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THE STUDY OF BAS COMPLEX IN CHLOROPHYLL-CONTAINING VEGETABLES AND DEVELOPMENT OF HEALTH-IMPROVING NANOPRODUCTS BY A DEEP PROCESSING METHOD

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Визначено комплекс БАР хлорофілвмісних овочів та розроблено оздоровчі нанопродукти з високим вмістом хлорофілу. Як інновацію використано комплексну дію на сировину процесів паротермічної обробки та механолізу із застосуванням нового покоління обладнання для теплової обробки та дрібнодисперсного подрібнення. Встановлено існування прихованих форм хлорофілів. Показано, що застосування зазначених інновацій дозволяє додатково вилучити хлорофіли із прихованої форми (в 2…2,3 рази більше)

Ключові слова: хлорофілвмісні овочі, комплекс БАР, оздоровчі нанопродукти, паротермічна обробка, механоліз, приховані форми хлорофілів

Определен комплекс БАВ хлорофиллсодержащих овощей и разработаны оздоровительные нанопродукты с высоким содержанием хлорофилла. Как инновацию использовано комплексное воздействие на сырье процессов паротермической обработки и механолиза с применением нового поколения оборудования для тепловой обработки и мелкодисперсного измельчения. Установлено существование скрытых форм хлорофиллов. Показано, что применение указанных инноваций позволяет дополнительно извлечь хлорофиллы из скрытой формы (в 2…2,3 раза больше)

Ключевые слова: хлорофиллсодержащие овощи, комплекс БАВ, оздоровительные нанопродукты, паротермическая обработка, механолиз, скрытые формы хлорофиллов

1. Introduction

The relevance of this work relates to the necessity of solving the global problem of falling people's immunity in many countries [1]. The problem is caused by shortage (about 50 %) of bioactive substances (BAS) in diets, famine (every eighth inhabitant of the Earth is starving). It is aggravated by general worsening of ecological situation in the world [2]. Improvement of immunity can be achieved by consuming BAS rich products (vitamins, β-carotene, proteins, minerals, chlorophylls, bioflavonoids, etc.) which contribute to immunity strengthening [3]. The main source of these BAS includes fruits, berries, vegetables, medicinal herbs, spice and aromatic plants, honey products. That is why health-improving products, especially those prepared from fruits, berries and vegetables are used to strengthen immune system in the leading countries of the world [4]. Scientists in the leading countries, in particular in Japan, the USA, Germany, the Netherlands, etc. are developing technologies for production of such products [1]. It is one of the priority, relevant and intensively developing scientific areas in the food industry.

Among the biologically active substances helping to strengthen immunity, chlorophylls occupy a special place. According to the latest data from Japanese scientists, "Vegetable food is the basis of human health and chlorophyll is the basis of vegetable food and the whole plant world. Chlorophyll is a product of solar energy that gives the human body impetus to movement and youth." The content of chlorophyll in plants is 0.008...0.8 % [5].

According to scientists from leading medical institutions in the USA, the Netherlands, India, Japan, Sweden, chlorophyll performs various protective and regulatory functions in the human body. Among such functions are, before all, cleaning (detoxification and removal of harmful and toxic substances from the body); regulation of acid-alkaline pH-balance and the level of hormones, strengthening of immunity as well as antibacterial, geroprotective effect and strengthening of heart and brain vessels [6].

Chlorophyll blocks action of carcinogens, protects DNA from the damage caused by toxins and free oxidizing radicals. It is a natural remedy for cancer prevention [7].

Protective properties of chlorophyll are explained by its structure. The chemical structure of chlorophyll is close to a haem in the human blood hemoglobin and is a porphyrin ring. The feature of its molecule structure consists in that there is an atom of iron in the central part of the haem, like magnesium in chlorophyll [8]. The protective effect of chlorophylls is explained by the fact that they belong to unsaturated reactively active conjugated compounds. In combination with L-ascorbic acid, β-carotene and phenolic compounds, they block negative processes occurring in the body under the action of carcinogens, free oxidative radicals, aging processes and other factors.

The sources of chlorophyll in the diet include fresh chlorophyll-containing vegetables (CCV): broccoli, spinach, salad, dill, parsley, etc. and the products prepared with their use [5]. It is known that there are significant losses of chlorophyll and other BAS during processing of CCV. Therefore, the search for technological methods ensuring preservation of BAS of chlorophyll-containing vegetables and development of health-improving nanoproducts with high content of chlorophyll are relevant.

2. Literature review and problem statement

Despite the unique healing properties of chlorophyll, chlorophyll-containing vegetables are not widely used in human diet. This is partially because of lack of advertising in the media about healing properties of the CCV chlorophyll and information on the recommended daily consumption rates. Among the CCV, green thick soups, green cocktail drinks, smoothies, fresh salads, etc. are popular. Conventionally, lettuce, parsley, dill and spinach are used mainly in a fresh form and various types of chlorophyll-containing cabbage, beans, green peas are used in a processed form. According to the published data, content of *a* chlorophyll in CCV is 380...420 mg in 100 g and content of *b* chlorophyll is 420... 580 mg in 100 g [9].

It should be noted that chlorophylls are unstable compounds which rapidly degrade during processing, get discolored or brownish and lose their useful properties [10]. Loss of chlorophylls during processing of CCO under influence of heat, light, oxygen, pH-media makes up from 20 to 100 %. Product darkening under the influence of these factors is explained by the reaction of substitution of hydrogen for complex-conjugated magnesium in chlorophyll molecules. In this process, a substance of brown color (pheophytin) is formed [9]. Therefore, the problem of preserving and stabilizing chlorophyll as well as green color of the products prepared from CCV is urgent.

In the scientific literature, there are disembodied data on the methods for extraction and stabilization of chlorophylls in processing CCV of certain types [8, 10]. For example, influence of various types of processing on preservation of chlorophylls in conversion of green spinach into food pigments and enriching additives is studied. Influence of processing with the use of enzyme preparations, surfactants, fatty components, etc. has been investigated. However, there are no established methods for such processing of CCV which would make it possible to preserve and stabilize chlorophylls of the starting stock [10]. According to the literature, loss of chlorophyll in thermal processing of CCV is from 40 to 100 % [6, 8]. It was shown that amount of chlorophyll is reduced by 35 % in frozen spinach and another 50 % is lost in its defrosting [9, 10]. Daily human demand in chlorophyll is 100...300 mg [1]. Therefore, consumption of green vegetables in a fresh, unprocessed condition is a reliable way to obtaining chlorophyll in the people's diet.

Conventional techniques of conversion of CCV and other types of fruit and vegetable stock to finished products result in significant losses of chlorophyll and other substances of fresh stock BAS. This results in inefficient use of the biological potential of the fresh stock. Processing losses amount to 20 to 80 % for various types of raw materials and BAS [5]. Therefore, the search for new technologies and development of high technologies that will help preserve and use quality of fresh raw materials are relevant.

Analysis of the present-day technologies for preparation of food products including CCV which were developed and implemented in the last 10 years is made in [11]. These technologies are based on application of new food making processes which are realized under the influence of high pressure, pulsed electric field, ultrasound and megasound, cavitation extraction at a negative pressure and hyperbaric storage. Compared to conventional technologies, they make it possible to materially intensify the process and reduce total time of product manufacture. In addition, they can significantly reduce energy costs and produce high-quality products. Scientists usually understand quality as the food safety and attractive appearance of the product but not the food and biological value [11].

Freezing belongs to the breakthrough methods of processing plant raw materials which preserve quality of fresh raw materials in BAS content [5]. There are conflicting results of studies for individual CCV types. Data from periodical literature relate mainly to the statement of BAS losses in freezing CCV and other types of plant raw materials. Besides, they are devoted to the study of influence of freezing conditions on cell structure and loss of cell juice while defrosting. For example, the effect of freezing storage on green beans and cauliflower was studied for 9 months at −10, −18, −26 °C. Loss of ascorbic acid and chlorophyll content and darkening of vegetables were also elucidated. Maximum losses and discoloring were observed in samples stored at –10 °C. It was found that the rate of loss of ascorbic acid in the samples stored at a temperature of –10 °C was three times higher than in the samples stored at –18 °C [12]. Influence of final temperature and duration of freezing spinach on its cell structure and loss of cellular juice during defrosting were studied. Studies were conducted in freezing periods of 0.5; 3.0; 5.5; 10.5 hours to the final product temperature of –4.0...–5.0 °C. It has been shown that the higher the speed and the duration of freezing the smaller damage of cell structure and loss of spinach cell juice during freezing-defrosting [13].

The problem of preservation and stabilization of BAS in CCV (broccoli, Brussels sprouts, spinach) was solved during development of a cryogenic deep processing method and technologies of frozen food products. The method is based on the use of cryogenic freezing using both refrigerant and inert medium of liquid and gaseous nitrogen [14]. The new method differed from the conventional freezing methods by the use of high freezing rates (2.0...10.0 °C/min) and lower final temperatures in the frozen product (–32.0...–35.0 °C) than those adopted in international practice (–18 °C). The use of cryogenic freezing has made it possible to fully preserve *a* and *b* chlorophylls of the raw material and also additionally extract chlorophylls from a hidden (bonded with biopolymers) form to a free form. It was established that the mass fraction of chlorophyll in CCV frozen by cryogenic method is 2.0...2.5 times more than in raw materials. This is controlled by chemical methods [14]. There is a conventional opinion that about 5...10 % of substances are found in vegetable raw materials in a bound with biopolymers (hidden) form. The obtained results have made it possible to conclude that the amount of useful substances in a bound form is significantly higher. Thus, it can be asserted that the predominant amount of chlorophyll is found in chlorophyll-containing vegetables in a bound with biopolymers (hidden) form. It was shown that the content of chlorophyll and other BAS in cryopreserved CCV is 2.0...2.5 times higher than that in fresh vegetables and known frozen counterparts. It has been established that the use of cryogenic shredding of frozen BAS leads to an additional (3.0... 3.5-fold) growth of content of *a* and *b* chlorophyll and other BAS, that is, the hidden, bound with biopolymers inactive forms are released [14]. With application of the deep processing methods based on freezing and fine-dispersed refining, nanotechnologies of health-improving products in a form of frozen puree with carotene-containing vegetables [15], mushrooms [16] and Jerusalem artichoke [17] were developed. In the BAS content, quality of the obtained frozen products is several times higher than that of fresh raw materials and known counterparts.

Heat treatment is the most common method of processing food raw materials [18, 19]. As was noted, conventional heat treatment methods, including heat treatment of CCV, result in significant losses of chlorophyll and other BAS. For example, destructive effect of blanching and various types of drying (with deep infrared radiation, vacuum, sublimation) on the content of carotene, ascorbic acid, chlorophyll was established for spinach leaves [20].

In recent years, a new generation of modern thermal equipment, steam convection ovens, has appeared and is widely used in food establishments, in particular in restaurants [21, 22]. The above equipment enables combination of three processes, e. g. steaming, frying and cooking in one apparatus and obtaining of high-quality products with an attractive appearance [23]. In literature, quality is usually understood as organoleptic and physicochemical characteristics of the product and not the BAS content [24]. For certain types of food raw materials, we have found that the use of steam convection processing in the manufacture of culinary products can almost completely preserve quality of raw materials in the content of carotenoids, ascorbic acid, phenolic compounds [25]. Quality of the products made from carotene-containing vegetables [26] and peas [27] exceeds quality of counterparts prepared with the use of conventional equipment. Thus, it was shown that processing in an automatic steam convection oven ensures better conservation and use of the biopotential stored in fresh raw materials.

However, a question remains about the impact of various types of heat treatment on the CCV quality as for the content of chlorophyll and other BAS in processing of fresh CCV. It was not determined which processing techniques allow producers to maximally preserve quality of the original chlorophyll-containing raw materials. Such data are necessary in formulation of dietary plans with the use of fresh chlorophyll-containing vegetables and products prepared from them for the health-improving purposes.

The tasks of this work included study of the BAS complex in chlorophyll-containing vegetables as raw materials for preparation of health-improving nanoproducts by the method of deep processing. The method is based on a combined action of the processes of steam treatment and mechanolysis during fine-dispersed refining. Application of this method in preparation of products from carotene-containing raw materials and mushrooms has made it possible to preserve the biological potential of fresh raw materials as much as possible. It was assumed that the method of deep processing will also ensure preservation of the CCV quality as for the content of chlorophyll and other BAS.

3. The aim and objectives of the study

The study objective was to determine the BAS complex of chlorophyll-containing vegetables and study influence of the combined action of the processes of steam thermal treatment and mechanolysis on chlorophylls and other BAS in the development of health-improving nanoproducts from chlorophyll-containing vegetables using deep processing. The following chlorophyll-containing vegetables were used: broccoli, spinach, Brussels sprouts, green beans. To achieve this objective, the following tasks had to be solved:

– to determine content of biologically active substances (*a* and *b* chlorophylls, β-carotene, low-molecular and high-molecular phenolic compounds, ascorbic acid, etc.) and prebiotics in chlorophyll-containing vegetables;

– to investigate influence of steam thermal treatment in modern devices, i.e. steam convection machines, on enzymatic and biochemical processes in chlorophyll-containing raw materials in comparison with conventional equipment;

– to scientifically substantiate parameters of steam thermal treatment and mechanolysis during preparation of finely dispersed puree in extraction of chlorophylls and other BAS from a hidden, bound in nanocomplexes with biopolymers form into a free form;

– to develop nanoproducts: fine-dispersed purees from chlorophyll-containing vegetables, thick green soup, nanodrinks, nanosorbets, dressings sauces; study quality as for the content of chlorophylls, other BAS and prebiotics and compare with the counterparts. Consider expediency of their application as products for health improving.

4. Materials and methods used in the study

4. 1. Materials and equipment used in the experimental studies

The studies were conducted at Kharkiv National University of Food and Trade (KhNUFT), Ukraine, the Department of Processing Fruits, Vegetables and Milk, in the research laboratory "Innovative Cryo- and Nanotechnologies for Plant Supplements and Health-improving Products". UNOX SPA XVC series steam convection oven (Italy) for steam thermal treatment was used. It had 70 programs differing in the modes of technological processing: temperature, intensity and amount of steam supply, air circulation or air blow (Fig. 1). Robot Coupe (France) shredder was used for fine-dispersed fragmentation (Fig. 2).

Fig. 1. UNOX SPA XVC series steam convection oven (Italy)

Fig. 2. Robot Coupe shredder (France)

As the study objects, chlorophyll-containing vegetables were used: broccoli (*Brassica oberacca*), Brussels sprouts (*Brassica oberacca, var gemmifera*), spinach (*Spinacia oberacca*), green beans (*Phaseolus*). Besides, health-improving nanoproducts of green color derived from chlorophyll-containing vegetables were used as the study objects: fine-dispersed purees, thick soups, drinks, sorbets, dressing sauces.

The study equipment and objects as well as the procedures used in experiments are presented in more detail in [28].

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

The content of BAS (*a* and *b* chlorophyll, β-carotene, low-molecular phenolic compounds as for chlorogenic acid and routine, high-molecular phenolic compounds as for tannin and L-ascorbic acid) were used as criteria for estimating quality of fresh and heat-treated CCV and health-improving nanoproducts prepared from them. Besides, total protein and pectin substances, soluble pectin, cellulose, total sugars, organic acids, minerals (K, Ca, Mg, P, Na) were monitored. Enzymatic processes were controlled by determining the enzymatic activity of oxidative enzymes, in particular peroxidase and polyphenol oxidase.

Besides the commonly used physicochemical, biochemical, spectroscopic study methods, an original method of determining assimilation of products by living organisms, namely the method of biotesting was used [5].

Experimental methods were conducted with a fivefold repetition. The obtained results are presented in units of the International SI system.

5. Study of the BAS complex in CCV and development of health-improving nanoproducts by the method of deep processing

Chlorophyll-containing vegetables known for their healing properties were selected as raw materials for preparation of health-improving nanoproducts. The BAS complex in chlorophyll-containing vegetables was determined. According to Table 1, the mentioned BAS complex in fresh CCV predominantly included *a* and *b* chlorophyll with mass fraction varying from 0.3 to 0.6 % depending on the type of raw material as well as polyphenols (240.0...400.0 mg per 100 g), low-molecular phenolic compounds (190.0...320.0 mg per 100 g), L-ascorbic acid (40.0...130.0 mg per 100 g), β-carotene (3.6...15.0 mg per 100 g). Chlorophyll-containing vegetables also feature a significant content of prebiotic substances, in particular, cellulose (1.8...5.2 %) and pectin substances (1.0...3.0%). In addition, CCV contain a significant amount of protein (4.2...5.5 %) and total sugar (4.8...7.6 %).

Thus, in authors judgment, availability of a unique BAS complex (chlorophyll, β-carotene, phenolic compounds, polyphenols, L-ascorbic acid) in 100 grams of fresh CCV in a quantity able to satisfy the daily demand of human organism imparts therapeutic and prophylactic properties to the fresh CCV. Inclusion of CCV in human diet gives antioxidant, detoxifying, antibacterial, antitumor action, promotes strengthening of the immune system and vessels of heart, brain, etc.

It should be noted that CCV (broccoli, Brussels cabbage, green beans, spinach) in diets are mainly in the processed form (after heat treatment, shredding, etc.).

It is known that conventional types of steam thermal treatment (cooking, blanching) of fruits and vegetables lead to destruction and oxidation of BAS (L-ascorbic acid, phenolic compounds, chlorophylls, carotenoids, etc.). In this case, the degree of BAS destruction depends on temperature of steam thermal treatment and occurs mainly because of action of oxidative enzymes of raw materials (peroxidase, polyphenol oxidase, ascorbinoxidase, etc.). According to [5], activation of oxidative enzymes takes place during steam thermal treatment. In comparison with the initial (fresh) raw material, this activity grows 4.0...5.5 times. Activation of enzyme molecules having protein nature is due to an increase in kinetic energy of molecules when the product is heated. In parallel, significant degradation and oxidation of low-molecular biologically active substances occurs. When the product undergoes heating, destruction (breakage, coagulation) of the protein component of oxidative enzymes, destruction of active centers and inactivation of enzymes are gradually taking place. At a complete inactivation of enzymes in the product, oxidative processes stop resulting in BAS destruction and degradation of its quality.

Influence of steam-thermal treatment in modern devices (such as steam convection ovens) on enzymatic and biochemical processes in CCV in comparison with steam-thermal treatment in conventional equipment has been studied. As can be seen from Tables 2 and 3, the enzymatic and biochemical processes in CCV occur in different ways.

Oxidative enzymatic processes in chlorophyll-containing vegetables during processing in an automatic steam convection oven occur with a significantly lower intensity than in conventional cooking and blanching (Table 2). As can be seen from Table 2, in the steam thermal treatment of CCV for 5 minutes in an automatic steam convection oven, enzymatic activity of peroxidase and polyphenol oxidase was at a level of 10 % in broccoli and Brussels spouts and almost completely exhausted in spinach. After treatment of CCV in an automatic steam convection oven for 10 minutes, complete inactivation of oxidative enzymes occurred. There was almost a 2-fold decrease in enzymatic activity during treatment of CCV for 10 minutes in the conventional equipment. Activity of peroxidase remained at 49…51 %.

The main thing in processing CCV into health-improving nanoproducts was to maximally preserve chlorophylls and other BAS of the starting (fresh) raw materials.

Influence of the processes of steam thermal and conventional heat treatment of CCV on the content of BAS (chlorophyll, β-carotene, ascorbic acid, phenolic compounds) was investigated. According to Table 3 and Fig. 5, during steam thermal treatment of CCV in a steam convection oven, not only preservation of *a* and b chlorophylls but also their more complete in comparison with fresh vegetables (1.33…1.4 times) extraction from the hidden form occured in 5 minutes. The mechanism of this process is connected with inactivation of oxidative enzymes and thermal destruction (thermolysis) of hydrogen and other bonds between hidden forms of chlorophylls in nanocomplexes with biopolymers (polysaccharides, proteins, etc.). Transformation into a free form took place which was defined by chemical research methods. As can be seen from Table 3 and Fig. 6, in comparison with fresh CCV, a 2-fold increase in extraction of β-carotene from the bound form occured during steam thermal treatment. For example, mass fraction of β-carotene in 100 g of fresh spinach was 10.5 mg. This figure grew to 21.2 mg after processing in a steam convection automatic oven for 5 minutes. For 100 grams of fresh and thermally treated broccoli, mass fraction of β-carotene was 9 mg and 17.5 mg, respectively, and 4.8 and 9.8 mg respectively for green beans (Table 2 and Fig. 1).

According to Table 2, the loss of vitamin C in CCV treated in an automatic steam convection oven for 5 minutes was much lower (10–15 %) than the loss in the conventional heat treatment (30…50 %). Loss of low-molecular phenolic compounds and polyphenols in CCV treated in an automatic steam convection oven for 5 minutes was 10…20 % (Table 3).

Table 1

Content of prebiotic and mineral substances in the BAS complex and physicochemical indicators of fresh chlorophyll-containing vegetables

Indicator name, mg per 100 g	Chlorophyll-containing vegetables			
	broccoli	Brussels sprouts	green bean	spinach
a chlorophyll	87.6…106.0	58.0…80.0	88.6…90.1	147.4…380.0
b chlorophyll	195.0…280.0	120.0…130.0	198.0…200.0	208.0…420.0
β-carotene	5.0…8.0	6.0…7.0	3.6…4.8	9.8…15.0
L-ascorbic acid	52.0…80.0	56.2…90.0	40.0…60.0	60.0…130.0
Phenolic compounds (by chlorogenic acid)	240.0…270.0	210.0…230.0	190.0…240.0	240.0…320.0
Phenolic glycosides (by rutene)	75.0…80.0	60.0…68.0	55.0…75.0	75.5…80.4
Tanning agents (by tannin)	340.0…380.0	310.0…400.0	240.0…245.0	310.0…340.0
Mineral substances (ash content), %	1.5…1.7	1.3…1.5	1.2…1.4	1.8…2.2
Potassium	490.0…510.0	380.0…420.0	410.0…440.0	775.0…820.0
Calcium	100.0…112.0	100.0…105.0	95.0…107.0	110.0…135.0
Magnesium	85.0…99.0	42.0…50.0	65.0…78.0	95.0…108.0
Phosphorus	86.0…92.0	80.0…90.0	82.0…95.0	85.0…99.0
Sodium	35.0…48.0	8.0…10.8	25.0…30.0	65.0…75.0
Total pectin, %	1.5…2.5	1.0…1.8	2.0…2.5	2.5…3.0
Protopectin, %	1.3…1.9	0.8…0.9	0.5…0.7	1.8…2.0
Soluble pectin, %	0.4…0.6	0.2…0.4	1.5…1.8	1.0…1.1
Cellulose, %	2.5…3.0	1.8…2.5	4.5…5.2	2.0…2.4
Proteins, %	4.9…5.5	4.8…5.0	4.2…5.0	4.6…5.2
Organic acids, %	0.7…1.1	0.8…1.0	0.5…0.6	0.6…0.8
Total sugar, %	7.0…7.6	6.8…7.4	6.5…7.0	4.8…6.8
Glucose+fructose, %	5.1…5.8	5.4…6.0	5.0…5.5	2.0…2.8
Dry matter, %	14.2…15.0	14.0…14.5	12.0…12.5	14.5…14.8

Table 2

Effect of steam thermal treatment, conventional boiling and fine-dispersed shredding on activity of oxidative enzymes and L-ascorbic acid of chlorophyll-containing vegetables

Product	L-ascorbic acid		Oxidative enzymes			
			polyphenoloxidase		peroxidase	
	mg in 100 g	% to the initial raw material	ml 0,01 N iodine to DS	% to the initial raw material	ml 0,01 N iodine to DS	% to the initial raw material
Broccoli						
Fresh	65.0	100.0	108.0	100.0	25.8	100.0
After steam-thermal treatment in an automatic steam convection oven during 5 min	60.2	90.2	105.8	10.0	2.6	29.8
during 10 min	52.4	80.9	0	0	0	0
Fine-dispersed puree from steam thermal treated raw material	102.3	155.0	0	0	0	0
After conventional boiling during 10 min	45.6	69.1	50.9	51.0	13.6	51.0
Brussels spouts						
Fresh	56.8	100.0	42.5	100.0	15.4	100.0
After steam thermal treatment in an automatic steam convection oven during 5 min	50.2	89.8	4.2	10.1	5.12	30.0
during 10 min	46.1	80.5	0	0	0	0
Fine-dispersed puree from steam thermal treated raw material	98.2	179.5	1.0	2.3	0.5	0.9
After conventional boiling during 10 min	40.3	68.8	20.0	48.5	7.5	50.0
Spinach						
Fresh	75.3	100.0	103.5	100.0	37.5	100.0
After steam thermal treatment in an automatic steam convection oven during 5 min	65.2	85.3	2.0	1.0	0.1	0.1
Fine-dispersed puree from steam thermal treated raw material	150.4	200.0	0	0	0	0
After conventional boiling during 10 min	38.6	50.0	39.4	40.0	18.5	49.2

Table 3

Influence of steam thermal treatment and fine-dispersed shredding of chlorophyll-containing vegetables on the content of biologically active substances

Product	Mass fraction							
	chlorophylls				β-carotene		phenolic compounds*	
	a		b					
	mg in 100 g	% to the starting raw material	mg in 100 g	% to the starting raw material	mg in 100 g	% to the starting raw material	mg in 100 g	% to the starting raw material
Broccoli								
Fresh	98.0	100.0	195.0	100.0	9.0	100.0	270.0	100.0
After steam thermal treatment in an automatic steam convection oven during 5 min	138.0	140.2	275.3	141.2	17.5	194.5	290.0	8.0
Fine-dispersed puree from steam thermal treated raw material	205.0	209.2	390.6	200.1	30.2	333.0	375.0	140.0
Brussels sprouts								
Fresh	60.2	100.0	125.5	100.0	6.2	100.0	210.0	100.0
After steam thermal treatment in an automatic steam convection oven during 5 min	81.4	135.0	175.6	140.2	12.6	200.0	185.0	88.1
Fine-dispersed puree from steam thermal treated raw material	121.4	200.1	261.2	208.5	20.6	330.0	315.0	150.0
Spinach								
Fresh	147.4	100.0	280.0	100.0	10.5	100.0	280.4	100.0
After steam thermal treatment in an automatic steam convection oven during 5 min	198.1	140.0	380.5	136.5	21.2	201.1	340.5	115.2
Fine-dispersed puree from steam thermal treated raw material	301.8	205.1	565.2	201.5	32.3	302.4	421.8	145.0
Green bean								
Fresh	88.6	100.0	198.0	100.0	4.8	100.0	190.2	100.0
After steam-thermal treatment in an automatic steam convection oven during 5 min	124.2	141.2	277.2	138.0	9.8	201.2	170.6	80.5
Fine-dispersed puree from steam thermal treated raw material	203.8	230.0	405.2	100.0	12.2	240.0	205.0	150.0

*Note: * – phenolic compounds (by chlorogenic acid), polyphenol oxidase: by 40...50 % (Table 2). It was established that activity of oxydative enzymes in obtained fine-dispersed puree after steam thermal treatment of CCV was completely absent in 10 minutes. Absence of action of oxidative enzymes will contribute to maintaining quality by the BAS contents and stability of the finished product*

Thus, not only preservation of *a* and *b* chlorophylls and β-carotene but also thermal destruction and extraction of hidden forms occured under certain conditions during steam thermal treatment of CCV in a steam convection oven. For example, mass fraction of chlorophyll increased in 1,33...1,4 times and in 2 times for β-carotene in comparison with fresh CCV (Table 3, Fig. 3, 4).

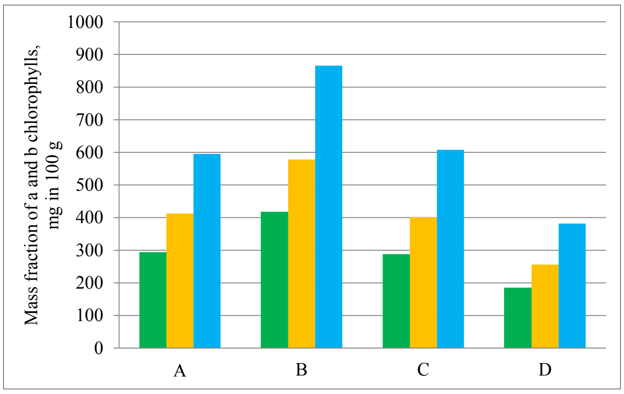

Fig. 3. Influence of combined action of steam thermal treatment and mechanolysis of chlorophyll-containing vegetables on preservation of *a* and *b* chlorophyll and transformation of its forms bound in nanocomplexes with biopolymers into a free form: fresh CCV (1), steam treated CCV (2), finely ground CCV (3); broccoli (A), spinach (B), green beans (C), Brussels sprouts (D)

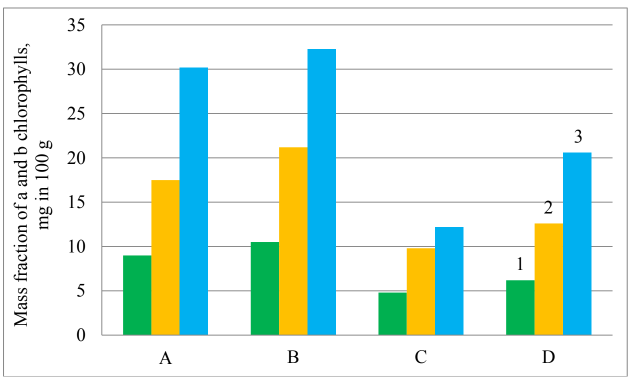

Fig. 4. Influence of combined action of steam thermal treatment and mechanolysis of chlorophyll-containing vegetables on preservation of β-carotene and its transformation from forms bound in nanocomplexes with biopolymers into a free form; fresh CCV (1), steam treated CCV (2), finely shredded CCV (3); broccoli (A), spinach (B), green bean (C), Brussels sprouts (D)

As it follows from Table 3, a significantly greater effect of extraction of hidden forms of chlorophyll, β-carotene, phenolic compounds and vitamin C was obtained in fine shredding of steam thermally treated CCV. It can be seen from Fig. 3, 4 that the bound form of heat-treated nanoproducts prepared from CCV contained 2.0...2.1 times more *a* and *b* chlorophylls and above 2.0...3.3 times more carotenoids than it was possible to extract to date from plant raw materials.

According to the data of Tables 2 and 3, during fine shredding of steam thermally treated CCV, a higher mass fraction of L-ascorbic acid (1.5...2.0 times) and phenolic compounds (1.3...1.5 times) and other BAS was observed compared with fresh raw materials. This increase compared with fresh raw material is due to the extraction during fine shredding of hidden forms.

6. Discussion of results obtained in the study of the BAS complex of chlorophyll-containing vegetables and development of health-improving nanoproducts

Content of biologically active substances (*a* and *b* chlorophylls, β-carotene, low-molecular and high-molecular phenolic compounds, ascorbic acid, etc.), prebiotics in chlorophyll-containing vegetables (broccoli, spinach, Brussels sprouts, green beans) was determined. Presence of the BAS complex (chlorophyll, phenolic compounds, L-ascorbic acid, β-carotene) in 100 grams of fresh CCV in a quantity capable of meeting daily demand of the human body was found. That is why CCV were used as raw materials for development of health-improving nanoproducts.

It was established that in comparison with boiling in conventional equipment, inactivation of oxidative enzymes during steam thermal treatment of CCV in an automatic steam convection oven occured more intensively. It was shown that there was a complete inactivation of enzymes after 10 minutes of steam thermal treatment of CCV. In this regard, it was assumed that treatment of CCV in an automatic steam convection oven ensures better preservation of biologically active substances.

This study advantage consists in that the innovations used have made it possible to discover hidden forms of chlorophylls and carotenoids in CCV. Conditions of steam thermal treatment of CCV were established in which there is not only preservation of *a* and *b* chlorophylls and β-carotene but also thermal destruction and extraction of hidden forms. In comparison with fresh CCV, mass fraction of chlorophyll increased in 1.33...1.4 times and β-carotene in 2 times. This was due to inactivation of oxidative enzymes and thermodestruction of hydrogen and other bonds between hidden forms of chlorophylls and biopolymers (polysaccharides, proteins, etc.) in nanocomplexes. Transformation of hidden forms of chlorophyll into a free form was detected by chemical methods. It has been established that significantly greater effect of extraction of hidden forms of BAS was achived in a case of fine shredding of thermally treated CCV. For chlorophyll and β-carotene, this increase in the production of fine-dispersed purees prepared from CCV amounts 2.0...2.1 times and 2.0...3.3 times, respectively. Thus, with the help of the innovations used, it was possible to establish existence of hidden forms of chlorophylls and carotenoids and transform the product into an easily digestible nanoform. This is the novelty, uniqueness and uncommonness of the study results presented in the paper.

It was established that quality of the fine-dispersed purees prepared from chlorophyll-containing vegetables obtained using these innovations exceeded quality of the raw material. In addition, it significantly exceeds quality of the counterpart purees prepared using conventional heat treatment and shredding methods in which loss of BAS is from 20 to 80 % [6, 8].

On the basis of fine-dispersed purees prepared from CCV, a wide range of health-improving green products in an easily digestible nanosized form has been developed. Thick purees, nanodrinks, nanosorbets, dressings sauces were developed. By content of the BAR complex (chlorophyll, ascorbic acid, β-carotene, phenolic compounds, etc.) the new types of nanoproducts exceeded existing ones. It was established that one portion of the product contained from 1/3 to one day human demand of BAS. By the BAS content, the new types of products can be defined as health-improving products and recommended for immune prophylaxis of people.

Disadvantages and peculiarities of processing chlorophyll-containing vegetables include darkening of products. This is caused by replacement of complex-bound magnesium with hydrogen in molecules of the green pigment of chlorophyll. Such transformations occur under the influence of heat, light, oxygen, medium pH or conventional heat treatment of CCV. In such conditions, a substance of brown color (pheophytin) is formed and the mass fraction of chlorophyll is significantly reduced. Loss of chlorophyll under the influence of these factors is from 20 to 100 %. The method of deep processing of CCV proposed by the authors makes it possible to preserve chlorophyll of the starting fresh raw material and prevent the processes of product darkening. In addition, the method enables extraction of hidden forms of chlorophyll and other BAS and obtaining products of green color with a high content of chlorophyll.

What is planned for the future is:

– to develop a green line of high-quality products of stable consistency and long shelf life with the use of the fine-dispersed purees prepared from CCV;

– to conduct a study with application of chemical, spectroscopic, microbiological methods;

– to study the influence of chemical composition of the formulation components, conditions of preparation and introduction of the purees prepared from CCV as well as the conditions and the terms of storage of new products on the content of chlorophyll and other BAS, physicochemical, microbiological, structural and mechanical characteristics.

It should be pointed out that the study was limited by seasonality of growing CCV and a short shelf life of fresh chlorophyll-containing vegetables (1 month). Most of the year, frozen CCV are available which have already undergone freezing and storage in a frozen condition. This significantly impedes conduction of studies with fresh CCV.

7. Conclusions

1. The BAS complex in fresh chlorophyll-containing vegetables was determined. This complex consists predominantly of *a* and *b* chlorophylls (0.3...0.6 %) as well as polyphenols (240.0...400.0 mg per 100 g), low-molecular phenolic compounds (190.0...320.0 mg per 100 g), L-ascorbic acid (40.0...130.0 mg per 100 g), β-carotene (3.6...15.0 mg per 100 g). Chlorophyll-containing vegetables also feature a significant percentage of prebiotics, in particular, cellulose (1.8...5.2 %) and pectins (1.0...3.0 %). In addition, they contain a substantial amount of protein (4.2...5.5 %) and total sugars (4.8...7.6 %). In the authors' opinion, presence of a unique BAS complex (chlorophyll, phenolic compounds, L-ascorbic acid, β-carotene) in 100 g of fresh CCV in an amount capable of satisfying the daily demand of human organism gives the fresh CCV therapeutic and prophylactic properties.

2. It was established that the enzymatic and biochemical processes in steam thermal treatment of CCV with the use of a steam convection oven compared with thermal treatment with the use of conventional equipment proceed in different ways. It has been shown that oxidative processes under the influence of enzymes (peroxidase and polyphenol oxidase) in CCV treated in an automatic steam convection oven occur with a significantly lower (2–3 times) intensity. It was established that a 5–10 min treatment brings about a complete inactivation of enzymes and it is completely absent in the puree. It has been shown that activity of peroxidase and polyphenol oxidase in CCV is maintained at a level of 40.0...50.0 % after boiling for 10 min with the use of conventional equipment.

3. It was established that during steam thermal treatment of CCV in an automatic steam convection oven during 5 minutes, not only preservation of chlorophylls but also their more complete (1.35...1.4 times) extraction from the hidden form take place in comparison with fresh vegetables. The mechanism of this process is associated with inactivation of oxidative enzymes and thermal destruction of hydrogen and other bonds between hidden forms of chlorophylls in nanocomplexes with proteins and polysaccharides. It was also shown that in comparison with fresh CCV, in steam thermal treatment both in an automatic steam convection oven and conventional equipment, extraction of hidden forms of β-carotene (2 times more) took place. It was shown that the loss of vitamin C in CCV treated for 5 minutes in a steam convection oven was considerably lower (10.0...15.0 %) than the loss in conventional heat treatment (30.0...50.0 % higher).

4. It was established that application of the combined action of steam thermal treatment and fine-dispersed shredding of CCV results both in preservation of chlorophyll and its additional (2.0...2.3 times more) extraction in a free state from its hidden form in which it is bound with proteins and other biopolymers. Health-improving nanoproducts have been obtained from CCV: fine-dispersed purees, thick soups, nanodrinks, nanosorbets, dressing sauces. Their quality has been studied and a comparison with counterparts was made. It has been established that new types of products made from chlorophyll-containing vegetables exceed existing counterparts in the content of the BAS complex (chlorophyll, ascorbic acid, β-carotene, phenolic compounds, etc.). The obtained nanoproducts can be considered the products of health-improving action.

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