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НОВЕ В ТЕХНОЛОГІЇ ЗАМОРОЖУВАННЯ ХЛОРОФІЛОВМІСНИХ ОВОЧІВ ІЗ РЕКОРДНИМИ ХАРАКТЕРИСТИКАМИ

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Розроблено кріогенну технологію замороження хлорофіловмісних овочів, яка включає кріогенне «шокове» заморожування з використанням рідкого та газоподібного азоту, що дозволяє не лише повністю зберегти хлорофіл a і b, каротиноїди та інші біологічно активні речовини, але й більш повно вилучити їх приховані форми та отримати заморожені овочі з принципово новими властивостями порівняно зі свіжими овочами.

Ключові слова: *кріогенне заморожування, інноваційні технології, хлорофіловмісні овочі, броколі, брюссельська капуста, азот.*

НОВОЕ В ТЕХНОЛОГИИ ЗАМОРАЖИВАНИЯ ХЛОРОФИЛЛСОДЕРЖАЩИХ ОВОЩЕЙ С РЕКОРДНЫМИ ХАРАКТЕРИСТИКАМИ

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Разработана криогенная технология замораживания хлорофиллсодержащих овощей, которая включает криогенное «шоковое» замораживание с использованием жидкого и газообразного азота, позволяет полностью сохранить хлорофилл a и b, каротиноиды и другие биологически активные вещества, более полно извлечь их скрытые формы и получить замороженные овощи с принципиально новыми свойствами по сравнению со свежими овощами.

Ключевые слова: *криогенное замораживание, инновационные технологии, хлорофиллсодержащие овощи, брокколи, брюссельская капуста, азот.*

THE NEW IN FREEZING TECNOLOGY OF CHLOROPHYLL-CONTAINING VEGETABLES WITH RECORD CHARACTERISTICS

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Developed cryogenic technology frozen chlorofill-containing vegetables (broccoli and Brussels sprouts), which includes cryogenic "shock" freezing with *high speeds and to lower the final temperature in products using liquid and gaseous nitrogen, allowing both to maintain chlorophyll a and b, carotenoids and other biologically active substances (BAS), but also allows them to more fully remove hidden form (linked to Nanocomplex of biopolymers). New way of preserving lets frozen vegetables with radically new properties compared to fresh vegetables. It was established that the use of cryogenic "shock" freezing chlorofill-containing vegetables with high speeds (5–10° C per minute) to a final temperature – 32 ... – 35° C will not only save chlorophyll, carotene, L-ascorbic acid, polyphenolic compounds, but also get frozen vegetables from different chemical composition, in particular content twice BAR and for some indicators, three times better than fresh raw materials (table 2). Thus, the mass fraction of chlorophyll after cryogenic freezing increased by 2–2,2 times, and carotenoids 2–3 times, that is their more complete extraction of raw materials from complex biopolymers nanokomponentiv BAR in free form, that there is the effect of "enrichment" of the product, and inactivation of oxidative and hydrolytic enzymes. New technology makes it possible to get frozen vegetables with record performance and quality of the contents ix BAR such as chlorophyll a and b, carotenoids, L-ascorbic acid, polyphenolic substances exceeding the initial fresh vegetables 2–3 times. The quality of vegetables exceeds domestic and foreign counterparts.*

Keywords: *cryogenic freezing, innovative technologies chlorophyll-containing vegetables, broccoli, Brussels sprouts, nitrogen.*

General formulation of the problem. At the current moment, the global problem in all countries is the lack of vitamins, valuable proteins, minerals and other biologically active substances (BAS) in a daily diet, the need in which in Ukraine is satisfied only for 50%. In addition, dietary nutrition is unbalanced: Ukrainian population suffers from the lack of meat, fish, milk, vegetables, fruits and other products promoting health strengthening. At the same time, environmental degradation and immunity reduction of the population are witnessed all over the world. In this connection, functional healthful products, especially from fruits and vegetables aimed to health strengthening are very popular in many countries of the world. Nowadays both domestic and foreign scientists give much attention to this problem in their works all over the world. The government invest the programs of health nutrition and health lifestyles, and implement them into the community in different forms at the national level. This is one of the most important ways of the science development, which is nowadays been rapidly developing in international practice.

Analysis of recent research and publications. One of the perspective ways for the manufacture of healthful food is the use of frozen vegetables and fruits containing a significant amount of biologically active substances, such as vitamins, carotenoids, chlorophylls a and b, phenolic compounds, which contribute to strengthening immunodefences of a human body with detoxicative and antioxidant effect on the human body. At present time in Ukraine, there is a shortage of domestic frozen vegetables.

Chlorophyll-containing vegetables (broccoli and Brussels cabbage) markedly stand out among the other, high in chlorophyll, plants, ascorbic acid, β-carotene, phenolic compounds with antioxidant and immunomodulatory effects. These vegetables are very popular among the people all over the world (especially in Japan, the USA, Brazil, etc.). It is known that unsaturated compounds of chlorophyll possess anti-radiation effect, antitumor activity and significantly increase the body defenses, especially in combination with ascorbic acid and β-carotene, which is found in broccoli and Brussels in large quantities. We know that they are poorly stored seasonal ingredients. Traditional recycling technologies lead to significant losses of BAS.

Difficulties in the processing of chlorophyll-containing vegetables to canned foods due to the fact that under the influence of heat treatment, light, oxygen, and pH, significant are the losses of chlorophyll, ascorbic acid, carotenoids and others BAS (from 20 to 80%). The loss of chlorophyll is accompanied by darkening or discoloration of the product. Under the influence of these factors, the reaction of substituting integrally conjugated magnesium to hydrogen occurs in the molecules of chlorophyll. This results in the formation of brown substance – pheophytin. In Ukraine, chlorophyll-containing vegetables (CCV) have not found proper application in the manufacture of food products.

There are practically no scientific works identifying the ways of processing chlorophyll-containing plant material, including freezing, which lead to the preservation of natural chlorophyll. The available scientific data are controversial.

We know that the most effective way of processing vegetables, fruits and berries is a quick freeze-quenching that ensures the best method of storing vitamins and other biologically active elements. However, during the defrost of plant material we can witness the loss of cell juice, vitamins and other substances. Warranty storage terms of frozen products are within 6 months. Cryogenic freeze-quenching, i.e. freezing with the use of cryogenic liquids (liquid nitrogen, liquid carbon dioxide, etc.) are widely applied abroad. In Ukraine, this method of freezing has not yet been applied or developed as cryogenic technology; biochemical and physical-chemical processes during freezing of chlorophyll-containing plant material have not been studied as well.

In this work, during the development of the technology of frozen chlorophyll-containing vegetables (broccoli and Brussels sprouts), it was innovatively suggested to use cryogenic "shock" fast freezing to lower final temperatures of the product compared to international practice. That allowed to elaborate a new way for obtaining frozen vegetables with qualitatively new consumer properties in comparison with outward raw material and traditional frozen vegetables. Until now, it was impossible to get them by using generally accepted methods. Under intensive cryogenic freezing with the use of high cooling velocity to lower temperatures than is accepted in international practice (-18° C) in a final product (-35...-40° C), crystal structure characteristic for frozen vegetables will form. Enzymatic and non-enzymatic processes, changes of BAS, cryodestruction of BAS, biopolymers and BAR Nano-associates – biopolymer or biopolymer – biopolymer and others – can occur. The enumerated processes using cryogenic freezing will take place otherwise than with traditional methods and different freezing temperatures, which requires additional research.

The feasibility of the development of frozen vegetables, which do not lose their cell juice and BAS, has developed due to the work of domestic and foreign research scientists such as B.I. Verkin, E.I. Almashi, E.I. Kvuhcheshvili, R.Y. Pavlyuk, V.V. Pogarska, N.Y. Orlov, I.A. Simahina, L.N. Telezhenko, S.A. Belinska and others.

The aim of the article is to develop a cryogenic technology of frozen chlorophyll-containing vegetables (broccoli and Brussels sprouts), which includes cryogenic freeze-quenching with high velocities, and to lower final temperatures in products with the use of liquid and gaseous nitrogen. This allows both to maintain chlorophyll a and b, carotenoids and other biologically active substances (BAS), and to remove their hidden forms (linked with biopolymers in Nano-complexes).

To consider this goal it is necessary to solve the following problems:

– examine the contents of biologically active substances such as chlorophyll a and b, β-carotene, vitamin C, tannins, organic acids in fresh chlorophyll-containing vegetables (broccoli and Brussels sprouts);

– to examine the activity of oxidative enzymes such as peroxidase and polyphenoloxidase in fresh broccoli and Brussels sprouts;

– to examine the impact of cryogenic freezing with high shock velocities to different final temperatures for storage of chlorophyll a and b, carotenoids, vitamin C and tannins of frozen broccoli and Brussels sprouts;

– to develop cryogenic technology of obtaining frozen chlorophyll-containing vegetables with the use of liquid and gaseous nitrogen with maximum preservation of chlorophyll a and b and other biologically active substances with minimal losses of cell sap during the defrosting;

– Examine the contents of BAS in frozen vegetables compared to outward materials and analogues.

The main material of the research. Academicians from KhSUFT together with specialists from Kharkiv trade-economic college of KNTEU proposed and developed cryotechnology of chlorophyll-containing frozen vegetables (broccoli and Brussels sprouts) with the use of liquid and gaseous nitrogen, including cryogenic freezing. The research was carried out in KhSUFT at the Department of fruits, vegetables and milk processing technology in two scientific and research laboratories: "Innovative cryo- and Nano-technologies of vegetable supplements and healthy products" and "Technologies and Biochemistry of phyto concentrates". The work is performed on the original modern equipment available at the department: cryogenic software refrigerant, which uses gaseous nitrogen as a refrigerant and liquid inert medium. The temperature in a fast-refrigerating chamber was -60° C. The products were frozen at various velocity (2, 5, 10° s per minute) to a final temperature: -18° C; -25° C; -30° C; -35° C. Thus from 0,5 to 1 kg of liquid nitrogen were used for the refrigeration of 1 kg of vegetables depending on raw material, thickness of a freezing product, etc.

The research carried out in this article is a continuation of the authors' works concerning the development of cryogenic technologies for processing different fruits and vegetables to fine supplements in the form of Nano-powders, frozen Nano-purees included in the work, which in 2016 was awarded the State Prize in Science and Engineering of Ukraine.

The main idea for the development of cryogenic technology of frozen chlorophyll-containing vegetables was complete elimination of the losses of cell sap during defrosting, elimination of heat treatment of raw material and full preservation of chlorophyll a and b, carotenoids, ascorbic acid and other biologically active substances both during freezing and during defrosting and storage for a year.

The study of BAS content in fresh vegetables showed that broccoli differed with a higher amount of chlorophyll a and b (almost 1.5 times higher) from the amount containing in Brussels (Table 1). Yes, mass fraction of chlorophyll was 90.5 mg, chlorophyll b – 198 mg per 100 g, and Brussels sprouts respectively contained 60.2 and 125 mg per 100 g.

Brussels sprouts differed with a higher amount of carotene compared to broccoli (respectively 10.8…12.3 mg per 100 g, and 9…10 mg per 100 g) and ascorbic acid (75…82 mg per 100 g and 54…59 mg per 100 g). Broccoli also differed in enzymatic system more active than with Brussels sprouts (2…2.4 times).

Table 1

The content of biologically active substances in fresh chlorophyll-containing vegetables

Indicator	Broccoli	Brussels Sprouts
Mass fraction of chlorophyll	Value	
a	90,5±10,5	60,2±10,0
b	198±20,4	125±15,0
Mass fraction of β-carotene, mg/100g	9,0±1,0	10,8±1,5
Mass fraction of L-ascorbic acid mg/100g	54±5	75±7
Polyphenols, mg/100g	380,2	310,4
Mass fraction of organic acids, %	0,3±0,05	0,4±0,05
The activity of polyphenol oxidase, 0,01N iodine solution	2,4±0,01	1,0±0,05
Peroxidase activity, 0,01N iodine solution	13,8±2,0	6,3±0,5
Moisture, %	85±0,5	83,6±0,5

Brussels sprouts differs in a higher content of carotene in comparison with broccoli (10.8…12.3 mg per 100 g and 9…10 mg per 100 g respectively), and ascorbic acid (75…82 mg per 100 g and 54…59 mg per 100 g respectively). Broccoli has more active enzymatic system than Brussels sprouts (2…2.4 times).

Thus, polyphenoloxidase activity in broccoli is around 2,4 0,01N of iodine solution, and in Brussels – around 1,0 0,01N of iodine solution, peroxidase activity 13.8 and 6,3 0,01N of iodine solution respectively.

It is also shown that chlorophyll-containing vegetables differ in a high content of phenolic compounds – oozes of tannin type with high antioxidant, immunomodulating and detoxifying properties. Therefore, chlorophyll-containing vegetables differ in high content of biologically active substances such as chlorophyll a and b, carotenoids, L-ascorbic acid and polyphenols differ by the amount of BAS.

It is established that the use of cryogenic freeze-quenching of chlorophyll-containing vegetables with high velocity (5…10° C per minute) to a final temperature -32...-35° C may both save chlorophyll, carotene, L-ascorbic acid, polyphenolic compounds, and get frozen vegetables of different chemical composition, better than fresh vegetables. In particular, by the content of BAS they are twice better and by some other indicators, three times better (table 2).

Thus, mass fraction of chlorophyll after cryogenic freezing increased 2…2,2 times, carotenoids 2…3 times, i.e. they are more completely extracted from raw materials from complex biopolymers of Nano-components of BAS to a free form, i.e. there is the effect of "enriching" the product and inactivation of oxidative and hydrolytic enzymes. The mechanism of this process is associated with significant cryodestruction of enzyme molecules and their active centers. More complete extraction mechanism of low BAS from frozen chlorophyll-containing vegetables is connected with the fact that during fast freezing inside the plant cells small ice crystals are formed. They destroy hydrogen bonds in Nano-complexes between the bound low-molecular BAS and Biopolymers. The number of BAS in their free state increases, which is recorded by chemical and spectroscopic methods. Besides, micro destruction of a cell biomembranes and destruction of Nano-complexes, cytoplasm biopolymers (namely, "protein-lipid-cellulose", etc.) occur, which promote better extraction of BAS from the bound state. At the same time, it is worth noting that during the vegetables defrosting no cell sap is lost. The obtained results became the basis for the development of cryogenic technology for obtaining frozen chlorophyll-containing vegetables – broccoli and Brussels sprouts.

It is shown that at freezing vegetables to the temperature of -18…-20° C slight losses of BAS occur (Table 2).

The increased extraction of BAS from frozen vegetables is confirmed in the study of infrared spectra. The changes are revealed in the frequency ranging from 3000-3650 cm-1, characteristic to stretching vibration of functional OH-groups in a free state, which participate in intramolecular and intermolecular hydrogen bonds , are included to free and bound moisture, phenolic compounds, tannins, proteins, sugars and so on during the comparison of fresh and chlorophyll-containing vegetables. It means that hydrogen bonds destroy in various Nano-complexes of biopolymer compounds from BAS and in biopolymers themselves. At this, the latter, transformed to their free state, extract fuller. Chemical methods of research specify and fix them by means of chemical methods of research.

Based on the experimental data, cryogenic freezing technology for chlorophyll-containing vegetables is elaborated. It differs from traditional technologies by activating high speed freezing to much lower temperatures than it is accepted in international practice (to -35° C).

Table 2

Effect of cryogenic freeze-quenching with the use of high speed freezing to different final temperatures of chlorophyll-containing vegetables per mass fraction of chlorophyll, carotenoids, L-ascorbic acid

Name of research objects	Mass fraction									
	Chlorophyll				Carotene		L-ascorbic acid		Polyphones	
	a		b							
	mg in 100 g	% to raw materials	mg in 100 g	% to raw materials	mg in 100 g	% to raw materials	mg in 100 g	% to raw materials	mg in 100 g	% to raw materials
Fresh broccoli	87,6	100,0	195,0	100,0	8,8	100	52,0	100,0	380,2	100
Frozen broccoli to -18° C	90,1	102,8	191,2	97,4	17,0	193,2	49,1	94,4	372	98,0
Frozen broccoli to -35° C	198,6	226,7	398,8	205,0	26,1	296,5	101,4	195,0	680,9	180,5
Fresh Brussels sprouts	58	100,0	120	100	10,5	100	75,6	100,0	310,4	100
Frozen Brussels sprouts to -18° C	57,4	99,4	118,4	98,7	18,7	178,1	74,2	98,7	302,6	97,5
Frozen Brussels sprouts to -18° C	116,2	200,4	258,7	215,6	25,9	246,7	140,4	185,7	579,7	187,2

New technology makes it possible to get frozen vegetables with record performance and quality in BAS contents, such as chlorophyll a and b, carotenoids, L-ascorbic acid, polyphenolic substances exceeding the initial fresh vegetables 2…3 times. The quality of frozen vegetables exceeds domestic and foreign counterparts.

Thus, frozen chlorophyll-containing vegetables obtained be means of cryogenic shock freezing with the use of liquid and gaseous nitrogen allows to obtain a new product that cannot be received based on traditional freeze-quenching during the refrigeration of fruit and vegetable raw materials. Various kinds of healthy foods are developed with the use of frozen vegetables (soups – mashed potatoes, vegetable stews, vegetable soups, hot salads, side dishes to meat dishes, etc.).

Conclusions. The authors proposed and developed cryogenic technology of frozen chlorophyll-containing vegetables, which differs from the traditional application of cryogenic freezer with the use of liquid and gaseous nitrogen.

The new technology makes it possible both to preserve all BAS, and fuller reveal biological potential of raw material, i.e. remove their hidden forms (linked to Nano-complex biopolymers, minerals and tannins) 2…3 times more comparing with fresh vegetables and all known analogues. New technologies are tested in the manufacturing environment in the "KRIAS" SME. Project documentation (TC and TI) for "Chlorophyll-containing frozen vegetables" are meant for healthy eating.

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