

18. Ishtvan, Ye. O. Kolorymetriya, matematychni osoblyvosti procesu obchyslennya kolirnyx xarakterystyk zrazka dlya realizaciyi ekspres metodu [Text] / Ye. O. Ishtvan // Aktualni problemy rozvytku xarchovyx vyrobnyctv, restorannogo ta gotel'nogo gospodarstv i torgivli, 2013. – P. 112–114.
19. Petrusha, O. Assessment of color of meat using the method of computer colorimetry [Text] / O. Petrusha, A. Niemirich // Eureka: Life Science. – 2016. – Vol. 3 (3). – P. 3–7. doi: 10.21303/2504-5695.2016.00141

*Вивчено вплив процесів глибокої переробки рослинної сировини, яка включає криогенне «шокове» заморожування та дрібнодисперсне подрібнення, на активацію важкорозчинних та важкозасвоєваних гетерополісахарид-білкових наноконкомплексів в розчинну форму. Установлено, що відбувається руйнування і трансформація їх значної частини в наноформу (на 45...55 %) при розробці нанотехнологій пюре з топинамбуру. Розкрито механізми процесів*

*Ключові слова: глибока переробка сировини, криомеханодеструкція, дрібнодисперсне подрібнення, топинамбур, наноконкомплекси, інулін, нанопюре*

*Изучено влияние процессов глубокой переработки растительного сырья, которая включает криогенное «шоковое» замораживание и мелкодисперсное измельчение, на активацию труднорастворимых и трудноусвояемых гетерополисахарид-белковых наноконкомплексов в растворимую форму. Установлено, что происходит разрушение и трансформация их значительной части в наноформу (на 45...55 %) при разработке нанотехнологий пюре из топинамбура. Раскрыт механизм процессов*

*Ключевые слова: глубокая переработка сырья, криомеханодеструкция, мелкодисперсное измельчение, топинамбур, наноконкомплексы, инулин, нанопюре*

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## THE EFFECT OF CRYOMECHANODESTRUCTION ON ACTIVATION OF HETEROPOLYSACCARIDE-PROTEIN NANOCOMPLEXES WHEN DEVELOPING NANOTECHNOLOGIES OF PLANT SUPPLEMENTS

**R. Pavlyuk**

Doctor of Technical Sciences, Professor,  
the State Prize laureate of Ukraine,  
Honored figure of Science and Technology in Ukraine\*

E-mail: ktpom@mail.ru

**V. Pogarska**

Doctor of Technical Sciences, Professor,  
the State Prize laureate of Ukraine\*

**K. Balabai\***

**V. Pavlyuk**

Doctor of Physical and Mathematical Sciences, Professor  
Department of Technology and  
Organization of Restaurant Business  
Kharkiv Trade and Economics Institute of  
Kyiv National University of Trade and Economics  
Otakara Jarosha alley, 8, Kharkiv, Ukraine, 61045

**T. Kotuyk**

Postgraduate Student\*

\*Department of Technology Processing of  
Fruits, Vegetables and Milk

Kharkiv State University of Food Technology and Trade  
Klochkivska str., 333, Kharkiv, Ukraine, 61051

### 1. Introduction

Deep processing of raw materials using the processes of cryomechanodestruction opens up a possibility of the more complete use of biological potential of plant raw materials (higher by 45–55 % than when using existing methods) and manufacturing a new generation of natural nanoproducts for healthy nutrition.

The relevance of development of nanotechnologies, based on applying the processes of cryomechanochemistry and cryomechanodestruction that make it possible to maximally preserve and extract biologically active substances (BAS) of the original raw materials, is caused by the need to address a global problem that is currently observed in many countries of the world. The problem is the imbalances and deficiency (by 50 %) in the food rations of population of vitamins, high-

grade proteins, mineral substances and other BAS [1, 2]. In addition, there is reduced immunity of the population, caused by general deterioration of ecological situation. According to the literature, the health condition of population, as well as the condition of the human immune system, depends by 80 % on the condition of the intestine [2–4]. Functional health foods with probiotic properties, which contain useful microflora in active state, help to maintain the necessary balance of intestinal microflora in the body. These products include pickled vegetables (cabbage, carrots, beets), sour-milk drinks (kefir, yoghurt, prostokvasha) and other fermented products. In addition, products that contain “prebiotics” help to support the balance in the body [2–5]. They stimulate development and metabolic and biological activity of one or more of the groups of their own bacteria in the human body, which make up the intestinal microflora of a human, positively affect the composition of microbiocenosis [4, 5].

In the developed countries, the problem of immunodeficiency is solved by introduction of health improving products and supplements to the diets, in particular, made of fruit and vegetable raw materials, which are distinguished by high BAS content that contribute to the increase of immunity. The substances that have prebiotic properties occupy a special place among them, along with antioxidant vitamins (vitamins C, E,  $\beta$ -carotene), phenolic compounds and mineral substances. These include indigestible components of food, first of all, ballast carbohydrates, including polysaccharides, inulin, pectin substances, dietary fiber, proteins, chitosans, fruit-oligosaccharides, lactulose and others [2–5]. The promising raw material for obtaining additives with prebiotic properties and their use in the manufacture of health improving products is Jerusalem artichoke [2, 6–8]. Its value for the food industry is defined, first of all, by hydrocarbon composition [9, 10]. It should be noted that 80 % of the dry substances in the tubers of Jerusalem artichoke are presented by the prebiotic inulin, the only natural polysaccharide that is made up by 95 % of fructose, the sugar that is harmless for diabetics [10]. Inulin has a form of linear polysaccharide, the main structural monomer of which is the fructose remains that are connected by  $\beta$ -fructose bonds. Jerusalem artichoke also contains pectin, fiber, protein, a wide range of minerals (potassium, calcium, manganese, etc.), vitamins (C, B<sub>1</sub>, B<sub>2</sub> and others), phenolic compounds, etc.

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## 2. Literature review and problem statement

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Data analysis of the periodic literature over the past 10 years has shown that the existing technologies of processing Jerusalem artichoke to various kinds of additives in the form of powders, pastes, flour, puree, extracts using the vapor thermal treatment, drying, extraction, do not make it possible to transfer inulin to easily digestible form [6, 8, 9, 11]. In this regard, it is relevant to find technological methods that allow obtaining supplements of Jerusalem artichoke with high quality with maximal preservation of raw materials' BAS and transfer inulin to easily digestible form. The conducted analysis of the literature data concerning the technology of processing Jerusalem artichoke to frozen finely dispersed and powdered supplements with the use of cryogenic processing showed the lack of such data in the periodic literature over the last 10 years [7, 8, 11, 12].

In this work we propose, in the development of nanotechnology of obtaining finely-dispersed puree from Jerusalem artichoke, to use the deeper processing of raw materials than that accepted today. As the innovation, we used comprehensive action on the raw materials of cryogenic «shock» freezing with higher speeds to lower temperatures of the product (to  $-35...-40$  °C) than it is accepted in international practice and low-temperature finely-dispersed grinding. These methods are accompanied by the processes of cryomechanodestruction (cryomechanochemistry, mechanoactivation, cryodestruction) [12, 13]. The Authors of this work understand cryomechanodestruction as the new technological process, which includes the action of freezing and mechanical grinding and leads to degradation, destruction of plant cells, nanocomplexes and nano associates of different sparingly soluble substance (ingredients), which they contain. The result is the fuller extraction of valuable components from the raw materials. The specified technological process is the alternative to fermentative treatment of plant raw materials. It should be noted that the use of processes of cryomechanodestruction is implemented in such industries as chemical, metallurgical, textile, aviation, in such countries as Japan, Russia and Kazakhstan. Thus, for example, applying the processes of cryo- and mechanochemistry allowed developing technologies of powder metallurgy, technology of plastic masses that do not have scratches, technology of textile products with water- and dirt-repellent properties. In the food industry, both in Ukraine and in international practice, these processes have hardly been explored [13, 14].

The conducted analysis of the data of the periodic scientific literature over the past 10 years, related to the study of the processes of cryomechanodestruction using cryogenic treatment and finely-dispersed grinding in processing of vegetable raw materials, including Jerusalem artichoke, revealed that in the scientific literature, except for the papers of the Authors of this article, the materials are absent [6, 14, 15]. Thus, in the Kharkiv State University of Food and Trade (Kharkiv, Ukraine), the specialists of the Department of Technology of Processing of Fruits, Vegetables and Milk proposed and designed cryogenic method of treatment and the nanotechnologies of obtaining nanopuree and nanopowders from Jerusalem artichoke with the use of liquid and gaseous nitrogen. It was discovered and shown for the first time in international practice that the comprehensive action of cryogenic «shock» freezing and finely-dispersed low-temperature grinding on the raw materials lead to not only complete preservation of all BAS, but also their fuller extraction from the raw materials from hidden bound forms with biopolymers (proteins, heteropolysaccharides) of the nanocomplexes and nano associates and transformation to free state. The mass fraction of BAS is 1,8...2,3 times higher than in the original raw materials. In parallel, it was found that during cryogenic treatment and finely-dispersed grinding of Jerusalem artichoke, the partial destruction of inulin to its individual monomers occurs – to fructose (by 45...55 %), protein to free amino acids (by 43...55 %), cellulose to sugars (by 43...55 %). It testifies to the destruction of sparingly soluble biopolymers and their transformation to the easily digestible nanodimensional form. However, the cited articles contain only assumptions concerning the mechanism of the influence of cryogenic treatment and finely dispersed grinding on the nanocomplexes of biopolymers (proteins and heteropolysaccharides). Conformational changes of protein

molecules, nanocomplexes of heteropolysaccharides together with proteins and their transformation to instant easily digestible form have not been examined. Not studied are the activation processes of hidden inactive forms of protopectin and their transformations to soluble form. Also not explored is the impact of the indicated processes on the degree of digestibility of nanosupplements made of Jerusalem artichoke compared to traditionally produced additives using modern method biotesting. In this regard, the study of regularities and mechanisms of influence of the processes of deep processing of raw materials, which are based on the use of the processes of cryomechanoactivation, cryomechanodestruction, on the nanocomplexes of heteropolysaccharide-protein, as well as biopolymers (proteins, heteropolysaccharides, in particular pectin) when developing nanotechnology of obtaining frozen supplements in the form of puree – prebiotics from Jerusalem artichoke in nanodimensional form, is relevant.

By the foregoing, it appears theoretically interesting and practically valuable to conduct a fundamental research into possibility of the fuller use of biological potential of carbohydrate-containing raw materials (in particular, Jerusalem artichoke), characterized by significant content of sparingly soluble biopolymers (inulin, pectins, cellulose, protein). These substances form nanocomplexes and nano associates between them. In this regard, they are related to the indigestible components of food that are difficult to transfer to soluble form in the process of technological treatment. A comprehensive action of cryogenic «shock» freezing and finely dispersed grinding on the raw materials was used for this purpose, accompanied by the processes of activation and cryomechanodestruction – cryomechanolysis (non-fermentative catalysis of polymers and nanocomplexes of biopolymers with BAS) of the original plant raw materials.

### 3. The purpose and objectives of the study

The aim of the work is to study the influence of the processes of cryomechanodestruction on the activation and destruction of biopolymers and heteropolysaccharide-protein nanocomplexes when designing cryogenic nanotechnology of nanopowders and frozen nanopuree made of Jerusalem artichoke. To achieve the set goal, the following tasks had to be solved:

- to study the influence of comprehensive action on the raw materials of cryogenic «shock» freezing and finely-dispersed grinding on the transformation of bound amino acids of protein to free form and conformational changes of protein molecules of Jerusalem artichoke (shape, volume, radius, radius of the molecule's nucleus, indicator of the nucleus filling by hydrophobic and hydrophilic amino acid remains);

- to examine the impact of the processes of cryomechanodestruction on the activation of heteropolysaccharides (pectins) of Jerusalem artichoke and their release from a hidden (inactive) form from nanocomplexes with biopolymers and their destruction and transformation from sparingly soluble to soluble form;

- to explore the impact of cryogenic treatment of raw materials, finely-dispersed grinding, cryodestruction on biological activity (degree of digestibility) of nanopowders and frozen nanopuree of Jerusalem artichoke using the express method of biotesting;

- to compare the quality of nanosupplements from Jerusalem artichoke with analogues and define the directions of their use in health improving and mass-market food products.

## 4. Materials and methods of research

### 4.1. Materials and equipment used in the experimental study

The study was conducted by using Jerusalem artichoke tubers (Fig. 1), frozen finely dispersed purees (Fig. 2) and the nanopowders made of them by freeze-drying (Fig. 3).



Fig. 1. Original raw material (Jerusalem artichoke tubers)



Fig. 2. Frozen finely-dispersed puree from Jerusalem artichoke



Fig. 3. Nanopowder made of Jerusalem artichoke by freeze-drying

Preparation of the samples. To study the influence of cryogenic treatment of raw material, low-temperature finely dispersed grinding and freeze drying, we performed preparation of the samples. They were ground into pieces with thickness of 0.5...1.0 cm and length of 4...5 cm, then put on trays for consequent freezing.

The treatment of samples was carried out using gaseous and liquid nitrogen in a quick-freezer.

More details on the method of the samples treatment can be found in the paper [16].

#### 4. 2. Methods for determining indicators of the studied samples

The method of determining the parameters (total nitrogen, protein structure and conformational changes, free and bound amino acids, biological activity, pectins, cellulose, vitamin C, phenolic substances, polyphenols (tannins), titrated (organic) acids) of the studied samples, in particular: Jerusalem artichoke tubers, frozen finely-dispersed purees and the nanopowders made by their freeze-drying, can be found in the paper [16].

#### 5. Study of influence of the cryomechanodestruction processes on activation and destruction of plant biopolymers, conformational changes of protein molecules, the degree of digestibility of plant supplements from Jerusalem artichoke in the development of nanotechnology of their manufacturing

The main thing in the development of nanotechnology of plant supplements from Jerusalem artichoke, using the cryogenic «shock» freezing and finely-dispersed grinding, was not only to increase the degree of extraction of hidden bound forms of BAS with biopolymers from the raw materials to free state, but to partially transform sparingly soluble polysaccharides, oligosaccharides and proteins to soluble form. It turns out to be possible due to cryodestruction and cryomechanoactivation, as well as mechanolysis.

The received plant supplements in the form of nanopowders and nanopuree are more technological in comparison with traditional powders and puree. They dissolve better and disperse in water and form a homogeneous suspension. The particles of nanopowders are not felt when consumed and they form a gel structure in aqueous solutions.

Study of mechanochemistry processes that occur at finely-dispersed grinding of supplements from Jerusalem artichoke suggests that comprehensive action of freezing and mechanical grinding leads to destruction of the protein biopolymers to individual monomers. In this regard, one might assume that the indicated technological methods may cause conformational changes in molecules, erasing molecules, changing their volume, shape, molecular mass reduction. It is known that a protein molecule consists of the hydrophobic nucleus and hydrophilic membranes and the molecules' form depends on the ratio of hydrophilic and hydrophobic amino acids remains. Hydrophilic amino acids determine colloidal properties of proteins and their ability to form gels. It is of great importance when using plant powders in manufacturing various food products. Thus, the dry mixes for juices and nanodrinks, made with their use, should at recovery in water form a stable colloidal suspension that does not stratify. Therefore, during finely dispersed grinding, in parallel with the decrease of the mass fraction of bound amino acids, the biopolymers of protein can undergo conformational changes of protein molecules, such as the redistribution of the ratio between the hydrophilic and hydrophobic amino acids remains. This may lead to changes not only in the volume, but also in the form of a protein molecule, depending on which amino acid remains (hydrophilic or hydrophobic) most-

ly remained in a bound state. In this regard, the task of this work was to study the influence of finely dispersed grinding on the mass fraction and the ratio of the polar (hydrophilic) and nonpolar (hydrophobic) remains of biopolymers amino acids, as well as conformational changes of molecules of protein of the dried Jerusalem artichoke and the nanopowders made of it.

To detect conformational changes of protein molecules at obtaining finely-dispersed nanopowders from Jerusalem artichoke is possible by the method of H. E. Fischer. To do this, one must define a mass fraction of the bound and free amino acids in the original raw materials – Jerusalem artichoke and the nanopowders made of it (Table 1). Then the mass fraction of the amino acids that are in a bound state is to be calculated per 100 g of protein. At the same time, to perform the division of amino acid into hydrophilic and hydrophobic remains, to determine their sums and ratio between the sum of the hydrophilic amino acids remains and the hydrophobic ones. In addition, to calculate by the known coefficients the degree of hydrophobicity of bound amino acids of protein. It is shown that during cryomechanodestruction, the destruction of the protein molecules into individual amino acids occurs (by 45...55 %), i. e., a partial destruction of the protein molecules occurs and the transformation of bound amino acids into easily digestible form (Table 1).

It was found that the hydrophilic and hydrophobic properties of dried Jerusalem artichoke and the nanopowder made of it are significantly different. For example, the mass fraction of the hydrophilic remains of amino acids of the Jerusalem artichoke nanopowder exceeds by 12.6 % the original dried Jerusalem artichoke. Accordingly, the mass fraction of hydrophilic amino acids remains of 100 g of protein of the nanopowder is 45.25 while in the raw materials – 36.15 g. decreases The mass fraction of hydrophobic amino acids remains in the nanopowder decreases in parallel (by 8.6 %). Accordingly, the mass fraction of hydrophobic amino acid remains in 100 g of protein of the nanopowder is 54.75 g, in the original raw material – 63.85 g. In this case, the degree of hydrophobicity of the bound amino acids of protein ( $\Delta F$ , kJ/mol) of the finely dispersed powder made of Jerusalem artichoke decreased by 10 %.

It is shown that after the finely dispersed grinding, the ratio of the sum of the polar to the sum of non-polar remains in the protein molecules of the nanopowder made of Jerusalem artichoke, in comparison with the original raw material, increases from 0.57 to 0.83. This testifies to the increase in the surface area of hydrophilic membrane of a protein molecule and parallel reduction of the molecule nucleus filling by hydrophobic remains. Using the resulting ratio of polar and nonpolar amino acid remains in a protein molecule, according to the method of H. E. Fischer, the radius, the volume and the shape of a protein molecule was calculated, as well as the indicator of nucleus filling with hydrophobic remains.

It was found that comprehensive action of cryogenic «shock» freezing and low-temperature grinding on the plant raw materials (Jerusalem artichoke) leads to a decrease in the radius, the volume of a protein molecule, the radius and the indicator of nucleus filling with hydrophobic remains (Table 2).



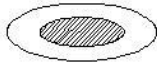

Table 1

Influence of cryomechanodestruction on the destruction of the protein molecules and the transformation of bound amino acids to free ones when obtaining nanopowders

Amino acid	Mass fraction of protein amino acids, %		ΔF, kJ/mol	Degree of hydrophobicity of bound amino acids of protein (ΔF, kJ/mol)	
	dried Jerusalem artichoke	Finely-dispersed nanopowder from Jerusalem artichoke		dried Jerusalem artichoke	Finely-dispersed nanopowder from Jerusalem artichoke
Hydrophilic amino acid remains					
Aspar acid	8,85	11,99	2,26	20,09	27,09
Alanine	3,39	4,13	3,05	10,34	12,59
Glutamic acid	10,28	13,22	2,50	25,70	33,05
Arginine	6,39	7,84	3,05	19,48	23,91
Threonine	2,02	2,37	1,84	3,72	4,36
Cistin	1,10	1,15	2,71	2,98	3,12
Serin	2,10	2,54	0,17	0,36	0,43
Glycine	2,02	2,01	0,0	0,00	0,00
Total:	36,15	45,25	–	82,67	104,55
Hydrophobic amino acid remains					
Lysine	8,68	9,14	6,27	54,23	57,37
Methionine	4,78	5,09	5,45	25,94	27,80
Tryptophan	0,89	1,20	12,50	10,88	15,13
Valine	3,77	3,94	7,06	26,51	27,88
Phenylalanine	6,36	7,04	11,10	70,37	78,26
Isoleucine	8,33	5,72	12,40	102,92	71,05
Leucine	7,50	5,70	10,10	75,45	57,67
Tyrosine	9,39	6,14	12,00	112,44	73,80
Proline	2,52	2,79	10,85	27,13	30,49
Histidine	11,63	7,99	5,85	67,86	46,80
Total:	63,85	54,75	–	573,73	486,25
Hydrophobic and hydrophilic amino acids remains					
Total:	100,0	100,0	–	656,40	590,80
Ratio of the sum of hydrophobic and hydrophilic amino acids remains	0,57	0,83	–	–	–

Table 2

Effect of comprehensive cryogenic «shock» freezing, drying and finely dispersed grinding on the conformational changes of protein molecules of the original Jerusalem artichoke during obtaining of nanopowder from it

Indicators	Jerusalem artichoke	
	original dried Jerusalem artichoke	nanopowder from Jerusalem artichoke
Content of polar amino acids remains, C <sub>n</sub>	36,15	45,25
Content of non-polar amino acids remains, C <sub>nn</sub>	63,85	54,75
Ratio C <sub>n</sub> / C <sub>nn</sub>	0,57	0,83
Radius of the globule, r <sub>o</sub> , microns	0,2705·10 <sup>-2</sup>	0,1816·10 <sup>-2</sup>
Radius of the globule, r, microns	0,3275·10 <sup>-2</sup>	0,2304·10 <sup>-2</sup>
Volume of the globule, V, mkm <sup>3</sup>	0,012·10 <sup>-5</sup>	0,074·10 <sup>-6</sup>
Indicator of molecule nucleus filling with hydrophobic, (b) by the schedule	1,48	0,26
The shape of protein molecule	 elongated ellipsoid	 supramolecular structures

It was found that in this case a change in the form of the protein molecules of the original raw materials occurs. For example, the radius of the protein molecule of finely-dispersed nanopowder made of Jerusalem artichoke is by 30 % less than the radius of the protein molecule of the dried Jerusalem artichoke (the original raw material) and it is  $0,2304 \times 10^{-2} \mu\text{m}$  (compared to  $0,3275 \times 10^{-2}$  in the original raw material), and its volume is by 1.7 times less and it is  $0,074 \times 10^{-6} \text{mkm}^3$  compared to  $0,012 \times 10^{-5} \text{mkm}^3$  in the source raw materials. The radius of the nucleus of the molecule is reduced by 1.5 times and the index of nucleus filling with hydrophobic remains – by 5.7 times (Table 2). The obtained data allowed us to establish the shape of a protein molecule according to the method of Fischer, raw materials and nanopowders. It is shown that protein molecules of the dried Jerusalem artichoke have the shape of elongated ellipsoids (Table 2), and while obtaining nanopowders, they acquire the shape of supramolecular structures. This itestifies to the fact that when obtaining the nanopowders from Jerusalem artichoke, the total surface area of the protein globules, which take the form of supramolecular structures is much larger than the surface area of protein molecules of the original raw materials in the form of elongated ellipsoids. This helps a larger capability for assimilation by the body, increasing the solution of proteins and the ability to gel formation. The obtained results will make it possible to imagine in a new way the impact of processes of deep processing of raw materials using cryogenic «shock» freezing and finely dispersed grinding on the conversion and transformation of biopolymers of plant raw materials to the instant nanoform.

Thus, it is shown that the use of finely dispersed mechanical grinding when obtaining nanopowders from Jerusalem artichoke leads to mechanodestruction and destruction of protein biopolymers, their larger availability for assimilation by the body, increasing the solubility of proteins and the larger capacity to gel formation.

The next task of this work was to study the influence of processes of cryomechanodestruction (cryogenic freezing and low temperature finely-dispersed grinding) on activation, extraction and transformation of pectins of Jerusalem artichoke to soluble active form, i. e., a fuller extraction of bound forms of pectins from associates and their nanocomplexes with biopolymers to free, active form. It should be noted that in the plant raw materials, including Jerusalem artichoke, pectin substances are in inactive form. In this regard, they have low gelling and adsorption properties. This is due to the fact that most carboxyl groups of polysaccharide chain of pectin in the plant raw materials have already been bound with either ions of metals (mostly with Mg and Ca) or the remains of methyl and ethyl alcohols. In addition, other polymer (arabans and galactans) and monomer molecules of polysaccharides and others inhibit access to the carboxyl groups of pectin. The methods that exist today of activation of extraction of pectins from nanocomplexes and nano associates of fruits, vegetables did not produce the desired results.

In this regard, of significant theoretical and practical interest is the development of technologies of plant supplements, including Jerusalem artichoke, with activation of pectins and obtaining dietary supplements with increased gelling properties and sorption abilities, which will make it possible to better utilize native properties of entire carbohydrate complex of raw materials as structure-creators, thickeners and detoxicants.

In this work we found that at high (2, 5, 10, 20 °C/min) and slow (0.1; 0.2; 0.5 °C/min) speeds of freezing to various

final temperatures in the product (in particular,  $-18...-20$  °C) and to lower temperatures in the product  $-32...-35$  °C, with further finely dispersed grinding (using the processes of cryomechanodestruction and cryomechanoactivation) of Jerusalem artichoke, the fuller extraction of pectin occurs from the bound state with other biopolymers and nanocomplexes to free active form (soluble form) (Table 3). It was revealed that a significant degradation and cryodestruction of protopectin occurs with its transformation from inactive to active soluble form. Thus, it was found that when obtaining nanopuree from Jerusalem artichoke, the fuller extraction of mass fraction of pectins from the nanocomplexes takes place, by 3,0...3,4 times larger than in the original raw materials, including protopectin (by 2 times) and its significant transformation to the soluble pectin (4.5 times larger). In general, 70 % of pectins in the nanopuree and nanopowder made of Jerusalem artichoke are in soluble form.

When obtaining nanopowders from Jerusalem artichoke, the same patterns of activation and transformation of sparingly soluble nanocomplexes of pectins to soluble form take place, as when obtaining the frozen nanopuree.

Thus, as a result of the experiments, we found that the use of cryofreezing, cryomechanodestruction and cryomechanoactivation processes leads to a full removal of pectins from inactive form to active, i.e., from a bound state in the nanocomplexes with other biopolymers to free soluble form (by 3.0–3.4 times larger than in the original raw materials) and transformation (or destruction of protopectin) to the soluble form (by 4.5 times larger than in the original raw materials). The mechanism of the fuller extraction of pectins from nanocomplexes and nano associates of plant raw materials is linked to their cryomechanocracking (destruction) and non-fermentative biocatalysis – cryomechanolysis.

It is known that soluble pectins are more highly-methoxylated and increase the degree of esterification and the amount of formation of hydrogen and ionic bonds. In this regard, it can be assumed that the gelling properties as well increase of finely dispersed frozen supplements from fruits that are manufactured using cryogenic freezing and the processes of mechanoactivation and mechanodestruction.

The obtained data enable to imagine anew the activation process and the fuller extraction of pectin substances from inactive hidden form to a soluble, easily digestible, form, which makes it possible to better use biological potential inherent in the plant raw materials.

The received nanosupplements (frozen nanopuree and nanopowders) from Jerusalem artichoke are in the nanoform in comparison with traditionally ground additives. In this regard, one could assume that their digestibility and biological activity can be significantly better and differ from traditional additives. In this regard, the task of this work was also to study the influence of cryotreatment of raw materials, finely dispersed grinding, the processes of cryodestruction on the biological activity (degree of digestibility) of the frozen nanopuree and nanopowders from Jerusalem artichoke in comparison to traditionally ground raw materials using the express method of biotesting.

As the objects of the study, we used:

- coarsely-ground supplements from fresh and dried Jerusalem artichoke with particle size 50... 250 microns;
- finely-dispersed frozen nanopuree and nanopowders.

In this case, the concentration of soluble and insoluble dietary and biologically active substances was controlled in parallel in the studied incubation live test systems (Fig. 4).

Table 3

The influence of cryogenic «shock» freezing and finely dispersed grinding of Jerusalem artichoke on the activation of sparingly soluble nanocomplexes of pectins and their transformation from inactive to active soluble form while obtaining the supplements in the form of nanopuree and nanopowder

Indicator name	Fresh Jerusalem artichoke	Frozen pieces of Jerusalem artichoke	Frozen finely-dispersed puree from Jerusalem artichoke	Dried pieces of Jerusalem artichoke	Finely-dispersed nanopowders from Jerusalem artichoke
pectin substances, %	1,9	2,7	6,5	10,8	30,0
protopectin, %	1,2	1,2	2,0	4,8	10,4
soluble pectin, %	0,7	1,5	4,5	6,0	23,0
organic acids, %	0,4	0,6	1,0	2,4	4,0

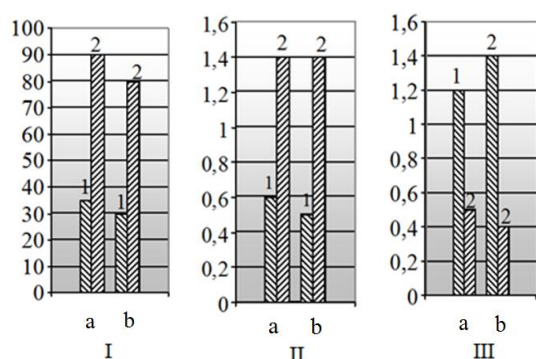


Fig. 4. Influence of the degree of finely dispersed grinding and cryodestruction while obtaining frozen nanopuree and nanopowders of Jerusalem artichoke on the generative activity of parametsiy (growth of young form, %) (I) and concentration of soluble (II) and insoluble substances (III) in biotest-systems; 1 – coarsely ground puree and powders; 2 – nanopuree (a) and nanopowders (b)

Comparison of generative activity in the test-systems of ciliates with the use of coarsely ground puree and powders and nanopuree and nanopowders from Jerusalem artichoke showed that the use of nanoadditives leads to a significant increase in generative activity by 2.7...3 times in comparison with coarsely ground (traditional foods). Thus, the growth of young forms in the test-systems of ciliates with coarsely ground supplements from Jerusalem artichoke amounted to 30...35 %, with finely ground nanoadditives – 85...90 %. It is shown that by using nanoadditives, the incubation system receives 2...2.4 times more of soluble substances and less – sparingly soluble (also by 2...2.3 times less).

Therefore, using the method of biotesting of the test-cultures of ciliates (one-cell by generative activity) shows that, in comparison with the coarsely dispersed ground Jerusalem artichoke, the assimilation of nanopuree and nanopowders from Jerusalem artichoke is better by 2.7...3 times. This is due to the higher removal (extraction) of soluble biologically active and food substances from the raw materials that are in the nanosoluble form at finely dispersed grinding.

Thus, to provide the human body with biologically active and food substances, Jerusalem artichoke is better to be consumed as a finely dispersed puree, in which all consumer

substances are in easily digestible form, than traditionally crushed fresh Jerusalem artichoke. In addition, the obtained results indicate that there occurs the fuller use of biopotential, inherent to the plant raw materials.

It was found that supplements from Jerusalem artichoke (nanopuree and nanopowders) by their chemical composition, content and dispersed state exceed the known world analogues and have a fundamentally new chemical composition than those obtained by traditional technology. A significant part of the substances (60...70 %) is in the nanosoluble form (Table 4).

Thus, for example, sparingly soluble biopolymers (proteins, inulin, cellulose) of Jerusalem artichoke transformed by 45.0...55.0 % to soluble form in the form of separate monomers (fructose, free  $\alpha$ -amino acids, glucose), which have a nanodimensional form. Nanopowders differ from analogues by high fructose (up to 25.0 %) and fruitoligosaccharides content. In addition, they are different in high content of low molecular phenolic compounds (5 times higher than in analogues), nanopowders contain 9...10 times more of flavonols glycosides than their analogues and 2.5...6 times more of tannins.

Thus, the use of cryomechanodestruction (cryogenic freezing and finely dispersed grinding) enables to obtain qualitatively new supplements in the form of frozen nanopuree and nanopowders from Jerusalem artichoke with a record content of BAS and biopolymers in the easily digestible nanoform that cannot be obtained by traditional methods of processing plant raw materials. According to the chemical composition, the new supplements (frozen nanopuree and nanopowders) from Jerusalem artichoke have potential prebiotic, immunomodulatory, antitumor and detoxic effect.

The obtained experimental data, presented in the article, were used as the base (foundation) in the development of cryogenic nanotechnology from Jerusalem artichoke in the form of frozen nanopuree and nanopowders.

New technologies were verified in the manufacturing process at NPP «KRIAS» (Kharkiv, Ukraine) and NPP «FIPAR» (Kharkiv, Ukraine), regulatory documentation was developed (TU U 15.3-01566330-304 and TI). Based on them, new kinds of health improving products were designed (dry instantly soluble fruit nanodrinks «Instant», dry juices (including of special purposes)), confectionery, new kinds of nanoicecream, biokefirs and bioyogurts with prebiotic properties, etc.).

Table 4

Content of biologically active and prebiotic substances (inulin, pectin, protein, phenolic and polyphenolic compounds) in nanopuree and nanopowders from Jerusalem artichoke in comparison with analogues (n=3)

Indicator name	Fresh Jerusalem artichoke	Nanopuree from Jerusalem artichoke	Nanopowder from Jerusalem artichoke	Analogue – powder from Jerusalem artichoke of convective vacuum drying (CVD)	Analogue – powder from Jerusalem artichoke of convective drying
Inulin, %	12,8±0,5	6,4±0,1	25,6±1,5	9,75±0,1	20,1±1,3
Fructose, %	–	7,4±0,2	25,6±1,5	0,0	0,0
Protein, %	1,2±0,01	1,4±0,1	9,1±0,2	8,9±0,1	8,5±0,1
Bound protein amino acids, mg in 100 g	1664,0	925,0	3698,0±0,2	–	–
Free protein amino acids, mg in 100 g	350,0	1353,0	5415,0±0,2	–	–
Basic pectin, %	1,9	6,5	30,0	9,2±0,1	8,0±0,1
Protopectin	1,2	2,0	10,4	–	–
Soluble pectin, %	0,7	4,5	23,0	–	–
Basic sugar, %	4,4±0,1	5,6±0,2	23,7±1,4	10,2±0,2	12,6±0,2
Vitamin C, mg in 100 g	10,3±0,1	19,8±0,5	78,2±2,4	16,4±1,1	12,2±0,3
Phenolic compounds (after chlorogenic acid), mg in 100 g	350,0±5,7	700,0±10,4	2800,0±12,4	640,0±10,2	520,0±12,4
Flavonol glycosides (by rutine), mg in 100 g	240,0±4,8	460,0±7,8	1800,0±12,4	200,0±5,2	162,0±2,6
Tannins, mg in 100 g	300,0±6,4	540,0±6,8	2160,0±14,0	840,0±10,2	360,0±11,7
Ash content, %	1,6±0,1	1,6±0,1	6,8±0,2	6,0±0,2	5,9±0,1
Organic acids, %	0,3±0,01	0,4±0,01	2,0±0,1	0,8±0,1	0,65±0,1
Moisture, %	76,4±1,2	75,5±0,1	5,5±0,1	7,9±0,1	7,3±0,1

## 6. Discussion of results of the study of influence of cryomechanodestruction on activation of heteropolysaccharide-protein nanocomplexes in the development of nanotechnologies of plant supplements

The influence was examined of cryomechanodestruction on activation of heteropolysaccharide-protein nanocomplexes that are contained by raw materials in inactive bound form, when developing nanotechnologies of plant supplements, particularly, puree and powders from Jerusalem artichoke.

The benefits of this research is that as a result of comprehensive use of cryotreatment and mechanodestruction of raw materials, the destruction of nanocomplexes and biopolymers occurs and their transformation from hidden bound form to soluble easily digestible form – the nanoform. In addition, the positive effect of the influence of the processes of cryomechanodestruction is that during cryogenic «shock» freezing, freeze drying and finely dispersed grinding of Jerusalem artichoke while obtaining nanopuree and nanopowders, not only preservation of all BAS is achieved, but also their fuller extraction from hidden bound forms of biopolymers (proteins, polysaccharides, oligosaccharides, etc.), which are in the form of nanocomplexes and nano associates, occurs and their transformation to free state (soluble form). This enables to obtain plant supplements with fundamentally new chemical composition and high consumer properties, which, in turn, can be used in the development of functional health products of mass catering, such as dry instantly soluble fruit nanodrinks “Instant”, dry juices, confectionery, new kinds of nanoicecream, biokefirs and bioyogurts with prebiotic properties, etc.

The shortcomings and peculiarities of the processing of Jerusalem artichoke to powders, syrups, purees may include availability in this raw material of active oxidative enzyme system (in particular, polyphenoloxidase, oxidase, etc.), which leads to its darkening. In this study, using such technological methods as cryogenic «shock» freezing with high speeds of freezing to lower temperatures in the product and finely dispersed grinding, this problem was solved. However, in future it is planned to search for other ways of inactivation of oxidative enzymes, namely, by regulating the medium pH, etc.

Development and continuation of research into this direction is to expand the range of products using the proposed frozen nanopuree and nanopowders from Jerusalem artichoke, in particular, design of products for special purposes (tourists, cosmonauts, submariners, soldiers of ATO zone, etc.). In addition, of interest is further conducting of microbiological, spectroscopic, chromatographic studies of new types of products and supplements, as well as exploring their compatibility with other food ingredients, selection of doses and technological modes of introduction of inulin containing plant supplements.

## 7. Conclusions

1. It was found that the freezing and cryomechanodestruction lead to non-fermentative biocatalysis – mechanolysis of protein molecules to separate monomers – free amino acids (45...55 %), their conformational changes. It was revealed that the ratio of the sum of hydrophilic to hydrophobic amino acids remains in protein globules of the nanopowders of Jerusalem artichoke, in comparison with the



original raw materials, increases by 40 %. This shows the increase in the surface area of hydrophilic membrane of protein globule and accompanying reduction of the nucleus filling with hydrophobic remains. It was also found that the radius, volume of protein globule, radius of the nucleus and the indicator of filling the nucleus with hydrophobic remains and form of a protein molecule are all reduced. We discovered the mechanisms of the indicated processes that are associated with mechanocracking.

2. It was found that with the comprehensive effect on Jerusalem artichoke from cryogenic «shock» freezing and finely dispersed grinding, there occurs activation of sparingly soluble inactive forms of pectins and their fuller extraction from nanocomplexes with other biopolymers by 3.0...3.4 times, including protopectin by 2 times larger than in the original raw material, which is controlled by traditional chemical methods and soluble pectin forms by

4.5 times more. In general, 70 % of pectins in the nanopowders and nanopuree are in soluble form. The mechanism of this process is linked with the non-fermentative biocatalysis – cryomechanolysis.

3. It was found that assimilation of nanosupplements (nanopuree and nanopowders) from Jerusalem artichoke is 2.7...3 times higher than the original raw material, which is determined by using the biotesting method of the test-cultures of ciliates (one-cell by generative activity) that is connected with peculiarities of chemical composition of the additives, BAS content and dispersed state. A significant part of the substances (60...70 %) is in the nanosoluble form.

4. With the use of nanosupplements, various kinds of health foods were developed (dry instantly soluble fruit nanodrinks “Instant”, dry juices (including for special purposes), confectionery, new kinds of nanoicecream, biokefirs and bioyogurts with prebiotic properties, etc.).

### References

1. FAO/WHO/UNU. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation [Text] // Food and agriculture organization of the united nations Rome. – 2013. – Vol. 92–57.
2. Kaprelyants, L. Prebiotics: chemistry, technology, application [Text] / L. Kaprelyants. – Kyiv: EnterPrint, 2015. – 252 p.
3. Gibson, G. Handbook of Prebiotics. Vol. 4 [Text] / G. Gibson, M. Roberfroid. – London: CRS Press, 2008. – P. 22–42. doi: 10.1201/9780849381829
4. Sousa, M. The importance of prebiotics in functional food and clinical practical [Text] / M. Sousa, E. Santos, V. Sgarbeeri // Food and Nutritional Science. – 2011. – Vol. 2, Issue 2. – P. 133–144. doi: 10.4236/fns.2011.22019
5. Roberfroid, M. Fructo-oligosaccharide malabsorption: benefit for gastrointestinal functions [Text] / M. Roberfroid // Curr Opinion Gastroenterology. – 2000. – Vol. 16, Issue 2. – P. 173–177. doi: 10.1097/00001574-200003000-00013
6. Pavlyuk, R., Pogarskaya, V., Pavlyuk, V., Radchenko, L., Yur'eva, O., Maksimova, N. (2015). Cryo- and Mechanochemistry in the food technology [Text] / R. Pavlyuk, V. Pogarskaya, V. Pavlyuk, L. Radchenko, O. Yur'eva, N. Maksimova. – Kharkov State University of Food Technology and Trade; Kharkov trade and economic Institute of Kyiv national University of trade and economy, 255.
7. Gaukel, V. Cooling and Freezing of Foods [Text] / V. Gaukel // Reference Module in Food Science. – 2016. – P. 1–3. doi: 10.1016/b978-0-08-100596-5.03415-6
8. Afoakwah, N. A. Characterization of Jerusalem artichoke (*Helianthus tuberosus* L.) powder and its application in emulsion-type sausage [Text] / N. A. Afoakwah, Y. Dong, Y. Zhao, Z. Xiong, J. Owusu, Y. Wang, J. Zhang // LWT – Food Science and Technology. – 2015. – Vol. 64, Issue 1. – P. 74–81. doi: 10.1016/j.lwt.2015.05.030
9. Kolida, S. Prebiotic effects of inulin and oligofructose [Text] / S. Kolida, K. Tuohy, G. Gibson // The British journal of nutrition. – 2002. – Vol. 87, Issue 2. – P. 193–197. doi: 10.1079/bjn/2002537
10. Galland, L. Functional Foods: Health Effects and Clinical Applications [Text] / L. Galland // Reference Module in Biomedical Sciences, from Encyclopedia of Human Nutrition. – 2014. – P. 366–371. doi: 10.1016/b978-0-12-375083-9.00130-6
11. Radovanovic, A. The use of dry Jerusalem artichoke as a functional nutrient in developing extruded food with low glycemic index [Text] / A. Radovanovic, V. Stojceska, A. Plunkett, S. Jankovic, D. Milovanovic, S. Cupara // Food Chemistry. – 2015. – Vol. 177. – P. 81–88. doi: 10.1016/j.foodchem.2014.12.096
12. Tu, J. Effects of different freezing methods on the quality and microstructure of lotus (*Nelumbo nucifera*) root [Text] / J. Tu, M. Zhang, B. Xu, H. Liu // International Journal of Refrigeration. – 2015. – Vol. 52. – P. 59–65. doi: 10.1016/j.ijrefrig.2014.12.015
13. James, S. J. Chilling and Freezing [Text] / S.J. James, C. James // Food Safety Management. – 2014. – P. 481–510. doi: 10.1016/b978-0-12-381504-0.00020-2
14. Balaz, P. Mechanochemistry in technology: from minerals to nanomaterials and drugs [Text] / P. Balaz, M. Balaz, Z. Bujnakova // Chemical Engineering & Technology. – 2014. – Vol. 37, Issue 5. – P. 747–756. doi: 10.1002/ceat.201300669
15. Zhao, X. Effect of superfine grinding on the physicochemical properties and antioxidant activity of red grape pomace powders [Text] / X. Zhao, H. Zhu, G. Zhang, W. Tang // Powder Technology. – 2015. – Vol. 286. – P. 838–844. doi: 10.1016/j.powtec.2015.09.025
16. 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, K. Balabai, S. Loseva // Eureka: Life Sciences. – 2016. – Vol. 3 (3). – P. 36–43. doi: 10.21303/2504-5695.2016.00145