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ВПЛИВ КРІОГЕННОГО ЗАМОРОЖУВАННЯ ТА НЕФЕРМЕНТАТИВНОГО КАТАЛІЗУ НА РУЙНУВАННЯ ІНУЛІН-БІЛКОВИХ НАНОКОМПЛЕКСІВ ТОПІНАМБУРА ДО МОНОМЕРІВ

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Вивчено вплив кріогенного «шокового» заморожування та неферментативного каталізу на руйнування інулін-білкових нанокомплексів до їх мономерів (відповідно залишків фруктози та амінокислот) під час отримання нанопюре з топінамбура. Установлено, що завдяки зазначеним процесам значна частина (45–55%) важкорозчинних та важкозасвоюваних нанокомплексів руйнується й трансформується в розчинну легкозасвоювану організмом людини форму.

Ключові слова: *кріогенне «шокове» заморожування, неферментативний каталіз, механодеструкція, топінамбур, інулін-білкові нанокомплекси, нанопюре.*

ВЛИЯНИЕ КРИОГЕННОГО ЗАМОРАЖИВАНИЯ И НЕФЕРМЕНТАТИВНОГО КАТАЛИЗА НА РАЗРУШЕНИЕ ИНУЛИН-БЕЛКОВЫХ НАНОКОМПЛЕКСОВ ТОПИНАМБУРА ДО МОНОМЕРОВ

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Изучено влияние криогенного «шокового» замораживания и неферментативного катализа на разрушение инулин-белковых нанокомплексов до их мономеров (соответственно остатков фруктозы и аминокислот) при получении нанопюре из топинамбура. Установлено, что благодаря указанным процессам значительная часть (45–55%) труднорастворимых и трудноусвояемых нанокомплексов разрушается и трансформируется в растворимую легкоусвояемую организмом человека форму.

Ключевые слова: *криогенное «шоковое» замораживание, неферментативный катализ, механодеструкция, топинамбур, инулин-белковые нанокомплексы, нанопюре.*

THE IMPACT OF CRYOGENIC FREEZING AND NON-ENZYMATIC CATALYSIS ON DESTRUCTION OF INULIN-PROTEIC NANOCOMLEXES OF TOPINAMBOUR TO MONOMERS

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The impact of cryogenic "shock" freezing and non-enzymatic catalysis on destruction of inulin-proteic nanocomlexes to their monomers (fructose and aminoacids) is studied during the obtaining of nanopuree from topinambour. It is determined that due to the mentioned processes the significant portion (45–55%) of these insoluble and hardly digestible substances is destructed and transformed to the soluble, easily digested form.

It was found that with the comprehensive effect on topinambour 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.

It was found that assimilation of nanosupplements (nanopuree and nanopowders) from topinambour 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.

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.).

Keywords: *cryogenic "shock" freezing, non-enzymatic catalysis, mechanodestruction, topinambour, inulin-proteic nanocomplexes, nanopuree.*

Statement of the problem. 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 cryomechano-destruction 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, β-carotene), phenolic compounds and mineral substances. These include indigestable 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 topinambour [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 topinambour 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 β-fructose bonds. Topinambour also contains pectin, fiber, protein, a wide range of minerals (potassium, calcium, manganese, etc.), vitamins (C, B_1, B_2 and others), phenolic compounds, etc.

Reviev of the latest researches and publications. Data analysis of the periodic literature over the past 10 years has shown that the existing technologies of processing topinambour 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 topinambour 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 topinambour 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 topinambour, 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 nanoassociates 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 nanoassociates 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 topinambour, 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 topinambour 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 topinambour 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, topinambour), 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.

The objective of the article. The purpose of research work is to study the impact of cryogenic freezing and non-enzymatic catalysis on destruction of inulin-proteic nanocomplexes to their monomers during the obtaining of nanopuree from topinambour. To achieve the set goal, the following tasks had to be solved:

– to study the impact 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 topinambour (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 topinambour 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 topinambour using the express method of biotesting;

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

Presentation of the research. The main thing in the development of nanotechnology of plant supplements from topinambour, 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 мechanolysis.

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 dispergate 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 topinambour 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) mostly 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 topinambour and the nanopowders made of it.

To detect conformational changes of protein molecules at obtaining finely-dispersed nanopowders from topinambour 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 – topinambour and the nanopowders made of it (table 1). Then the mass fraction of the amino acids that are in a bound state is to 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).

Table 1

Impact of cryogenic freezing and non-enzymatic catalysis on destruction of inulin-proteic nanocomlexes to monomers and the transformation of bound aminoacids to free ones when obtaining nanopowders of topinambour decreased by 10%

Amino acid	Mass fraction of protein amino acids, %		ΔF, kJ/mol	Degree of hydrophobicity of bound amino acids of protein (ΔF, kJ/mol)	
	dried topinam-bour	finely-dispersed nanopowder from topinambour		dried topinam-bour	finely-dispersed nano-powder from topinambour
Hydrophilic aminoacid 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 aminoacid 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 aminoacids remains	0,57	0,83	–	–	–

It was found that the hydrophilic and hydrophobic properties of dried topinambour and the nanopowder made of it are significantly different. For example, the mass fraction of the hydrophilic remains of amino acids of the topinambour nanopowder exceeds by 12,6% the original dried topinambour. 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 (ΔF, kJ/mol) of the finely dispersed powder made of topinambour decreased by 10%.

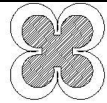
It is shown that after the finely dispersed grinding, the ratio of the sum of the polar to the sum of non-polar remains in the protein molecules of the nanopowder made of topinambour, in comparison with the original raw material, increases from 0,57 to 0,83. This testifies to the increase in the surface 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 (topinambour) 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).

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 topinambour is by 30% less than the radius of the protein molecule of the dried topinambour (the original raw material) and it is $0{,}2304 \cdot 10^{-2}$ μm (compared to $0{,}3275 \cdot 10^{-2}$ in the original raw material), and its volume is by 1,7 times less and it is $0{,}074 \cdot 10^{-6}$ mkm^3 compared to $0{,}012 \cdot 10^{-5}$ 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 topinambour 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 topinambour, 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.

Table 2

Impact of comprehensive cryogenic “shock” freezing, drying and finely dispersed grinding on the conformational changes of protein molecules of the original topinambour during obtaining of nanopowder from it

Indicators	Topinambour	
	original dried topinambour	nanopowder from topinambour
Content of polar amino acids remains, Cn	36,15	45,25
Content of non-polar amino acids remains, Cнп	63,85	54,75
Ratio $C_п / C_{нп}$	0,57	0,83
Radius of the globule, ro, microns	$0{,}2705 \cdot 10^{-2}$	$0{,}1816 \cdot 10^{-2}$
Radius of the globule, r, microns	$0{,}3275 \cdot 10^{-2}$	$0{,}2304 \cdot 10^{-2}$
Volume of the globule, V, mkm^3	$0{,}012 \cdot 10^{-5}$	$0{,}074 \cdot 10^{-6}$
Indicator of molecule nucleus filling with hydrophobic, (b) by the schedule	1,48	0,26
The shape of protein molecule	 elongated ellipsoid	 supramolecular structure

Thus, it is shown that the use of finely dispersed mechanical grinding when obtaining nanopowders from topinambour 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 topinambour 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 topinambour, 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 topinambour, 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 ºC) and to lower temperatures in the product –32…–35 °C, with further finely dispersed grinding (using the processes of cryomechanodestruction and cryomechanoactivation) of topinambour, 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 topinambour, 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 topinambour are in soluble form.

When obtaining nanopowders from topinambour, 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.

Table 3

The impact of cryogenic "shock" freezing and finely dispersed grinding of topinambour 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 topinambour	Frozen pieces of topinambour	Frozen finely-dispersed puree from topinambour	Dried pieces of topinambour	Finely-dispersed nano-powders from topinambour
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

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 topinambour 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 topinambour 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. 1).

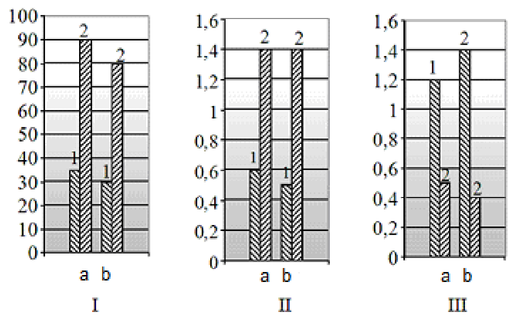


Fig. 1. Influence of the degree of finely dispersed grinding and cryodestruction while obtaining frozen nanopuree and nanopowders of topinambour on the generative activity of parametsiy (growth of young form, %) (I) and concentration of soluble (II) and insoluble substances (III) in biotest-systems; 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 topinambour 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 topinambour 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 topinambour, the assimilation of nanopuree and nanopowders from topinambour 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, topinambour is better to be consumed as a finely dispersed puree, in which all consumer substances are in easily digestable form, than traditionally crushed fresh topinambour. 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 topinambour (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 topinambour transformed by 45,0–55,0% to soluble form in the form of separate monomers (fructose, free α-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 topinambour 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 topinambour 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 topinambour in the form of frozen nanopuree and nanopowders.

New technologies were verified in the manufacturing process at NPP "KRYAS" (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 topinambour in comparison with analogues (n=3)

Indicator name	Fresh topinam-bour	Nanopuree from topinam-bour	Nano-powder from topinambour	Analogue – powder from topinambour of convective vacuum drying (CVD)	Analogue – powder from topinam-bour of convective drying
1	2	3	4	5	6
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 aminoacids, mg in 100 g	1664,0	925,0	3698,0±0,2	–	–
Free protein aminoacids, 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	–	–

Continuation of table 4

1	2	3	4	5	6
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

The impact 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 topinambour.

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 topinambour 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 topinambour 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 topinambour, 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.

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 topinambour, 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 topinambour 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 topinambour 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.).

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