

This paper reports the development of nanotechnology for processing vegetables (spicy and carotene-containing ones) into frozen cryoadditives – enrichers with biologically active substances (BAS), as well as natural protein health snacks containing them. An innovative method proposed for obtaining vegetable nano additives involves the use of deep processing of raw materials. The method is based on a complex effect exerted on raw materials by cryo-processing and cryomechanodestruction, making it possible not only to preserve the BAS of fresh vegetables but also to transform them into a nano-structured form in order to fully reveal the biological potential of the raw materials. The resulting nano additives made from vegetables have no analogs. The BAS mass fraction in the produced frozen cryoadditives is 3.0...3.2 times larger than that in the starting fresh raw materials. Vegetable-based cryoadditives that are used in the manufacture of snacks are not only the BAS carriers but also perform the functions of structure-forming agents, gel-forming agents, colorants, thereby making it possible to produce high-quality health products and eliminate the need for food additives.

Applying the vegetable-based frozen cryoadditives enrichers with biocomponents has made it possible to devise a new generation of natural protein snacks for healthy eating. The protein base used included nano additives made from legumes (peas) and soft salt cheese, which are distinguished by a high content of complete protein and are easily digestible. The new protein-vegetable snacks differ from conventional ones in the high content of β -carotene, phenolic compounds, tannins; 100 g of the product may satisfy about 30 % of the daily protein needs. The devised snacks are a new type of natural wellness products that are made without the use of artificial food additives and are recommended for industrial production

Keywords: processing of vegetables, cryoadditives, nano additives made from legumes, protein-vegetable snacks, health products

DEVISING NANOTECHNOLOGY FOR VEGETABLE CRYOFROZEN ENRICHERS WITH BIOCOMPONENTS AND THE NATURAL PROTEIN HEALTHY SNACKS CONTAINING THEM

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1. Introduction

The relevance of our study is predetermined by attempts to address a global problem of the reduced immunity of the population [1], caused by unbalanced nutrition [2], and a deficiency of biologically active phytochemicals [3] and protein in diets (2 times less than the norms recommended by the International Health Organization FAO/WHO) [1, 4].

There are also issues related to hunger [5]. Every eighth inhabitant of the globe is starving [6]. The problem is complicated by the general deterioration of the environmental situation [7] and the spread of the global pandemic related to COVID-19. It is possible to increase the immunity of the population by eating healthy foods with a high content of biologically active substances, in particular vitamins (especially, antioxidant series: C, E, β -carotene), chlorophyll

a and c, phenolic compounds, tannins, and minerals (especially potassium, iodine, selenium, etc.), complete proteins, α amino acids, and prebiotic substances (pectin, cellulose, inulin, etc.) [8]. The main source of those BASs is fresh and frozen fruits, berries, vegetables, medicinal and spicy-aromatic plant raw materials, as well as additives and products made from them [9].

2. Literature review and problem statement

It was established that one of the priority areas for resolving the issue of immunity reduction is the development of functional products [10] for health nutrition [6]. The main requirement for such products is the maximum naturalness, the presence of components and substances in the composition that contribute to health [11], as well as the absence of harmful food impurities for the human body [12]. The important products for immunoprophylaxis and strengthening the health of the population include combined products (primarily dairy and vegetables) [8]. The health properties of such products are due to a high content in their composition of complete protein, as well as biologically active phytochemicals of plant raw materials (vitamins, minerals, etc.) [13]. The base and the source of protein used in the manufacture of such products, along with skimmed milk powder and dry whey, increasingly include flour or additives made from legumes (primarily soybeans) [14] and nut crops [15]. Other types of legumes, such as peas, chickpeas, mash, have not yet been widely used, which requires additional fundamental and applied research. Vegetable additives made from various types of vegetables, fruits, spices in the form of powders, pastes, concentrates [16] are used to enrich protein products. In recent years, pasty protein snacks based on legumes and melted fermented milk cheeses have gained popularity among other combined products [16]. The main disadvantage of such products is the high fat content [17], low content of protein and BAS [18], as well as the presence in their composition of various types of food additives [19], responsible for organoleptic indicators and extended shelf life of the product [20].

The manufacture of protein snacks based on legumes (in particular peas, nut, etc.) and cheeses faces certain difficulties due to the following:

- a shortage of commercially available high-quality natural vegetable and vegetable protein fillers that could form the base for protein-vegetable snacks [8];
- the widespread use of various types of food additives (structure-forming agents, stabilizers [21], colorants, flavors, transgenic fats, etc.[22]), which can adversely affect the human body [16];
- the use of vegetable additives (pastes, powders, frozen pastes), manufactured according to conventional technologies, as natural enrichers, which have low quality in terms of their BAS and protein content [23];
- a short shelf life (28 days at a temperature of $+4...+6$ °C) [16].

These difficulties in the production of snacks remain unresolved [17]. An option to overcome these difficulties in the manufacture of protein paste-like snacks for healthy eating can be the application of natural vegetable and protein plant raw materials and natural spices, which are known for their therapeutic and prophylactic properties. Among the promising types of plant raw materials are spicy vegetables (in particular sweet pepper, parsley root, garlic), as well as

carotene-containing vegetables (carrots, pumpkin, tomatoes), which are in great demand by the population [18]. This is due to their high taste properties, as well as the capability to improve the defenses of the human body, to exert antitumor, detoxifying, bactericidal, anti-oxidative effect. These properties are due to the presence in their composition of unsaturated conjugated low-molecular phenols and tannins, carotenoids, aromatic substances, and L-ascorbic acid [19]. In addition, to solve the task of manufacturing protein herbal snacks for healthy eating, it is necessary to produce unique vegetable supplements, BAS enrichers, with a high content of those substances. New additives should not only carry BAS but also simultaneously act as structure-forming agents, colorants, flavorings, etc. That would reduce or eliminate the need to use artificial food additives in the manufacture of snacks.

The main disadvantage of those vegetables is their seasonality and short shelf life. Difficulties in processing vegetables into food products using conventional methods are associated with significant BAS losses (from 20 to 80 %) [24]. One of the promising areas in the processing of fruit and vegetable raw materials and obtaining health products is freezing [25]. The most effective freezing technique, which ensures a high degree of preservation of vitamins and other BASs, is a rapid “shock” freezing by cold airflow, followed by storing the frozen food at a temperature of -18 °C [26]. The disadvantage of freezing fruits and vegetables by the flow of cold air is a significant loss of vitamins and cell juice when defrosting frozen foods after storing for 6 months [27]. Currently, the leading countries increasingly use cryogenic “shock” freezing, which is characterized by the application of cryogenic liquids (liquid and gaseous nitrogen, carbon dioxide) [25]. The data from the scientific literature addressing the quality of frozen products of plant and animal origin were analyzed in [28]. The authors considered the influence of freezing temperature regimes on the texture, organoleptic, thermophysical indicators, and BAS content, regarding losses in them [29].

It was found that the higher the freezing rate, the less BASs are lost [30], as well as cell juice [31], in fruits and vegetables during defrosting [32], and the lower the cell structure destruction [33].

It should be noted that the scientific and technical literature contains little information regarding the influence of cryogenic processing, both at “shock” freezing and at finely-dispersed grinding [34]. The biochemical, microbiological, physicochemical processes, the processes of cryodestruction, cryomechanodestruction during the cryo-freezing and grinding of fruits, vegetables, as well as purees made from them [35], have not been studied in detail. The exception is the results of fundamental and applied research in this area, which we reported in [18].

Thus, the issues related to making high-quality health protein snacks and nanostructured plant-based supplements-fillers for their enrichment remain unresolved. This requires in-depth fundamental and applied research.

3. The aim and objectives of the study

The purpose of this study is to devise nanotechnology for processing spicy and carotene-containing vegetables into cryo-frozen additives – BAS enrichers, involving the application of cryogenic “shock” freezing and non-fermentative

catalysis during grinding. That would make it possible to produce a new generation of natural protein health snacks that contain them.

To accomplish the aim, the following tasks have been set:

- to study the comprehensive effect of cryo-processing involving the use of liquid and gaseous nitrogen and finely-dispersed low-temperature grinding of spicy and carotene-containing vegetables on the activity of oxidative enzymes, preservation of BAS; to design the nanotechnology of vegetable cryo-frozen additives-enrichers;

- to determine the chemical composition of nano additives made from dried peas as the base for protein snacks, in particular the mass fraction of protein, its amino acid composition, the bound and free soluble α amino acids, and to study their quality compared to analogs;

- to determine the chemical composition for the content of protein, the bound and free amino acids of soft salted cheese as the protein base for the manufacture of healthy pasty snacks; to study the effect of freezing and mechanical destruction on the destruction of casein-phosphate-protein lipid complex;

- to devise a new generation of natural protein health snacks that are based on the protein nano additives made of peas and soft cheese, as well as cryo-frozen additives-BAS enrichers made from spicy and carotene-containing vegetables and natural spices.

4. Materials and methods used in the development of nanotechnology for vegetable cryoadditives – enrichers, and protein snacks containing them

4.1. Materials and equipment used in the experimental study

Our research was conducted at Kharkiv State University of Nutrition and Trade (KSUNT, Ukraine), Department of Food Technologies of Products from Fruits, Vegetables, and Milk, and Innovations in Wellness Nutrition. The experimental study was carried out at this department's research laboratory "Innovative cryo- and nanotechnology of plant-based supplements and health products".

The study results reported here are a continuation of the work "Creation and implementation of advanced technologies and effective equipment for obtaining new functional wellness products".

The materials to be examined in the development of the nanotechnology of vegetable cryoadditives, enrichers with biocomponents, were spicy and carotene-containing vegetables (sweet pepper, parsley root, garlic, carrot, pumpkin, tomato).

The base for the natural protein health snacks was nano additives made from peas, in the form of puree and powder, and soft salt cheese. Nano additives from cheese were made using the processes of non-fermentative catalysis of a casein-calcium-phosphate complex to manufacture easily digestible products.

To prolong the shelf life, the composition of snacks was supplemented with the formulation components that included natural spices in the form of powders and water-alcohol extracts (rosemary, oregano, black pepper, nutmeg). It has been shown that these types of powders and water-alcohol extracts from natural spices act as phytoncides with bacteriostatic, bactericidal, and fungicidal properties, as well as antioxidant action [20].

Our research was carried out using different types of equipment. Cryogenic freezing involved a cryogenic fast-freezing apparatus using liquid and gaseous nitrogen as a refrigerant [9]. For finely-dispersed grinding, the homogenizers-shredders "Robot Couper" and an innovative food processor "ThermoMix", manufactured in France [18], were used. Steam thermal treatment in the manufacture of protein snacks was carried out using modern Italian-made equipment – UNOX steam-convection furnace of the XVC series [9].

4.2. Research methods used in the development of nanotechnology and when devising natural protein snacks

The criteria for assessing the quality of the raw materials and products were the following:

- protein (according to the content of total nitrogen in the samples studied), identified by the Kjeldahl method;

- free and bound amino acids, detected by the chromatographic method followed by the calculation of the peak area of each amino acid according to the external standard method;

- organic acids, identified by titrimetric method, based on neutralization of acids contained in the product with NaOH solution in the presence of phenolphthalein;

- dry substances, detected by drying the batch to a constant mass.

We determined the quality of the raw materials and additives made from spicy and carotene-containing vegetables and natural spices in terms of BAS content, in particular:

- β -carotene, using a colorimetric method of Muri after extracting carotene from the product with organic solvent and purifying carotene from related dyes using column chromatography;

- L-ascorbic acid, by visual and potentiometric titration with a solution of 2,6-dichlorophenolindophenolate Na;

- low-molecular phenolic compounds (for routine and chlorogenic acid separately), using a colorimetric method of Folin-Denis recalculated for routine and, separately, for chlorogenic acid;

- polyphenolic compounds, using a titrimetric method based on the properties of polyphenolic compounds to oxidize in the presence of the indicator indigo carmine, the calculation of tanning substances was carried out in terms of tannin;
- aromatic volatile substances, using a titrimetric method by the number of flavors.

We treated the results from the experimental study by using methods of mathematical processing employing the software Mathcad and Microsoft Excel.

5. Development of the nanotechnology for vegetable cryoadditives-enrichers and protein snacks containing them

5.1. Determining the effect of cryo-processing and finely-dispersed grinding on the oxidative enzymes and biologically active substances of spicy and carotene-containing vegetables

In the development of the nanotechnology for vegetable cryo-frozen additives-enrichers from spicy and carotene-containing vegetables (CCV) in the form of frozen finely dispersed puree, we applied cryogenic "shock" freezing and finely-dispersed low-temperature grinding. The cryogenic "shock" freezing differs from conventional techniques

by high freezing rate (from 1 to 20 °C/min) and lower end temperatures in a frozen product (from –32 to –35 °C). The freezing modes of various fruits or vegetables were determined depending on their architectonics, chemical composition, the enzymatic activity of oxidative enzymes, a slicing or finely dispersed grinding technique, etc. The conventional “shock” freezing involves freezing a product to –18 °C inside it. The finely-dispersed grinding of frozen vegetables was performed at a temperature not higher than –10 °C.

We investigated patterns in the enzymatic processes at the cryogenic “shock” freezing and finely-dispersed grinding of sweet pepper, a mixture of carotene-containing vegetables (carrot, pumpkin, and tomato), as well as parsley roots (Table 1).

It was established that enzymatic processes in vegetables during cryogenic “shock” freezing at different freezing speeds and to different end temperatures occur in products differently (Table 1). We detected the activation of oxidative enzymes (peroxidase and polyphenol oxidase) in vegetables when using different freezing speeds to a temperature of –18 °C. The activation of oxidative enzymes was

1.3...1.4 times higher than in the starting vegetables (Table 1). It was established that at the subsequent finely-dispersed grinding of frozen vegetables, their activity was 2.5...4.0 times higher than that in the fresh vegetables. It was found that at cryogenic “shock” freezing involving high speeds (in particular, 2 °C/min) to a temperature inside the product of –32...–35 °C, complete inactivation of oxidative enzymes occurs (Table 1). The mechanism of this process is associated with the denaturation and destruction of the protein part of enzymes and the inactivation of the active centers of enzymes. The study results were taken into consideration in the development of the nanotechnology for nano additives made from spicy and carotene-containing vegetables.

The influence of cryo-processing and fine-dispersed grinding of spicy and carotene-containing vegetables on the content of BAS during the manufacture of cryo-frozen additives was studied. In the raw materials and resulting cryoadditives, the mass fraction of L-ascorbic acid, β-carotene, phenolic compounds, flavonol glycosides, polyphenols (Table 2) was determined.

Table 1

Influence of the high rates of cryogenic “shock” freezing, the end temperature in frozen vegetables, and finely-dispersed additives made from them on the activity of oxidative enzymes

Product	Activity			
	peroxidase		polyphenol oxidase	
	ml 0.01 N iodine to DM	% of the starting raw materials	ml 0.01 N iodine to DM	% of the starting raw materials
A mixture of carotene-containing vegetables (carrot, pumpkin, and tomato)				
Fresh vegetables (starting raw materials)	35.6	100.0	30.4	100.0
Frozen to –18 °C	49.2	138.2	40.1	132.1
Crypaste made from a mixture of CCV frozen to –18 °C	89.0	250.0	99.0	301.0
Cryogenically «shock» frozen to –35 °C	0	0	0	0
Crypaste from the CCV «multicarotene» frozen to –35 °C	0	0	0	0
Sweet pepper				
Fresh (starting raw material)	55.0	100.0	42.4	100.0
Frozen to –18 °C	74.3	135.2	58.8	140.1
Crypaste from sweet pepper frozen to –18 °C	209.0	380.0	147.0	350.0
Cryogenically «shock» frozen to –35 °C	0	0	0	0
Crypaste from sweet pepper frozen to –35 °C	0	0	0	0
Parsley root				
Fresh (starting raw material)	29.5	100.0	25.4	100.0
Frozen to –18 °C	41.3	140.0	35.0	138.0
Crypaste from parsley root frozen to –18 °C	94.4	320.0	71.1	280.0
Cryogenically «shock» frozen to –35 °C	0	0	0	0
Crypaste from parsley root frozen to –35 °C	0	0	0	0

*Note: the high freezing rate of –2 °C/min was used in the experiments

Table 2

Effect of cryo-processing and finely-dispersed grinding of spicy and carotene-containing vegetables on the content of biologically active phytochemicals

Product	Mass fraction, mg per 100 g				
	L-ascorbic acid	β-carotene	phenolic compounds (for chlorogenic acid)	flavonol glycosides (for rutin)	polyphenolic tannins (for tannin)
Fresh sweet pepper	310.0...335.0	5.0...5.4	132.0...150.1	28.5...32.4	118.3...126.2
Cryporee of pepper	720.2...800.4	12.8...15.5	204.2...210.6	41.0...46.3	190.2...195.4
A mixture of fresh CCV (carrot, pumpkin, tomato)	18.2...20.4	8.2...10.3	170.1...182.3	48.2...51.4	240.3...250.2
Cryporee from CCV «multicarotene»	40.1...45.6	30.5...32.4	295.3...304.2	108.2...125.4	390.1...425.3
Fresh parsley root	28.4...30.1	0.2...0.3	305.0...321.4	50.6...56.7	380.2...385.4
Cryporee from parsley	58.6...62.5	0.3...0.4	506.2...529.2	82.7...90.3	556.0...570.2

We have revealed an error in the generally accepted ideas regarding the amount of (from 5 to 10 %) hidden, inactive (bound in nanocomplexes with biopolymers) low-molecular BAS (β -carotene, ascorbic acid, phenolic compounds, etc.) in vegetable raw materials. It has been shown that the application of the cryogenic “shock” freezing to spicy and carotene-containing vegetables to a temperature of $-32...-35\text{ }^{\circ}\text{C}$ makes it possible to additionally extract the previously unknown forms of BAS from raw materials into a free form. Compared to fresh raw materials, the mass fraction of those BASs in cryo-frozen vegetables is 2.0...2.5 times larger. It is also shown that with the further finely dispersed crushing of cryo-frozen vegetables, the mass fraction of BAS in cryoadditives is larger by 3.0...3.2 times than that in fresh vegetables. We have revealed the mechanism of processes that result from the application of cryomechanodestruction processes, mechanocracking when processing vegetables, and their transformation into easily digestible form. The study results were used in the development of nanotechnology for vegetable cryo-frozen additives, which have no analogs in terms of BAS content. The resulting vegetable cryoadditives are recommended to be used in the production

of protein snacks as “five in one”: BAS carriers, structure-forming agents, gel-forming products, colorants, and flavoring.

5.2. Determining the chemical composition of the nano additives made from dried peas in terms of protein content, the content of bound and free amino acids

A new generation of natural protein wellness snacks for breakfasts has been developed using pea-based nano additives and the soft salt cheese-based nano additives as a protein base. When compared to conventional additives, they differ by the content of complete protein, 50...55 % of which are in the easily digestible form – certain monomers of α amino acids, the size of whose particles is from 1.0 to 1.5 nm (Table 3). The additives made of peas in the form of powder and puree are produced using nanotechnology based on the use of non-fermentative catalysis – mechanolysis, mechanodestruction, which make it possible to make additives from them in easily digestible nanoscale form.

It has been shown that the resulting nanopuree and pea-based nanopowders contain 21.5...23.0 % of complete protein, of which 51 % of amino acids are free and 49 % are in a bound state.

Table 3

The content of the mass fraction of bound and free amino acids in the protein of finely-dispersed nanopowders made from dried pea puree and nanopuree and the starting raw material – dried peas

Amino acid name	Amino acid mass fraction, %					
	bound			free		
	dried peas	finely-dispersed nanopuree made from dried peas in the form of		dried peas (starting raw material)	finely-dispersed additives made from dried peas in the form of	
	powder	puree		powder	puree	
Essential protein amino acids						
Lysine	1.91	1.10	0.43	0.15	0.98	0.48
Tryptophan	0.65	0.10	0.20	0.06	0.55	0.15
Phenylalanine+tyrosine	1.44	0.72	0.46	0.10	0.75	0.53
Valine	1.36	0.68	0.29	0.14	0.73	0.34
Threonine	0.96	0.43	0.22	0.09	0.54	0.25
Leucine	1.81	0.90	0.45	0.13	0.94	0.47
Isoleucine	1.05	0.48	0.29	0.10	0.59	0.28
Methionine	0.39	0.15	0.06	0.05	0.18	0.08
Total:	9.57	4.56	2.40	0.96	5.26	2.58
Non-essential amino acids of protein						
Asparagine acid	2.28	1.24	0.62	0.25	1.29	0.65
Arginine	2.15	1.62	0.80	0.16	0.70	0.15
Glutamine acid	3.98	1.80	1.01	0.43	1.80	0.22
Serine	1.10	0.49	0.26	0.11	0.52	0.32
Glycine	0.80	0.35	0.20	0.06	0.34	0.22
Histadine	0.75	0.30	0.15	0.05	0.40	0.42
Proline	0.95	0.44	0.45	0.10	0.42	0.32
Alanine	1.10	0.45	0.24	0.11	0.55	0.33
Cystine	0.70	0.30	0.16	0.05	0.48	0.19
Total:	13.75	6.97	3.48	1.32	6.50	2.67
In general:	23.28	11.53	5.88	2.28	11.76	5.25

5.3. Determining the chemical composition of soft brined cheese in terms of protein content, the content of the bound and free amino acids

We studied the amino acid composition of soft brined cheese, which, together with pea-based additives, was used as a protein base to produce natural protein snacks for healthy eating (Table 4). When using cheese, special mechanodestruction treatment was also applied.

Table 4

Effect of mechanodestruction processes on the content of the bound and free amino acids in the casein-calcium-phosphate complex of soft brined cheese when obtaining cheese mass in a nanoscale form

Amino acid name	Amino acid mass fraction, %			
	Bound		Free	
	Soft cheese	Cheese mass	Soft cheese	Cheese finely-dispersed mass
Essential protein amino acids				
Lysine	1.56	0.78	0.15	0.93
Tryptophan	0.95	0.48	0.09	0.56
Phenylalanine	1.08	0.52	0.11	0.63
Valine	1.10	0.49	0.11	0.60
Threonine	0.85	0.34	0.09	0.43
Leucine	1.55	0.62	0.16	0.78
Isoleucine	0.92	0.46	0.09	0.55
Methionine	0.70	0.35	0.07	0.42
Total:	8.71	4.04	0.86	4.90
Non-essential protein amino acids				
Tyrosine	1.20	0.60	0.12	0.72
Aspartic acid	1.22	0.62	0.12	0.74
Arginine	0.80	0.38	0.08	0.46
Glutamic acid	4.08	2.00	0.41	2.61
Serine	1.20	0.55	0.12	0.77
Histidine	1.32	0.66	0.13	0.79
Proline	1.92	0.92	0.19	1.09
Alanine	0.65	0.32	0.06	0.36
Cystine	0.20	0.10	0.02	0.12
Total:	12.59	4.90	1.30	7.7
In general:	21.30	8.94	2.16	12.6

It is shown that the soft brined cheese contains proteins in a bound form before special treatment. The application of mechanodestruction leads to the transformation of 50...55 % of the bound amino acids into a protein molecule in α amino acids that are in a free easily digestible form (Table 4).

5.4. Development of a new generation of natural protein health snacks

A new generation of natural protein health snacks has been developed. Nano additives made of peas and soft salt cheese were used as a protein base and the carriers of complete protein in easily digestible form. Frozen cryoadditives-BAS enrichers made from spicy and carotene-containing vegetables, as well as additives from natural spices in the form of powders and nano extracts, were used as an innovation to produce protein snacks. It has been shown that vegetable cryoadditives in the manufacture of snacks also perform the functions of natural structure-forming agents, gel-forming agents, colorants, while additives made from

natural spices – natural preservatives. The use of these additives made from spicy and carotene-containing vegetables, as well as additives made from natural spices, makes it possible to obtain high-quality health products and eliminate the need for harmful food additives. Protein snacks are in a nanoscale form and differ from analogs by the high content of β -carotene, phenolic compounds, tannins; 100 g of the product can satisfy about 30 % of the daily protein needs (Table 5).

Table 5

The content of protein and biologically active phytochemicals that strengthen the protective mechanism of the human body in natural wellness protein snacks

Name of the indicator, mg per 100 g	Name of health protein vegetable and dairy snacks			
	«Bohatyr»	«Karoton»	«Svitlyachok»	Analog
β -carotene	6.5 \pm 0.1	5.0 \pm 0.1	3.0 \pm 0.1	0
L-ascorbic acid	75.0 \pm 0.5	52.1 \pm 0.8	41.6 \pm 0.2	0
Phenolic compounds (for chlorogenic acid)	150.1 \pm 10.2	120.2 \pm 5.1	112.3 \pm 0.5	2.0
Phenolic compounds (for rutin)	28.2 \pm 0.4	25.4 \pm 0.1	22.6 \pm 0.1	0
Tannins (for tannin)	165.2 \pm 10.5	144.3 \pm 5.5	138.5 \pm 0.4	8.1
Protein, %	18.5 \pm 0.5	18.2 \pm 0.4	18.0 \pm 0.5	–
Free amino acids, %	10.5 \pm 0.1	11.2 \pm 0.3	11.0 \pm 0.4	–
Bound amino acids, %	8.0 \pm 0.1	7.0 \pm 0.2	7.0 \pm 0.3	–
Fat, %	7.5 \pm 0.1	6.0 \pm 0.1	5.0 \pm 0.1	28.0
Total pectin, %	1.1 \pm 0.1	1.0 \pm 0.05	1.1 \pm 0.05	0
Soluble pectin, %	1.0 \pm 0.1	0.9 \pm 0.05	0.9 \pm 0.05	0
Protopectin, %	0.1 \pm 0.05	0.1 \pm 0.05	0.1 \pm 0.05	0
Cellulose, %	0.5 \pm 0.05	0.6 \pm 0.05	0.5 \pm 0.05	0
Total sugar, %	4.5 \pm 0.1	4.2 \pm 0.1	5.0 \pm 0.2	0
Dry matter	70.0 \pm 0.5	68.1 \pm 0.5	65.2 \pm 0.5	38.5

Based on the content of the listed BASs, new snacks meet the criteria for healthy foods recommended by FAO/WHO and the Ministry of Health of Ukraine. The additives and snacks that we devised are completely natural and exceed analogs in terms of quality; they are recommended for industrial production.

6. Discussion of the study results in the development of the nanotechnology for vegetable cryoadditives and protein snacks containing them

A new generation of combined protein-vegetable snacks for healthy eating with a high content of healing natural biologically-active phytochemicals has been produced. They differ from conventional ones by the content, per 100 g of product, of plant-based β -carotene and vitamin C (from 0.5 to 1 daily need), tannins and phenolic compounds, as well as a low fat content (from 5 to 7 %). One serving (100 g) of the product can satisfy about 30 % of the daily needs of the human body in protein. The snacks we devised are new natural wellness products made without the use of food additives, their quality exceeds that of analogs.

The special feature of making healthy protein snacks is that nano additives made from peas and soft brined cheese are used as a protein base. The latter differ from conventional additives in that they contain complete protein, which 50...55 % is in easily digestible form – separate α amino acids, the particle size of which ranges from 0.5 to 1.5 nm. Protein additives were made using nanotechnology based on the processes of mechanodestruction, mechanoactivation, non-fermentative catalysis, which makes it possible to produce nano additives in a nanoscale form. In addition, protein nano additives made from peas, when manufacturing snacks, are not only the carriers of complete protein and free α amino acids but also simultaneously act as structure-forming agents, gel-forming agents. That makes it possible to obtain the finished product – high-quality protein snacks without the use of harmful food additives.

The feature of the proposed method for making protein snacks is the use of cryoadditives from spicy and carotene-containing vegetables as BAS enrichers and, at the same time, natural structure-forming agents, colorants, flavoring. The vegetable cryoadditives were obtained using cryogenic nanotechnology and have a record BAS content. It has been shown that the resulting vegetable cryoadditives, in terms of BAS content (β -carotene, L-ascorbic acid, phenolic compounds, etc.), exceed fresh vegetables by 3.0...3.2 times (Table 2). The cryoadditives from spicy and carotene-containing vegetables that we have devised have no analogs.

The resulting new types of natural protein health snacks are unique in chemical composition and have no analogs.

Our research results make it possible to develop affordable natural protein plant-based snacks of high biological value without the use of food additives harmful to the human body.

7. Conclusions

1. It has been established that the enzymatic processes during the cryogenic treatment of vegetables (carotene-containing and spicy ones) occur differently depending on the freezing rate and the final temperature in frozen products. We have found that the activation of oxidative enzymes (peroxidase and polyphenol oxidase) when freezing vegetables using different freezing speeds up to -18°C inside a product is 1.3...1.4 times larger than that in the starting raw materials); an even greater activation of enzymes was detected when frozen vegetables were crushed, by 2.5...4.0 times. Complete inactivation of oxidative enzymes was established when freezing vegetables to the final temperature inside a product of -32°C ... -35°C . The mechanism of inactivation of oxidative enzymes has been revealed, which is associated with the denaturation and destruction of the protein part of

enzymes and the inactivation of active enzyme centers. The study findings were used in the development of nanotechnology for frozen nano additives made from vegetables.

2. An error in the generally accepted ideas regarding the amount, in vegetable raw materials, of (from 5 to 10 %) hidden, inactive (bound in nanocomplexes with biopolymers) low-molecular BAS (β -carotene, ascorbic acid, phenolic compounds, etc.) has been revealed. We have shown that the use of cryogenic “shock” freezing of spicy and carotene-containing vegetables to the temperatures of -32°C ... -35°C makes it possible to additionally extract the previously unknown forms of BAS from raw materials in a free soluble easily digestible form (2.0...2.5 times more than fresh). It is also shown that with further low-temperature finely-grained grinding of vegetables, the mass fraction of BAS in cryoadditives in the form of cryopuree increases by 3.0...3.2 times compared to fresh vegetables (carotene-containing and spicy ones). The mechanism of processes has been revealed.

3. A new generation of natural wellness protein pastes has been developed – breakfast snacks using, as a protein base, the nano additive that we devised, made from peas and soft cheese. As regards conventional additives, ours differ by the content of complete protein, 50...55 % of which are in an easily digestible form – individual monomers of α amino acids, the size of whose particles is from 1.0 to 1.5 nm. The additives made from peas, in the form of powder and puree, were produced using nanotechnology based on the application of non-fermentative catalysis, specifically mechanolysis, mechanodestruction, which make it possible to manufacture additives from them in an easily digestible nanoscale form.

4. We have prepared the formulations, manufacturing scheme, and technology for a new generation of natural protein health snacks based on the nano additives made from peas and soft brined cheese. Frozen cryoadditives-BAS enrichers made from spicy and carotene-containing vegetables, as well as additives made from natural spices in the form of powders and nano extracts, are our innovation. It has been shown that vegetable cryoadditives in the manufacture of snacks also perform the functions of natural structure-forming agents, gel-forming agents, colorants, while additives from natural spices – natural preservatives. The use of these plant-based supplements makes it possible to obtain high-quality health products and eliminate the need for harmful food additives. Protein snacks are in a nanoscale form and differ from analogs by the high content of β -carotene, phenolic compounds, tannins; 100 g of the product can satisfy about 30 % of the daily protein needs. Based on the content of the listed BAS, new snacks meet the criteria for healthy foods recommended by FAO/WHO and the Ministry of Health of Ukraine. The additives and snacks we created are completely natural and exceed analogs in terms of quality; they are recommended for industrial production.

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