

Вивчено процеси кріомеханохімії при розробці харчових нанотехнологій добавок в формі заморожених паст в нанорозмірній формі із хлорофілмісних овочів (ХВО): шпинату, зелені селери та петрушки. Нанотехнології засновані на використанні як інновації комплексної дії кріогенного «шокового» заморожування та низькотемпературного дрібнодисперсного подрібнення, що супроводжуються процесами кріомеханодеструкції та кріомеханохімії. Розроблені нанотехнології дають змогу не тільки зберегти хлорофіли а і b, β-каротин та інші БАР вихідної сировини, а також більш повно вилучити приховані неактивні зв'язані з біополімерами (білком, полісахаридами) форми БАР у вільну легкозасвоювану форму. Виявлено зв'язані неактивні форми хлорофілу та інших БАР в ХВО при переробці за кріотехнологією в оздоровчі продукти – заморожені кріопасту в нанорозмірній формі. Встановлено, що в ХВО в зв'язаній формі знаходиться в 3,0...3,5 раз більше хлорофілу та інших БАР, ніж можна екстрагувати із свіжих хлорофілмісних овочів. Показано, що масова частка БАР в кріопастах із ХВО в 2,5...3,5 рази більше ніж у свіжих овочах. Розкрито механізм процесів збільшення.

Встановлено, що активність окислювальних ферментів (пероксидази та поліфенолоксидази) ХВО кріозаморожених з високою швидкістю залежить від кінцевої температури заморожування продукту. Встановлено, що заморожування до температури в середині продукту $-35...-40$ °C призводить до повної інактивації окислювальних ферментів, в той час як заморожування до -18 °C призводить до збільшення активності ферментів в 1,4...1,5 раз, як у випадку активації ферментів під дією теплової обробки сировини при $+35$ °C. Розкрито механізми процесів кріомеханохімії, які пов'язані з механокрекінгом та повною інактивацією окислювальних ферментів.

Розроблені кріопасту із ХВО не мають аналогів, є джерелом унікального комплексу БАР (хлорофілів а і b, β-каротину, L-аскорбінової кислоти, фенольних сполук та ін.). Із застосуванням отриманих кріопаст розроблена зелена лінійка оздоровчих продуктів, де добавки із ХВО виступають як натуральні барвники, згущувачі, стабілізатори текстури

Ключові слова: кріомеханохімія, кріогенне «шокове» заморожування, хлорофілмісні овочі, хлорофіл, оздоровчі продукти, приховані форми БАР

INVESTIGATION OF CRYOMECHANOCHEMISTRY PROCESSES IN THE DEVELOPMENT OF NANOTECHNOLOGIES OF SUPPLEMENTS PREPARED FROM CHLOROPHYLL CONTAINING VEGETABLES AND DISCOVERY OF HIDDEN CHLOROPHYLL FORMS

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1. Introduction

The relevance of this study relates to a global problem to be addressed today. This problem consists in degradation of

population immunity caused by deficiency (about 50 %) of vitamins and other biologically active substances (BAS) in diets, protein deficiency, unbalanced diet complicated by general worsening of environmental situation in the

world [1–3]. Immunity can be improved by consuming health improving products with high content of BAS (vitamins, minerals, phytochemicals), proteins and prebiotic substances (pectins, cellulose, etc.) [2–4]. These substances help to strengthen the body's protective force as they have detoxifying, antioxidant and adaptogenic action [4, 5]. Fruits, vegetables, and health improving products are the BAS source.

Immunodeficiency is being addressed in many countries around the world. In particular, health improving products prepared from fruits and vegetables that improve health are popular [5].

It should be noted that the need for fresh vegetables and fruits in the population diet is satisfied by just 50 % for today [1, 3]. The deficiency problem is partially solved by consuming frozen produce. Unlike the leading countries such as the USA, Germany, France, or Japan where annual consumption of frozen fruits and vegetables is 40...100 kg per capita, only 300 g is consumed in Ukraine [6]. This is caused by underdeveloped segment of the food industry engaged in production of frozen fruits, vegetables, and convenience foods.

Chlorophyll-containing vegetables (CCV) are promising raw materials for the production of frozen green food products and health improving supplements. CCV includes spinach, broccoli, Brussels sprouts, green peas, celery, dill, parsley and more [6]. These CCV are a source of chlorophyll in the population diet [7, 8]. Chlorophyll weight fraction in them ranges from 0.1 to 0.8 % [6, 9]. For today, shortage of natural green supplements and health improving products derived from CCV takes place. Development of frozen chlorophyll-containing supplements and products with high chlorophyll content using CCV is promising.

2. Literature review and problem statement

According to the latest data from leading oncologists and vitologists in the USA, Japan, the Netherlands, Sweden, and others, antitumor, immunomodulatory, detoxifying, antioxidant and antiradiation properties of chlorophyll have been proven [7, 10]. The discovered protective properties of chlorophyll are explained by chemical structure of its molecules. By their structure, chlorophyll molecules are close to the structure of human blood hemoglobin and are unsaturated porphyrin rings [4, 6]. Difference in the molecule structure consists in that an iron atom is in the central part of the blood heme while magnesium is in the center of the chlorophyll molecule [11, 12]. Chlorophyll molecules, like blood hemoglobin, belong to reactive unsaturated conjugated compounds that block negative processes occurring in the human body. The negative processes are related to the action of carcinogens, free oxidizing radicals, allergens, aging processes, and other negative factors [4, 6].

Despite the unique healing properties of chlorophyll, chlorophyll-containing vegetables have not been properly used in the population diet [6]. This relates to the lack of information on the healing properties of chlorophyll, recommended daily consumption rates and promotion of CCV products. Fresh chlorophyll-containing vegetables are used in diets. This is explained by the fact that there are objective difficulties in processing CCV into food products caused by the fact that *a* and *b* chlorophylls are unstable, easily destroyed compounds discoloring or turning brown

and losing their properties during processing [4, 6]. It has been shown that chlorophyll losses in processing of CCV are from 20 to 100 % under the influence of heat treatment and up to 35 % in freezing plus about 50 % in defrosting [12–14].

“Shock” freezing is one of the progressive methods used in processing and preserving products of vegetable and animal origin [5, 6, 14]. It is based on the use of cryogenic liquids as refrigerants. Liquid nitrogen, carbon dioxide, etc. are used as cryo-agents. Direct dependence of the degree of preservation of vitamins and other BAS, loss of cellular juice during defrosting and preservation of products made of fruits and vegetables on the freezing rate have been established [15, 16].

Analysis of published data for the last 10 years has shown that the problem of preservation and stabilization of chlorophyll and other BAS during processing including freezing of CCV into finished products was not solved completely in international practice [14, 16]. Solution of this problem requires new non-standard approaches, ideas and innovations. An option of solving the problem of preserving and stabilizing chlorophyll and other BAS in processing CCV to frozen fine-dispersed supplements consists in application of an innovative method of in-depth processing of raw materials based on a complex action of cryogenic “shock” freezing and fine-dispersed grinding of raw materials [15, 16]. The use of this method of in-depth processing provides the highest degree of preservation of vitamins and other BAS [17, 18]. Disadvantage of the existing cryogenic method of freezing foods to –18...–25 °C consists in a significant (up to 50 %) loss of vitamins and other BAS during defrosting after storage of the frozen product for six months. Besides, cellular juice losses up to 10 % during defrosting are observed [20–22].

Scientific studies of cryogenic processes of freezing fruits and vegetables are devoted mainly to the study of crystallization and thermophysical processes [14, 20], development of mathematical models of regulation of these processes, study of conventional physicochemical parameters of products [23] as well as ascertaining cell juice [22, 24] and vitamin losses [21, 25].

Exceptions include results of previous fundamental and applied studies of the effect of complex action of cryogenic “shock” freezing and fine-dispersed grinding of fruits, vegetables, and mushrooms [15–17]. The results obtained in previous scientific studies became the basis for the development of cryogenic nanotechnology and cryogenic equipment which were implemented at a number of enterprises of Ukraine, Latvia, and other countries. In particular, cryogenic nanotechnologies of frozen fruits and vegetables and fine-dispersed paste-like fruit supplements [27–29], fine-dispersed cryopastes of carotene-containing vegetables and berries [26], sun root [29], mushrooms [27], spicy vegetables and natural spices [30]. The developed cryogenic technologies have made it possible to discover hidden BAS forms (vitamins, carotenoids, phenolic compounds) in products of fruits and vegetables. Their weight fraction in the obtained products is 2.0...3.5 times more than in original plant raw materials [15, 16, 27]. Mechanisms of increasing low molecular weight BAS found in frozen products in a nanoscale form have been disclosed [6]. This is the approach used in the present study.

Analysis of published data shows that an issue remains unresolved for today as to why so significant losses of

chlorophyll and other BAS occur in frozen and further defrosted products. The losses measure 25...35 % when using "shock" freezing of CCV and 50...60 % in defrosting the products [6, 13, 28]. Cryogenic technologies of freezing CCV and cryopastes produced of them which would allow to store chlorophyll and other BAS during freezing, storage and further defrosting of the product have not been developed. The processes occurring in cryogenic "shock" freezing and fine-dispersed grinding of CCV have not been studied. This requires in-depth knowledge of biochemical, enzymatic and mechanochemical processes occurring during cryo-freezing and fine-dispersed grinding of CCV into health improving supplements such as semi-finished and finished products. The above processes will occur in a different way in cryogenic shock freezing and low-temperature fine-dispersed grinding than with conventional methods of rapid shock freezing and grinding at -18°C . This requires further studies.

3. The aim and objectives of the study

This study objective consists in studying the processes of cryomechanochemistry occurring in development of a nanotechnology of cryo-frozen health improving supplements of chlorophyll-containing vegetables in a nanosized form with the use of liquid and gaseous nitrogen at a high degree of preservation of chlorophyll and other BAS, without loss in defrosting and with no use of artificial food supplements.

To achieve this objective, the following tasks were set:

- to study the processes of cryomechanochemistry and influence of high-speed cryoprocessing during "shock" freezing and fine-dispersed grinding of CCV on preservation and transformation of chlorophyll and other BAS;
- to carry out modeling experiments to study the effect of high-speed cryogenic "shock" freezing and final temperature of CCV products on enzymatic processes (activity of oxidizing enzymes) and their inactivation in comparison with conventional freezing;
- to develop a cryogenic nanotechnology of frozen fine-dispersed supplements with CCV applying cryogenic "shock" freezing with the use of liquid and gaseous nitrogen, processes of cryomechanochemistry and cryomechanical destruction in low-temperature grinding without the use of artificial supplements;
- to study the content of chlorophyll and other BAS in cryo-frozen nano supplements with CCV in comparison with counterparts and substantiate feasibility of using structure-forming agent as a source of chlorophyll and other BAS in the production of products for healthy nutrition.

4. Materials and study methods used in the development of a nanotechnology for cryo-frozen supplements prepared from chlorophyll-containing vegetables

4.1. Materials and equipment used in the experimental studies

Scientific studies were carried out at the Department of Fruit, Vegetable and Milk Processing Technologies, Kharkiv State University of Food and Trade, Ukraine. Experimental studies were conducted in Scientific Research Laboratory of

Innovative Cryo- and Nanotechnologies of Herbal Supplements and Health Products of the above department.

The study presented in this paper is a continuation of the scientific research work named Creation and Implementation of Advanced Technologies and Efficient Equipment for the Production of New Functional Foods which was awarded the State Prize of Ukraine in Science and Technology in 2006.

Chlorophyll-containing vegetables were taken as research materials: spinach leaves (Victoria, Rembor, Krepys, Motodor), parsley (Astra, Gospodynia, Alba, Lystova Kucheriava), celery (Diamant, Kaskad, President, Prazsky Gigant), their frozen fine dispersed semi-finished supplement products as well as health improving products using the supplements obtained.

Cryogenic "shock" freezing was carried out in a fast-freezing experimental apparatus using liquid and gaseous nitrogen both as a refrigerant and an inert medium. The appliance was designed for freezing hard-shell and liquid products in special containers. Temperature of the gas medium in the freezer was manually set and automatically maintained constant throughout the experiment. Temperature of -60°C was chosen for model studies as an optimum temperature in the freezer. Freezing of CCV was performed at a high rate from 0.5 to 10°C per minute to end temperatures of -18°C ... -40°C in the middle of the product.

Robot Couper homogenizer shredder (France) and Paco-Jet low-temperature shredder (France) were used for fine grinding of chlorophyll-containing vegetables [6].

4.2. Experiments conducted to study influence of cryomechanochemistry, cryomechanodestruction on chlorophylls and other BAS in the processing of chlorophyll-containing vegetables

Quality in terms of BAS content was determined in fresh and cryo-frozen chlorophyll-containing vegetables (spinach leaves, celery, and parsley) as well as frozen puree, in particular:

- chlorophyll: by spectrophotometric method through determining optical density of acetone extracts of the test specimens;
- L-ascorbic acid: by the method of visual and potentiometric titration with a solution of Na 2,6-dichlorophenoldiphenate;
- low molecular weight phenolic compounds (for routine and chlorogenic acid separately): by Folin-Denis colorimetric method in terms of routine and separately for chlorogenic acid;
- polyphenolic compounds: by titrimetric method based on properties of polyphenolic compounds oxidized in the presence of indigo-carmin indicator; calculation of tannins was performed in terms of tannin;
- β -carotene: by the Mourie colorimetric method after extracting carotene from the product with an organic solvent and purifying it from accompanying dyes by column chromatography;
- oxidizing enzymes: by the method based on quinone property to oxidize ascorbic acid.

Sampling, solids content, organoleptic parameters, total sugar and pectin content in the raw material, nanosupplements obtained from CCV and the health improving products prepared with their use were determined according to the current standards.

Generally adopted standard and special research methods were applied in this study, that is the physicochemical,

biochemical, spectroscopic methods. The experimental study results were processed by mathematical methods using Mathcad and Microsoft Excel software.

5. Results obtained in the studies of influence of cryomechanodestruction processes on chlorophyll and other CCV BAS in development of nanotechnologies of cryo-frozen supplements

A number of varieties of fresh vegetables with high content of chlorophyll (spinach leaves, celery and parsley) cultivated in various regions of Ukraine were used as raw materials for development of nanotechnologies of cryo-frozen supplements (semi-finished products in a form of frozen pastes). CCV are known for their healing, curative and prophylactic properties due to their chemical composition. The results of influence of the processes of cryomechanochemistry and cryoprocessing using high speeds of “shock” freezing and fine-dispersed grinding of chlorophyll-containing vegetables on conservation and transformation of chlorophyll and other BAS are presented in Table 1.

A unique method of preserving and additional extraction of CCV chlorophyll naturally occurring in plant cells was developed during the study. It was found that the weight fraction of chlorophyll extracted in fine dispersed supplements is 3.2...3.8 times higher than that found in fresh chlorophyll-containing vegetables (Table 1, Fig. 1).

Biochemical, chemical, and spectroscopic methods of investigation on the example of chlorophyll-containing vegetables have established fallacy of the common ideas of the amount of hidden (bound) inactive forms of chlorophyll, carotenoids, and other BAS (5 to 10 %). It was shown that the use of cryogenic “shock” freezing makes it possible to extract and transform BAS from the hidden form into a freely digestible form. Weight fraction of BAS in cryo-frozen CCV is 2.2...2.5 times greater than that in fresh chlorophyll-containing vegetables. The mechanism was established.

Model studies have shown that inactivation of oxidizing enzymes (peroxidase and polyphenol oxidase) does not occur in cryogenic “shock” freezing of CCV using high and superhigh rates of freezing to -18 °C (Table 2). The laboratory equipment used in the studies had an error of 0.0001 to 0.05 % depending on the device type.

Table 1

The results obtained in the study of influence of mechanochemistry during complex effect of cryoprocessing for “shock” freezing and fine grinding of CCV on preservation and extraction of chlorophyll and other BAS in a free active form (n=3, P≥0.95)

Product	Weight portion									
	chlorophylls				β-carotene		L-ascorbic acid		polyphenols (in terms of tannin)	
	a		b		mg/100 g	% of raw material	mg/100 g	% of raw material	mg/100 g	% of raw material
	mg/100 g	% of raw material	mg/100 g	% of raw material						
Spinach leaves (Victoria variety)										
Fresh	190.2	100.0	550.0	100	7.5	100.0	78.2	100.0	380.2	100.0
Frozen to -18 °C	202.5	106.0	575.0	104.5	15.0	200.0	77.1	99.0	375.0	98.2
Frozen to -18 °C and fine-dispersed	275.2	151.5	650.0	118.1	18.7	250.0	102.0	140.2	517.5	138.0
Frozen to -40 °C	380.1	200.0	1115.0	202.3	19.0	251.4	195.5	250.0	577.6	152.0
Frozen to -40 °C and fine-dispersed	657.0	341.0	1680.0	300.0	23.3	310.0	274.7	301.0	710.6	187.0
Celery (President variety)										
Fresh	88.2	100.0	264.0	100.0	3.5	100.0	98.0	100.0	350.4	100.0
Frozen to -18 °C	92.4	101.4	270.2	102.1	7.6	206.0	96.2	98.0	352.0	101.0
Frozen to -18 °C and fine-dispersed	150.2	160.5	369.6	148.0	8.0	228.0	139.0	142.0	490.0	140.0
Frozen to -40 °C	176.0	200.0	554.4	210.0	8.8	250.0	200.0	205.0	497.0	142.0
Frozen to -40 °C and fine-dispersed	265.2	301.1	792.0	301.0	10.7	306.0	278.0	284.0	612.0	175.0
Parsley (Lystova Kucheriava variety)										
Fresh	124.6	100.0	364.8	100.0	3.9	100.0	250.0	100.0	270.0	100.0
Frozen to -18 °C	130.5	104.0	378.2	103.9	7.7	198.0	252.5	101.8	264.0	98.0
Frozen to -18 °C and fine-dispersed	186.0	150.2	527.8	145.0	7.8	200.0	350.0	140.0	356.0	135.0
Frozen to -40 °C	272.8	220.0	800.0	221.0	9.7	250.0	575.0	230.4	540.0	200.0
Frozen to -40 °C and fine-dispersed	347.2	280.1	1274.0	350.1	11.8	280.4	800.0	320.4	756.0	280.0

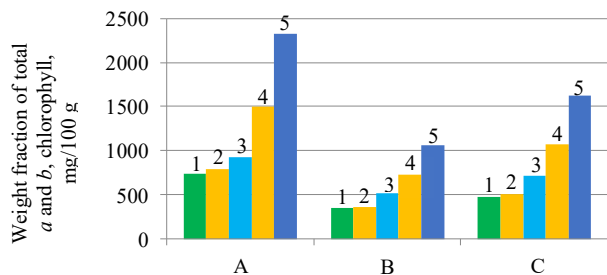


Fig. 1. Preservation of a and b chlorophyll and extraction of its hidden forms from nanocomplexes and nano-associates with biopolymers during cryogenic “shock” freezing and fine-dispersed grinding of CCV: A – spinach leaves; B – celery; C – parsley; 1 – fresh CCV; 2 – frozen to –18 °C; 3 – finely dispersed CCV; 4 – frozen to –35 °C; 5 – finely dispersed CCV

The cryogenic nanotechnologies for the production of a green line of health improving fine-dispersed supplements from chlorophyll-containing vegetables (spinach leaves, celery and parsley) with high content of chlorophyll and other BAS have been developed (Fig. 2).

New technologies differ from conventional ones by using liquid and gaseous nitrogen as an environmentally friendly natural refrigerant and inert medium. Cryotechnology innovations include the use of cryogenic high-speed ‘shock’ freezing (from 1 to 10 °C per minute) and freezing to a still lower temperature in the middle of the product (–32...–35 °C) than in conventional freezing (up to –18...–20 °C) (Fig. 2). In addition, cryotechnologies include low-temperature fine-dispersed grinding of frozen CCV. Rational technological conditions of cryogenic “shock” freezing and low-temperature grinding have been elaborated (Fig. 2).

The amount of liquid nitrogen used per 1 kg of frozen cryopaste prepared from chlorophyll-containing vegetables ranged from 0.5 to 1.0 l.

Fine-dispersed frozen chlorophyll-containing vegetable pastes obtained by the cryogenic nanotechnology have a record high content of natural chlorophyll, β-carotene, L-ascorbic acid, phenolic compounds and exceed world known counterparts and the raw material 3.0 to 3.5 times (Fig. 3). Cryopastes prepared from CCV have a fundamentally new chemical composition compared to those obtained by conventional technology and are in a nanosized form easily assimilated by the human body. For example, weight fraction of chlorophyll in 100 g of product ranges from 1,057 mg (in celery cryopaste), 1,620 mg (in parsley cryopaste) and up to 2,320 mg (in spinach cryopaste). Weight fraction of β-carotene in 100 g of cryopaste is from 10.7 mg (in celery cryopaste) to 23.0 mg (in spinach cryopaste). Corresponding figures for L-ascorbic acid are from 274.5 mg (in spinach cryopaste) to 800 mg (in parsley cryopaste). The results obtained for an increased weight fraction of chlorophyll and other BAS are common and independent of the CCV variety.

Table 2

Influence of the rate of cryogenic “shock” freezing of CCV and final temperature in the middle of the product and fine-dispersed grinding on activity of oxidizing enzymes (n=3, P≥0.95)

Product	Activity			
	peroxidase		polyphenol oxidase	
	0.01 N iodine solution	% raw material	0.01 N iodine solution	% raw material
Spinach leaves (Victoria variety)				
Fresh (raw material)	25.6	100.0	6.2	100.0
Frozen to –18 °C	33.2	130.0	9.0	145.1
Cryopaste of spinach frozen to –18 °C	75.1	300.0	16.2	353.3
Cryofrozen to –35 °C	0	0	0	0
Cryopaste of spinach frozen to –35 °C	0	0	0	0
Celery (President variety)				
Fresh (raw material)	13.2	100.0	2.8	100.0
Frozen to –18 °C	18.6	140.0	4.2	150.0
Cryopaste of celery frozen to –18 °C	35.2	304.5	8.1	407.8
Cryofrozen to –35 °C	0	0	0	0
Cryopaste of celery frozen to –35 °C	0	0	0	0
Parsley (Lystova Kucheriava variety)				
Fresh (raw material)	6.8	100.0	2.4	100.0
Frozen to –18 °C	9.6	141.2	3.5	145.8
Cryopaste of parsley frozen to –18 °C	20.2	320.6	7.2	329.2
Cryofrozen to –35 °C	0	0	0	0
Cryopaste of parsley frozen to –35 °C	0	0	0	0

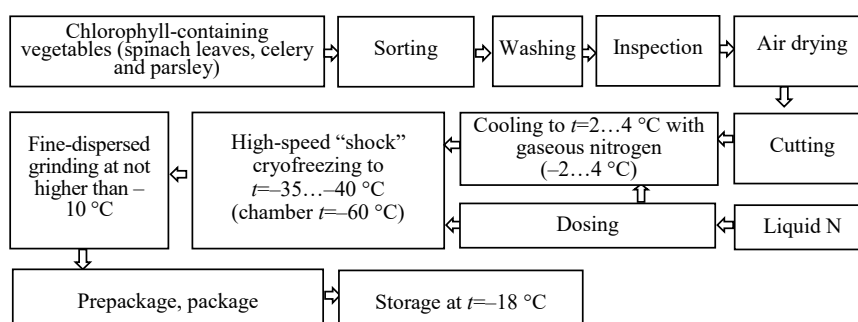


Fig. 2. Schematic diagram of production of a green line of health improving fine-dispersed frozen supplements from chlorophyll-containing vegetables with a record content of chlorophyll using the cryogenic nanotechnology

Using the cryopastes obtained, a green line of health improving products and dishes (nanosorbets, cheese desserts, nanodrinks, jellies, biscuits, pastries, sauces, etc.) were developed. New types of CCV supplements act as natural dyes, thickeners, and texture stabilizers. The use of cryopastes produced from CCV in the manufacture of health improving

products makes it possible to refuse from the use of harmful food supplements in food production.

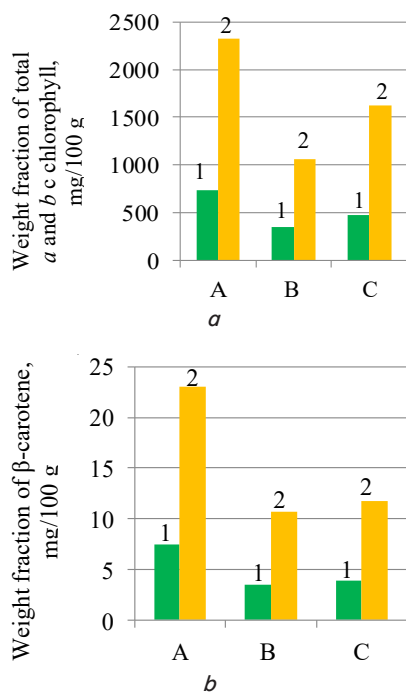


Fig. 3. Content of chlorophyll and β-carotene in cryo-frozen pastes prepared from chlorophyll-containing vegetables: A – spinach leaves, B – celery, C – parsley; 1 – fresh CCV, 2 – cryo-frozen CCV pastes; weight fraction of total amount of *a* and *b*: *a* – chlorophyll and *b* – β-carotene

6. Discussion of results obtained in the study of influence of cryomechanodestruction processes on chlorophylls and other CCV BAS in the development of nanotechnologies for cryo-frozen supplements

Chemical composition of CCV including the content of chlorophyll and other BAS was investigated. It was found that spinach (0.74 %) had the highest content of *a* and *b* chlorophyll among all CCV species under study. Weight fraction of chlorophyll in parsley and celery was 1.5...2 times lower (0.49 % and 0.35 %, respectively), see Table 1. It has been shown that spinach can be considered as a source of β-carotene. Weight fraction of β-carotene in 100 g of spinach leaves was at the level of carrots being the conventional source of β-carotene in the diet of population of Ukraine. For example, weight fraction of β-carotene in 100 g of spinach was 7.5 mg and 8 to 10 mg in carrots which corresponds to two daily needs of the human body in β-carotene [4, 6].

It was also shown that parsley has the highest content of L-ascorbic acid in 100 g of product (250 mg), then celery (98 mg) and spinach leaves are in the third place (72.8 %) (Table 1). Green chlorophyll-containing leafy vegetables exceed citrus fruits in content of L-ascorbic acid. It has been established that parsley is close to dog rose hips, black currant and sweet pepper which have a record content of L-ascorbic acid among berries and vegetables [4, 6].

It was established that CCV also have a considerable content of natural antioxidants, detoxifiers, in particular

polyphenolic compounds amounting 0.35...0.38 %, except for parsley (0.27 %), see Table 1.

It was shown that regularities of influence of cryomechanodestruction processes on chlorophylls and other BAS found for different types of CCV are common and do not depend on variety of the tested specimens.

Thus, presence of a unique complex of BAS phytochemicals (*a* and *b* chlorophyll, β-carotene, L-ascorbic acid, low molecular weight and high molecular weight phenolic compounds, for example tannins) was established in the study of chemical composition of fresh CCV. It has been shown that 100 g of chlorophyll-containing vegetables are capable of meeting several daily human needs for chlorophylls, β-carotene, phenolic compounds and L-ascorbic acid (Table 1). This complex of natural BAS phytochemicals gives the fresh CCV therapeutic and prophylactic properties, in particular, antitumor, immunomodulatory, detoxifying, antioxidant action, helps to strengthen vessels of heart, brain, etc [10–12].

The main issue in developing the new method of deep CCV processing to obtain frozen health improving nano-supplements consisted in as much as possible preservation of chlorophyll and other BAS. Losses of chlorophyll when using existing methods of CCV processing depend on the type of processing and range from 25 to 100 %. Exceptions include the products of sublimation drying and shock freezing: losses of chlorophyll are 25...35 % in freezing plus 50...60 % in defrosting. The cause of such losses has not been determined.

A preservation method and a cryotechnology of producing frozen fine dispersed supplements from CCV have been proposed and scientifically substantiated. The proposed method makes it possible not only to retain chlorophyll, carotenoids, and other BAS in raw materials but also to more fully convert hidden biopolymer-related forms into a free form. The method is based on the use of an innovative cryogenic “shock” freezing with liquid and gaseous nitrogen and fine dispersed grinding accompanied by the processes of cryomechanochemistry, cryomechanodestruction and mechanical activation.

It was shown that use of the complex effect of cryogenic “shock” freezing to –35...–40 °C and fine grinding on raw materials results in a high degree of extraction of hidden bound forms of BAS (chlorophyll, carotenoids, phenolic compounds, L-ascorbic acid) in chlorophyll-containing vegetables. Weight fraction of these BAS in frozen supplements is 3.2...3.5 times higher than in fresh CCV (Table 1, Fig. 1).

The mechanism of extraction of hidden (bound in nano-complexes) forms of chlorophyll, carotene and other CCV BAS during cryogenic “shock” freezing and fine dispersed grinding was disclosed. The latter is the result of the processes of cryocracking, mechanocracking (destruction) of nanocomplexes and nano-associates of biopolymers (protein, polysaccharides) with low molecular weight BAS and minerals and leads to BAS transformation into a free form.

One of the main factors affecting quality of final products in processing of fruits and vegetables into health improving supplements and products using various technologies including freezing technologies is inactivation of oxidizing and hydrolytic enzymes. As regards CCV, these processes were not studied in processing of chlorophyll-containing vegetables.

In this regard, it is relevant to study features of enzymatic, biochemical and cryomechanical processes when developing the nanotechnology of fine dispersed supplements in a

form of pastes produced from CCV using cryogenic “shock” freezing and fine-dispersed grinding.

Large, up to 50...60% chlorophyll losses and decomposition of CCV during defrosting of fine-dispersed supplements prepared from CCV and frozen to -18°C were found after 30–40 min. It was shown that when defrosting CCV and fine-dispersed supplements prepared from them and cryogenically frozen to a lower temperature in the middle of the product, up to $-35...-40^{\circ}\text{C}$, no losses of chlorophyll occur (Table 1, Fig. 1). In this regard, it was assumed that freezing of CCV to -18°C does not result in inactivation of oxidizing enzymes (peroxidase, polyphenol oxidase). On the contrary, there is a growth of enzymatic activity when defrosting the product. This indicates activation of enzymes during freezing to -18°C similar to activation of enzymes during heat treatment. The processes of activation and inactivation of oxidizing enzymes of fruits and vegetables during heat treatment are reflected in numerous monographs and textbooks on biochemistry of fruits and vegetables [31]. Data on the effect of freezing on enzymatic activity of fruits and vegetables have not been found in literature. In this regard, experimentation with and elucidation of freezing conditions leading to inactivation of oxidizing enzymes in the cryoprocessing of CCV are relevant.

It was shown that a 1.3...1.5-time higher activation of oxidizing enzymes occurs when freezing CCV to -18°C . It was revealed and shown that further low-temperature grinding when preparing frozen homogeneous pastes into a nanoscale form further increases activity of oxidizing enzymes. Activity of enzymes in cryopaste warming is 3.5...4.0 times greater than that in fresh CCV (Table 2). Mechanisms of activation of oxidizing enzymes have been established.

It was found that a complete inactivation of oxidizing enzymes occurs during cryogenic “shock” high-speed freezing of CCV to temperatures of $-35...-40^{\circ}\text{C}$ which is connected with irreversible denaturation of the protein structure in the latter.

It was shown that activity of oxidizing enzymes is not restored in further fine-dispersed grinding and preparation of pastes as well as during defrosting (Table 2). The results of scientific studies of oxidative enzymatic processes occurring during cryo-freezing and fine-dispersed grinding of CCV will allow us to re-consider the processes of cryoprocessing and cryomechanicochemical processes in development of cryogenic technologies.

Complete inactivation of oxidizing enzymes in CCV cryo-frozen to $-35...-40^{\circ}\text{C}$ and in fine dispersed supplements prepared from them provides a longer shelf life of 12 months without loss of chlorophyll and other BAS during storage and defrosting.

A characteristic feature of the obtained results consists in that the proposed approach provides not only conservation of the biological potential of plant raw materials but also a possibility of realizing the prospects for its fuller use.

This is a new trend in deep processing of food raw materials. The study is of practical importance because by the content of chlorophyll and other BAS, efficiency of obtained nanosupplements and CCV products is 3.0... 3.5 times higher than those obtainable by means of existing technologies. The proposed method of processing CCV into nanosupplements and health improving products opens up new opportunities for the food technologies and will find its consumer. It is generally known that significant losses of useful vegetable food products (fruits and vegetables) have occurred globally in

recent years. Total losses during processing, transportation and storage amount to hundreds of billions of tons. What is more, the fact that there are large amounts of hidden bound inactive BAS in fruits and vegetables that are the hidden reserves of raw materials non-consumed by mankind has not been paid attention. This paper sheds light on this very issue. Its solution will open the prospects of processing CCV into nanosupplements and health improving products.

Thus, a significant amount of hidden, bound inactive forms of chlorophyll and other BAS as hidden reserves of raw materials were revealed in the course of studying CCV processing into frozen health improving products, i. e. fine dispersed pastes by means of the cryogenic technology. It was established that CCV contain 3.0...3.5 times more chlorophyll in a bound hidden form than can be extracted from fresh vegetables.

A method of deep processing of CCV into frozen health improving products, for example cryopastes has been developed. The method is based on application of cryogenic “shock” freezing and low-temperature grinding. Their complex application results in a complete inactivation of oxidizing enzymes and cryo- and mechanodestruction of nanocomplexes and nano-associates of chlorophyll and other BAS containing biopolymers and minerals and facilitates preservation and additional extraction and stabilization of chlorophyll and other BAS.

The CCV cryopastes having a unique texture are produced without the use of food supplements. They are recommended for the use in manufacture of a wide range of health improving products such as natural dyes, thickeners, texture stabilizers, gel formers and are a unique source of various phytochemicals.

7. Conclusions

1. Hidden bound inactive forms of chlorophyll and other BAS in chlorophyll-containing vegetables using cryo-mechanochemistry and cryomechanical destruction in development of cryotechnology of health improving products, frozen fine dispersed cryopastes were discovered. It has been found that CCV in a bound form contain 3.0...3.5 times more chlorophyll than that extractable from fresh vegetables. It has been shown that the use of the complex effect on cryogenic “shock” freezing and fine-dispersed grinding of CCV leads to a high degree of extraction of hidden bound forms of BAS from raw materials (3.2...3.5 times more than in fresh vegetables). The mechanism of the process was established.

2. It was found that activity of oxidizing enzymes (peroxidase and polyphenol oxidase) in chlorophyll-containing vegetables rapidly cryo-frozen by means of liquid and gaseous nitrogen depends on the final freezing point in the middle of the product. Freezing to $-35...-40^{\circ}\text{C}$ results in a complete inactivation of oxidizing enzymes while freezing to -18°C results in a 1.4...1.5-fold increase in activity. The mechanism of influence of final cryo-freezing temperature in the middle of CCV on activity of oxidizing enzymes was established.

3. Cryotechnology of frozen fine-dispersed supplements prepared from CCV using liquid and gaseous nitrogen has been developed. The nanotechnologies include fine-dispersed grinding of pre-crystallized CCV to a nano scale. This leads to a 3.2...3.5 times greater weight fraction of chlorophyll and other BAS and enables the use of obtained frozen

supplements as natural BAS concentrates and dyes, natural structure forming agents and thickeners in production of health improving products without the use of synthetic admixtures.

4. It was shown that fine-dispersed frozen pastes prepared from chlorophyll-containing vegetables by means of cryogenic nanotechnology have a record content of natural chlorophyll, β -carotene, L-ascorbic acid, phenolic compounds and exceed

the world known counterparts 3.0...5.0 times. The cryopastes produced from CCV have a fundamentally new chemical composition compared to those obtained by the conventional technology and are in a nanosized form easily assimilated by the human body. For example, weight fraction of chlorophyll in 100 g of product ranges from 1,057 mg (in a celery cryopaste), 1,620 mg (in a parsley cryopaste) to 2,320 mg (in a spinach cryopaste).

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