can get acquainted with the method of determination of the indices of the studied samples in more detail in [20].

5. Studies on the development of technology of healthy processed cheese products without melting salts

In the course of developing nanotechnologies of healthy cheese products with the use of freezing and non-fermentative catalysis – cryomechanolysis and solid rennet cheeses, the most important task is to maximally reduce the amount of melting salts in the preparation of solid rennet cheeses (SRC) for melting, and to destroy low-soluble lipid-protein nanocomplexes and nanoassociates, to extract protein from the bound state, to carry out peptization and the melting process with a minimum amount of melting salts or without them. It appears possible when using low-temperature finely dispersed grounding and freezing, accompanied by processes of cryodestruction and non-fermentative catalysis.

The obtained healthy processed cheese products compared with traditional products do not contain melting salts, harmful to a human organism, because they can cause renal diseases, diseases of bile system, joints, allergies, etc. The obtained nano semi-finished products (or bases) from rennet cheeses in the form of a homogeneous elastic mass are more technological, compared with the traditional ones. They are better dissolved, dispersed in water and in the fat and vegetable disperse systems and form a homogenous stable gel structure.

Various samples of solid rennet cheese "Rosiisky" were used as a base in manufacturing processed cheese products. It was shown that hard rennet cheese, as it should have been expected, contains a significant amount of protein and fat (29,6±1,5 % and 28,8±1,2 % respectively in the ratio of 1:1) and is characterized by the high content of mineral substances (4 %).

It was shown, that proteins of rennet cheese were presented by bound and free amino acids (Table 2). So, the total number of α-amino acids in rennet cheese is – 29,7 g in 100 g, in which free amino acids make up 25…26 % of the total number of α-amino acids (respectively 6,2 g in 100 g and 23,4 g in 100 g), bound amino acids – 74…75 %. It is shown that hard rennet cheese contains all essential α-amino acids (lysine, tryptophan, threonine, valine, methyonine, isoleucine, leucine, phenylalanine), which according to the scale of the FAO/WHO are in a balanced state.

It was determined that in processing hard rennet cheeses before melting process with the use of freezing and fine dispersed crushing, there is a destruction of low-soluble lipid-protein nanocomplexes and nanoassociates and the release (extraction) of protein from the bound, hidden, and inactive status with lipids and mineral substances to the free state by 33,3…35,0 % more than in the original raw material (Table 1, Fig. 3).

Table 1

Influence of cryomechanolysis and freezing of solid rennet cheese on the content of α-amino acids in free and bound state

Name of amino acids	Bound amino acids of solid rennet cheese				Free amino acids of solid rennet cheese			
	in original cheese, mg in 100 g	After mechanolysis and freezing, mg in 100 g	% of original	increase to original, times	in original cheese, mg in 100 g	After mechanolysis and freezing, mg in 100 g	% of original	increase to original, times
Valine	450	1080	240,0	2,4	340	960	282,3	2,8
Isoleucine	920	2100	228,0	2,3	650	1250	192,0	1,9
Leucine	2300	2550	111,0	1,1	130	250	192,0	2,0
Lysine	1240	3140	253,2	2,5	360	550	153,0	1,5
Methionine	1040	1120	108,0	1,1	400	600	150,0	1,5
Threonine	710	1240	175,0	1,7	120	240	200,0	2,0
Tryptophan	700	700	100,0	1,0	400	400	100,0	1,0
Phenylalanine	1070	1480	138,0	1,4	230	640	278,0	2,8
Arginine	1910	1300	67,9	–	430	870	202,0	2,0
Asparatic acid	1330	2310	174,0	1,7	200	490	245,0	2,5
Histidine	1010	1240	123,0	1,2	80	150	188,0	1,9
Glycine	410	560	137,0	1,4	70	140	200,0	2,0
Glutamic acid	4410	4700	107,0	1,1	1620	1790	110,0	1,1
Proline	1720	2760	160,0	1,6	60	150	250,0	2,5
Serine	1100	1840	167,0	1,7	310	370	119,0	1,2
Tyrosine	2210	1630	73,7	–	240	340	142,0	1,4
Cysteine	300	300	100,0	1,0	540	420	–	–
Alanine	580	1200	207,0	2,1	90	260	289,0	2,9
Total	23410	31250	33,5	1,33	6270	9870	57,4	1,57

Thus, for example in the original rennet cheese there are 23,4 g of bound amino acids, and in frozen and finely dispersed ground – 31,3 g in 100 g. The mechanism of this process, which is connected with the cryomechanocracking, mechanic destruction of the bonds between lipids and proteins was established. This process of destruction of nanocomplexes and nanoassociates of biopolymers is connected with non-fermantative catalysis (destruction), cryodestruction and cryomechanolysis. This indicates that protein is freed from the state, bound with nanocomplexes, to free state. According to Authors, nanocomplexes and nanoassociates are complex difficultl to solve lipidoproteincalciumphosphate complexes (LPCPC). In these, lipids and polypeptide chains are intertwined, stitched together using calcium bridges, disulphide and phosphoramid bonds, hydrogen bonds, and intermolecular interactions. This prevents their peptization and dissolution in the process of melting cheese and getting a homogeneous fluid mass [3, 6, 7]. Thus, one of the main technological operations in the technology of processed cheeses is softening of cheeses and getting a homogeneous fluid mass in the course of heating in presence of melting salts. In this case, peptization and pasteurization take place simultaneously.

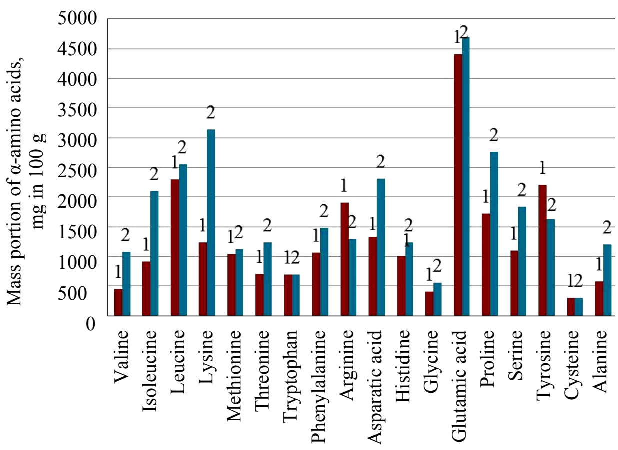


Fig. 3. Influence of freezing and non-fermentative catalysis – cryomechanolysis on destruction of lipidoparacaseinatcalciumphosphate nanocomplexes and nanoassociates and release of protein from bound state with lipids to free state (by the number of bound α-amino acids in protein molecules) in original rennet cheese (1 – ■) compared with frozen and finely dispersed ground (2 – ■)

It was also found that in the course of the specified processing of rennet cheeses there is non-fermentative cryocatalysis (destruction) of proteins to monomers of free α-amino acids by 55–60 % (Table 1, Fig. 4). It was found that in the process of cryomechanolysis and freezing, a part of α-amino acids from the bound state is transformed in a free form. So, the mass fraction of free α-amino acids in the frozen finely dispersed ground pasty cheeses increased by 1,1…2,9 times (up to their quantity in the original solid rennet cheese to low temperature grinding).

It was shown that the largest increase in the mass fraction of amino acids is by 2.8…2.9 times and it is observed for such α-amino acids as alanine, valine, and phenylalanine. For proline and aspartic acid, the mass fraction increases by 2,5 times, for threonine, glycine, isoleucine, leucine, histidine and arginine the mass fraction increases by 1,9…2,0 times. The lowest increase in mass fraction of free α-amino acids is observed for glutamate acid, tryptophan, serine and cysteine.

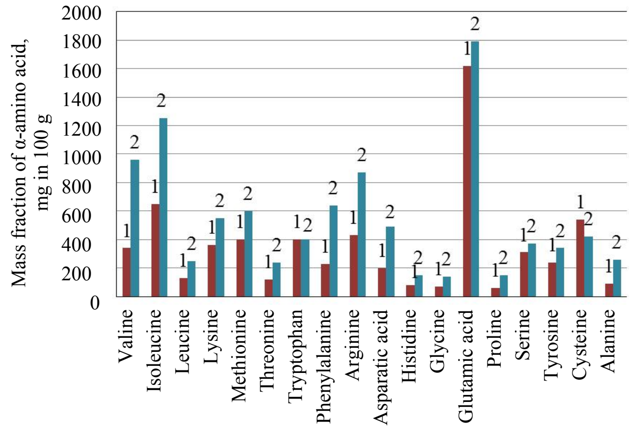


Fig. 4. Influence of freezing and non-fermentative catalysis – cryomechanolysis on cryodestruction – destruction of protein of rennet cheeses (1 – ■) to individual monomers (α-amino acids) and their transformation to free form while preparing them to melting, peptization and pasteurization and manufacturing cheese products without melting salts (2 – ■)

It was displayed that cryomechanolysis and freezing of solid rennet cheeses greatly intensifies the process of destruction of protein-lipid complexes and promotes mechanical destruction (mechanolysis) of proteins to separate free amino acids. The mechanism of this process is linked to the fact that during mechanical grinding, as a result of mechanocracking there occur such critical energy tensions in areas of biopolymeric chain of protein, that lead to a rupture, destruction of the peptide, as well as hydrogen bonds, calcium bridges and considerable destruction of protein molecules into free amino acids. Different degree of increase in mass fraction of different types of α-amino acids in the process of cryomechanolysis of protein is connected with the specificity of amino acid composition of proteins of solid rennet cheeses.

The studies of the processes of mechanolysis and freezing that occur during cryogenic "shock" freezing and low temperature grinding, indicate that the integrated action of freezing and mechanical grinding leads to the destruction of biopolymers of proteins to individual monomers – amino acids (by 55…60 %). It is known that the dimensions of molecules of α-amino acids vary from 0,4 to 1,5 nm [3]. In other words, cheese products that are obtained from the use of cryomechanolysis are in nano-dimensional form. In this regard, it is possible to assume that these technological methods may cause conformational changes in molecules, erasing of molecules, changes in their volume, shape, and an increase in their molecular weight. It is known that colloidal properties of proteins, their ability to form gels depends on the hydrophilic properties of residues of amino acids that make up their composition. It has a fundamental importance for melting and peptization of the main low soluble protein of solid rennet cheese – paracasein [3, 10–12, 14].

The tasks of the work included studying the influence of freezing and cryomechanodestruction of solid rennet cheese on the conformational changes of protein molecules (volume, radius, radius of nucleus, the index of filling the molecule core with hydrophobic residues) using the method of Y. G. Fisher.

The residues of amino acids that are the part of polypeptin chain, as it is known, can be divided into non-polar and polar. Organic compounds containing polar groups, soluble in water and capable to enter dipole – dipole interaction with water molecules and form hydrogen bonds with them, are hydrophilic. Analysis of literature data showed, that hydrophobic interaction lies at the basis of protein – protein complex formation, as well as of complex formation with salts. Protein molecules consist of the hydrophilic (polar) and hydrophobic (non-polar) residues of amino acids. Polar residues tend to the maximum contact with the aqueous environment and non-polar – to the minimal contact. Therefore, a flexible protein molecule is minimized to globule. In this case, a compact body – a ball with the hydrophobic nucleus and hydrophilic surface.

We studied the effect of freezing and cryomechanodestruction on the content of hydrophilic and hydrophobic residues of amino acids in protein molecules in solid rennet cheese (Table 2).

It was shown that in the process of freezing and cryomechanodestruction along with the destruction of a part of protein to amino acids and simple peptides there is a decrease in the mass fraction of the hydrophilic (HFL) residues of amino acids (C_n), an increase in hydrophobic (HF) residues (C_{hn}) in protein molecules and the increase in the correlation between them (C_n/C_{hn}).

It was found that compared with the original raw materials, in the process of freezing and low temperature grinding, the mass fraction of hydrophilic residues of amino acids in 100 g of protein decreases by 6,3 % and concurrently the mass fraction of hydrophobic residues increases by 5,3 % (Table 3).

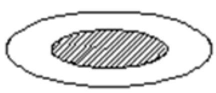
Table 2

Influence of cryomechanolysis and freezing of solid rennet cheese on the content of hydrophilic and hydrophobic residues of amino acids in protein molecules

Amino acid	Mass fraction of bound amino acids of protein, mg		Degree of hydrophobicity (ΔF, kJ/mol)	Degree of hydrophobicity of bound amino acids of protein (ΔF, kJ/mol)	
	Original solid rennet cheese	solid rennet cheese after cryomechanolysis and freezing		Original solid rennet cheese	solid rennet cheese after cryomechanolysis and freezing
Hydrophilic residues of amino acids					
Alanine	2,48	3,84	3,05	7,56	11,71
Arginine	8,16	4,16	3,05	24,89	12,69
Cysteine	1,28	0,96	2,71	3,47	2,60
Glutamic acid	18,84	15,04	2,50	47,10	37,60
Asparatic acid	5,68	7,39	2,26	12,84	16,70
Threonine	3,03	3,97	1,84	5,58	7,30
Serine	4,7	5,89	0,17	0,80	1,00
Glycine	1,75	1,78	0,0	0	0
Total	45,92	43,03		102,24	89,60
Hydrophobic residues of amino acids					
Valine	1,92	3,46	7,06	13,55	24,43
Isoleucine	3,93	6,72	12,4	48,73	83,33
Leucine	9,82	8,16	10,10	99,18	82,42
Lysine	5,3	10,05	6,27	33,23	63,01
Methionine	4,44	3,58	5,45	24,20	19,51
Tryptophan	3,0	2,24	12,50	37,50	28,0
Phenylalanine	4,57	4,74	11,10	50,73	52,61
Histidine	4,31	3,97	5,85	25,21	23,22
Proline	7,35	8,83	10,85	79,75	95,80
Tyrosine	9,44	5,22	12,00	113,28	62,64
Total	54,08	56,97		525,37	534,97
Hydrophilic and hydrophobic residues of amino acids					
Total	100,0	100,0		627,61	624,57

Table 3

Comparative characteristic of protein molecules of original solid rennet cheese after cryomechanolysis and freezing

Indices	Solid rennet cheese	
	original	After processing
Content of polar residues of amino acids, C_n	45,92	43,03
Content of non-polar residues of amino acids, C_{hn}	54,08	56,97
Ratio C_n/C_{hn}	0,85	0,76
Radius of molecule, r_o, μm	$0{,}2265 \cdot 10^{-2}$	$0{,}2474 \cdot 10^{-2}$
Radius of molecule nucleus, r, μm	$0{,}1765 \cdot 10^{-2}$	$0{,}1974 \cdot 10^{-2}$
Volume of molecule, V, μm ^{3}	$0{,}04 \cdot 10^{-6}$	$0{,}08 \cdot 10^{-6}$
Index of filling core of molecule with hydrophobic residues, (b) by diagram	0,90	0,45
Shape of protein molecule	 ($b>b_s$) stretched ellipsoid	 ($b<b_s$) supermolecular structure

In addition, the ratio between hydrophilic and hydrophobic residues changes from 0,85 to 0,76. The obtained results made it possible to compare the dimensions and shapes of protein molecules of original solid rennet cheese and after freezing and low temperature grinding according to the theory of Y. G. Fisher (Table 3).

It was established that freezing and cryomechanodestruction lead to an increase in radius, volume of protein molecules, radius of its nucleus, and to a decrease in the index of filling of nucleus with hydrophobic residues. In addition, the shape of protein molecules also changes. Thus, the radius of the protein molecule of solid rennet cheese after cryomechanolysis and freezing increases by 9,2 % and makes $0{,}2265 \cdot 10^{-2}$ μm (compared to $0{,}2474 \cdot 10^{-2}$ μm in the original solid rennet cheese). Its volume increases by 2 times and is $0{,}0810^{-6}$ μm^3 (compared to $0{,}04 \cdot 10^{-6}$ μm^3 in the original cheese). The ratio of the sum of the hydrophilic and hydrophobic residues of amino acids of original rennet cheese before cryogenic processing and finely dispersed grinding of cheese makes respectively 0,85 and 0,76. In this case, the radius of the nucleus of molecule increases by 1,1 time, and the index of filling the nucleus with hydrophobic residues decreases by 2 times.

According to the theory of Y.G. Fisher, it was found that the molecules of the original solid rennet cheese have the shape of ellipsoids, and after cryomechanolysis and freezing take the form of supermolecular structures (Table 3). This helps to increase availability, solubility, peptization of protein molecules in the process of preparation of solid rennet cheeses before melting and obtaining the homogeneous fluid gel texture of cheese mass.

Based on the experimental research, Authors developed nanotechnology of processed cheese products with the use of rennet cheeses, which excludes melting salts and is different from the traditional ones by using freezing to the temperature of 18 °C and finely dispersed grinding at temperatures not less than 10 °C up to the dimensions of the particles, which are several times as small as in traditional grinding. The technology also includes the process of pasteurization (at the temperature of 70...75 °C) and melting (without melting salts) and homogenization. Enrichment of cheese products involves the introduction of natural vegetable carotenoid additives in the form of nanopowder (from carrots or pumpkin), nanopowder from garlic, as well as nanopowders, nanoextracts from natural spices (black pepper peas, sweet pepper, cilantro, etc.). In addition, the technology involves packing and packaging in gas – moisture-light proof packaging or landing the finished filling on a pastry pancake «PanCake» (Fig. 5).

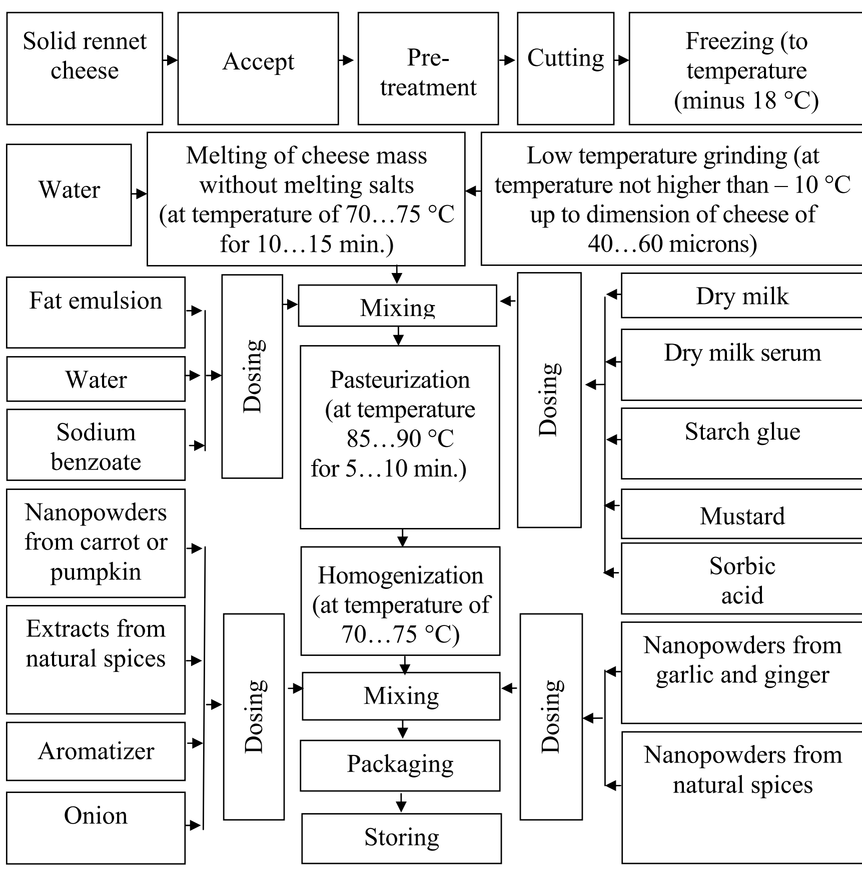


Fig. 5. Fundamental technological scheme of production of processed cheese products with the use of freezing, finely dispersed grinding and nanopowders from spices, spicy and carotene-containing vegetables

The introduction of listed vegetable additives with high content of BAS, such as terpenoids (aromatic substances, essential oils, carotenoids, etc.) and phenolic compounds in processed cheese products allows enriching the cheese products with vegetable BAS. This allows increasing the shelf life by 2,5–3,0 times rather than without using them.

As analogues, we manufactured model systems from rennet cheeses, using a different dose of melting salts, which are commonly used in traditional technologies of manufacturing processed cheese (0,5 %; 1,0 %; 1,5 %; 2,0 %). It was established that the integrated use of freezing and cryomechanodestruction makes it possible to completely exclude, rather than decrease the amount of melting salts.

Authors developed the technologies and formulations of healthy processed cheese products with the use of solid rennet cheeses as raw materials, and the innovation – processes of its freezing and non-fermentative catalysis in the course of their preparation to melting. These are the following products: cheese fillings for confectionery product "PanCake", pasty processed cheeses, cheese dressing sauces and dipping sauces. The technology of cheese fillings for confectionery products "PanCake" was implemented into industrial production at TOV PKG "Lisna Kazka", Kharkiv (Fig. 7).

Four formulations of cheese fillings for confectionery products "PanCake", enriched with vegetable nanoadditives, were developed: "Cheese with garlic", "Cheese with garlic and bacon", "Cheese with mushrooms", "Cheese with vegetables". They are different in the way they look and the amount of the introduced nanopowders with carrots or pumpkin (2,5...5 %), as well as nanopowder with garlic and natural spices (1,0 and 3,0 %) and extracts from natural spices (1,5 and 2,0 %). Nanopowders from carrots, pumpkin, spicy vegetables and natural spices were developed by Authors of the paper. Nanopowders are finely dispersed powders in nanodimensional form. In this case, 60...70 % of the product have the dimension of particles up to a few nanometers, in particular, the dimensions of molecules of α-amino acids, glucose, fructose, galacturonic acid, L-ascorbic acid, fatty acids, carotene, etc. are in the range from 0,5 to 1,5 nm. A part of biopolymers and nanocomplexes is in the low-soluble state. The dimensions of molecules of biopolymers and nanocomplexes are in the range of 50 nm to 5...10 microns. The quality of new cheese filling by the content of BAS and physical and chemical indicators was studied (Table 6).

Table 4

Content of biologically active substances, protein and essential amino acids in cheese fillings for confectionery products "PanCake" enriched with vegetable nanoadditives, obtained by nanotechnology

Index	Processed cheese fillings enriched with vegetable nanostructured additives and produced by nanotechnology			
	«Cheese with garlic»	«Cheese with garlic and bacon»	«Cheese with mushrooms»	«Cheese with vegetables»
Protein, %	28,7	26,6	24,6	22,5
Essential amino acids, mg in 100 g:				
Valine	1428	1326	1224	1122
Isoleucine	2345	2177,5	2010	1842,5
Leucine	1960	1820	1680	1540
Lysine	2583	2398,5	2214	2029,5
Methionine	1204	1118	1032	946
Threonine	1036	962	888	814
Tryptophan	770	715	660	605
Phenylalanine	1484	1378	1272	1166
β-carotin, mg in 100 g	3,0	3,1	3,5	3,2
Phenolic compounds (by chlorogenic acid), mg in 100	25,1	27,8	26,4	25,8
Flavonolic glycosides (by rutine), mg in 100 g	5,3	6,5	5,5	6,7
Free catechins (by d-catechin)	5,4	6,1	5,2	6,5
Tannins (by tannin), mg in 100 g	61,1	33,8	45,6	38,4
Aromatic substances, mg $Na_2S_2O_3$	37,5	15,3	28,4	32,9
Fat, %	20,0	21,2	23,4,	24,6
Dry substances, %	69,9	70,0	65,4	63,8

Fig. 6. Section of technological production line of confectionery products "PanCake" with cheese filling (industrial production at TOV PKG "Lisna Kazka", Kharkiv)

It was shown that new kinds of cheese fillings are characterized by high content of BAS, including those that have antioxidant properties (Table 4). Thus, 100 g of filling of aromatic substances contain 15,3...37,5 mg of sodium thiosulfate, 25,1...27,8 mg of total phenolic compounds, 5.3...6.7 mg of flavonolic glycosides, 5.2...6.5 mg of free catechins, 33,8...61,1 mg of tannins, 3,0...3,5 mg of β-carotene. The listed BAS are missing in the reference samples – the processed cheese and vegetable filling without enriching additives. In the course of studying physical and chemical indices, it was established, that in the cheese and vegetable fillings the protein content amounts to 22.5...28.7 %, the amount of fat is 20...24,6 %, the amount of moisture is 30,0...34.6 %. Thus, the new cheese and vegetable fillings along with proteins (22,5...28,7 %) and fats (20,0...24,6 %) contain a considerable amount of BAS of vegetable raw materials with immunomodulatory and antioxidant properties, namely ½ of the daily needs in β-carotene and the daily norm of phenolic compounds. The analysis of chemical composition allows referring new processed cheese products to healthy food products.

New technologies of healthy processed cheese products were tested under industrial conditions at a number of enterprises of Ukraine (TOV VKG "Lisova kazka", NVP "FIPAR", BVP "KRIAS-1"). The regulatory documentation (TU, TI for "cheese and vegetable fillings for confectionery products "PanCake" and "cheese dressing sauces") was developed and approved.

6. Discussion ofresults of research into the course of development of nanotechnologies of healthy processed cheese products without melting salts

We explored the influence of cryomechanolysis and freezing on the amino acid composition of solid rennet cheese during the preparation for melting, the content of α-amino acids in free and bound state, conformational changes of protein molecules, which are in low-soluble form in the original raw cheese, during the development of nanotechnologies of healthy processed cheese products.

The merits of this study is that for the first time it was shown, that as a result of the complex use of cryomechanolysis and freezing of rennet cheeses before melting, there occur the destruction, degradation, disaggregation of nanocomplexes and biopolymers and the release of protein from the hidden bound forms to the free form. In addition, a new effect from the influence of the processes of cryomechanolysis and freezing is the fact that in the process of cryogenic "shock" freezing and low temperature grinding of solid rennet cheese during obtaining healthy processed cheese products, there occurs more complete extraction of protein from lipid-protein nanocomplexes (by 30...35 %), an increase in accessibility, peptization, destruction of protein to separate monomers (by 50...55 % more than in the original state). In addition, there is a formation of supermolecular structures in the process of preparation of solid rennet cheese for melting and obtaining homogeneous gel cheese mass in melting without melting salts.

This allows obtaining healthy processed cheese products based on solid rennet cheese with a fundamentally new chemical composition and high consumer properties without melting salts and with higher assimilation by the human organism. In turn, they can be used in developing the functional healthy food products of mass nutrition based on solid rennet cheese food, such as snacks, fillings, sauces, etc.

The disadvantages of processed cheese products and of processing solid rennet cheese include the presence of this raw material of slightly soluble paracaseinatcalciumphosphate complexes, in which polypeptide chains are tightly interweaved with calcium bridges, disulfide, phosphoamidic and other links, which prevents peptization and dissolution of proteins and obtaining a homogeneous fluid cheese mass during melting. In addition, the peptization of proteins is slowed down by the high content of lipids, which with proteins form a complex lipid–protein calciumphosphate complexes, in which protein is located in the hidden form (by 30...35 %).

In this study, using such technological methods as cryogenic "shock" freezing of the product and finely dispersed grinding, this problem has been solved. Besides, the introduction of vegetable enriching additives from natural spices and spicy vegetables with a considerable amount of BAS with immunomodulatory and antioxidant properties allowed obtaining processed cheese fillings, 100 g of which contain ½ of the daily needs of β-carotene and the daily norm in phenolic compounds. Introduction of nanoextracts from natural spices and nanopowders from spices and spicy vegetables made it possible to increase the shelf life of processed cheese fillings by 2 times compared with traditional products. The analysis of the chemical composition allows including new processed cheese products to healthy food.

However, in the future it is planned to search for other ways of activation, disaggregation of inactive hidden forms of nanocomplexes of biopolymers of solid rennet cheeses during their preparation for melting, namely by regulating pH of the environment, enriching with various vegetable additives with high content of BSA, etc.

7. Conclusions

1. It was established that during comprehensive influence of freezing and finely dispersed grinding on solid rennet cheeses, there is a destruction of low-soluble lipid-protein nanocomplexes and the release of protein from the state bound with lipids to free state (by 33,5...35 % more). The mechanisms of this process, which are connected with cryomechanodestruction of the bonds between lipids and protein and non-fermentative catalysis was revealed.

2. It is established that during freezing and finely dispersed grinding of solid rennet cheeses, there is cryomechanodestruction and non-fermentative catalysis (destruction) and conformational changes of protein molecules to separate monomers – α-amino acids by 55...60 %. We revealed the mechanism of this process, which is connected with the cryomechanic cracking of protein molecules due to the destruction of the peptide bonds in protein to separate α-amino acids and their transformation to a free form.

3. Authors proposed and developed the nanotechnology of manufacturing processed cheese products based on solid rennet cheeses without melting salts, which includes comprehensive influence of freezing and finely dispersed grinding. The mechanisms of the processes, related to cryomechanodestruction of bonds between lipids and protein and non-fermentative catalysis of protein to separate α-amino acids were revealed.

4. It was established that cheese fillings for confectionery products “PanCake”, produced by nanotechnology and enriched with herbal additives, exceed the known analogs in chemical composition and are characterized by 2 times longer shelf life. Besides, a considerable part of substances (as BAS and biopolymers) in cheese fillings is in the nanostructured form (55...60 % of protein) in the form of free amino acids. Dressing sauces, dipping sauces and cheese snacks were also developed.

References

1. FAO/WHO/UNU. Global’naya strategiya po pitaniyu, fizicheskoj aktivnosti i zdorov’yu – 2004 [Text]. – RezolyuciyaWHA.55.23 prinyata sessiej Vsemirnoj assamblei zdravoohraneniya (VAZ), World Health Organization, ZHeneva, 2004.
2. FAO/WHO/UNU. Dietary protein quality evalution in human nutrition. Report of an FAO Expert Consultation [Text] // Food and agriculture organization of the united nations Rome. – 2013. – Vol. 92-57.
3. Pavlyuk, R. Yu. Krio- i mekhanokhimiya v kharchovykh tekhnolohiyakh [Text]: monohrafyya / R. Yu. Pavlyuk, V. V. Pohars’ka, O. O. Yur»yeva, V. A. Pavlyuk et. al. – Kharkiv: Finart, 2014. – 260 p.
4. Apurba, G. Effect of phytosterols on textural and melting characteristics of cheese spread [Text] / G. Apurba, K. K. Suresh, R. Avneet // Food Chemistry. – 2014. – Vol. 157. – P. 240–245. doi: 10.1016/j.foodchem.2014.01.127
5. Rudavs’ka, H. B. Ozdorovchi produkty KhKhI storichchya [Text] / H. B. Rudavs’ka, B. O. Holub // Visnyk KDTEU. – 1999. – Vol. 4. – P. 42–45.
6. Boisard, L. The salt and lipid composition of model cheeses modifies in-mouth flavour release and perception related to the free sodium ion content [Text] / L. Boisard, I. Andriot, Ch. Martin, Ch. Septier, V. Boissard, Ch. Salles, E. Guichard // Food Chemistry. – 2014. – Vol. 145. – P. 437–444. doi: 10.1016/j.foodchem.2013.08.049
7. McCarthy, C. M. Effect of salt and fat reduction on proteolysis, rheology and cooking properties of Cheddar cheese [Text] / C. M. McCarthy, M. G. Wilkinson, P. M. Kelly, T. P. Guinee // International Dairy Journal. – 2016. – Vol. 56. – P. 74–86. doi: 10.1016/j.idairyj.2016.01.001
8. Hougaard, A. B. Emulsifying salt increase stability of cheese emulsions during holding [Text] / A. B. Hougaard, A. G. Sijbrandij, C. Varming, Y. Ardö, R. Ipsen // LWT – Food Science and Technology. – 2015. – Vol. 62, Issue 1. – P. 362–365. doi: 10.1016/j.lwt.2015.01.006
9. Amamcharla, J. K. Prediction of process cheese instrumental texture and melting characteristics using dielectric spectroscopy and chemometrics [Text] / J. K. Amamcharla, L. E. Metzger // Journal of Dairy Science. – 2015. – Vol. 98, Issue 9. – P. 6004–6013. doi: 10.3168/jds.2015-9739
10. Katarzyna, K. Czynniki kształtujące teksturę serów topionych [Text] / K. Katarzyna // ŻYWNOŚĆ. Nauka. Technologia. Jakość. – 2008. – Vol. 3, Issue 58. – P. 5–17.
11. Cichosz, G. Technologia serów topionych [Text] / G. Cichosz. – Warszawa: Hoża, 2000. – 255 p.
12. Surmacka-Szcześniak, A. Texture is a sensory property [Text] / A. Surmacka-Szcześniak // Food Quality and Preferences.– 2002. – Vol. 13. – P. 215–225. doi: 10.1016/s0950-3293(01)00039-8
13. Barambojm, N. K. Mekhanohimiya vysokomolekulyarnyh soedinenij [Text] / N. K. Barambojm. – Moscow: Himiya, 1978. – 384 p.
14. Allen Foegeding, E. Sensory and mechanical aspects of cheese texture [Text] / E. Allen Foegeding, J. Brown, M. A. Drake, C. R. Daubert // International Dairy Journal. – 2003. – Vol. 13, Issue 8. – P. 585–591. doi: 10.1016/s0958-6946(03)00094-3
15. Tamime, A. Y. Processed Cheese and Analogues: An Overview [Text] / A. Y. Tamime // Processed Cheese and Analogues, 2011. – P. 1–24. doi: 10.1002/9781444341850.ch1
16. Surówka, K. Tekstura żywności i metody jej badania [Text] / K. Surówka // Przem. Spoż. – 2002. – Vol. 10. – P. 12–17.
17. Pavlyuk, R. Yu. Novye tekhnologii vitaminnyh uglevodsoderzhashchih fitodobavok i ih ispol’zovanie v produktah profilakticheskogo dejstviya [Text]: monografiya / R. Yu. Pavlyuk, A. I. Cherevko, I. S. Gulyj et. al. – Kharkiv; Kyiv, 1997. – 285 p.
18. Pogarskaya, V. V. Aktivaciya gidrofil’nyh svojstv karotinoidov rastitel’nogo syr’ya [Text]: monografiya / V. V. Pogars’ka, R. Yu. Pavlyuk, A. I. Cherevko, V. A. Pavlyuk, N. F. Maksimova. – Kharkiv: Finart, 2013. – 345 p.
19. Pavlyuk, R. Yu. The development of technology of nanoextracts and nanopowders from herbal spices for healthful products [Text] / R. Yu. Pavlyuk, V. V. Pohars’ka, L. O. Radchenko, O. O. Yur”yeva, H. E. Hasanova, T. S. Abramova, T. M. Kolomiyets’ // Eastern-European Journal of Enterprise Technologies. – 2015. – Vol. 3, Issue 10 (75). – P. 54–59. doi: 10.15587/1729-4061.2015.43323
20. Pavlyuk, R. Elaboration of the new method of the melt cheese products manufacturing without salts-smelters using cryomechanolysis [Text] / R. Pavlyuk, V. Pogarska, O. Yurieva, L. Skripka, T. Abramova // EUREKA: Life Sciences. – 2016. – Vol. 4 (4). – P. 49–56. doi: 10.21303/2504-5695.2016.00193
21. Pavlyuk, R. The development of cryogenic method of deep treatment of inulin-containing vegetables (topinambour) and obtaining of prebiotics in the nanopowders form [Text] / R. Pavlyuk, V. Pogarska, V. Pavlyuk, R. Balabai, S. Loseva // EUREKA: Life Sciences. – 2016. – Vol. 3 (3). – P. 5–9. doi: 10.21303/2504-5695.2016.00145