

EFFECT OF BLANCHING AND TREATMENT WITH A SALT SOLUTION ON THE BIOLOGICAL VALUE OF BROCCOLI

S. Belinska

Doctor of Technical Sciences, Professor,
Head of Department*

E-mail: prodtknteu.kiev.ua

N. Kamienieva

PhD, Associate Professor*

E-mail: nataliakameneva32@gmail.com

S. Levytska

Postgraduate student*

E-mail: levitskaya_stan@ukr.net

S. Rogalskiy

PhD, Associate Professor

Department of Crop Production

Uman National University of Horticulture

Institutska str., 1, Uman, Ukraine, 20305

E-mail: shark32008@yandex.ua

*Department of commodity science, safety
and quality management

Kyiv National University of

Trade and Economics

Kyoto str., 19, Kyiv, Ukraine, 02156

Досліджено та проведено аналіз складових біологічної цінності районованого в Україні сорту капусти броколі – Партенон. Наведено результати досліджень, проаналізовані зміни вмісту аскорбінової кислоти, ізотіоціанатів, пігментного складу, хлорофілу та β -каротину у свіжозібраній капусті броколі та залежно від способів її попередньої обробки перед заморожуванням: бланшуванням та витримуванням у розчині кухонної солі.

Доведено, що кращим способом попередньої обробки капусти броколі перед заморожуванням, який дозволяє максимально зберегти її вихідні споживні властивості, є витримування в 3 % розчині кухонної солі протягом 20 хв. Витримування у розчині солі сприяє максимальному збереженню біологічної цінності капусти броколі порівняно з бланшуванням.

Встановлено, що капуста броколі сорту Партенон містить 116,4 мг/100 аскорбінової кислоти. Після бланшування втрати становлять 19,3 %. Підтверджено значне зниження кількісного вмісту ізотіоціанатів – на 43 % порівняно з вмістом у вихідній сировині. Втрати хлорофілу становлять 26 %, зокрема *a*-хлорофілу – 10 %, *b*-хлорофілу – 16 %. Змінюється також співвідношення форм *a*- і *b*-хлорофілу.

Результати засвідчують, що при витримці у розчині кухонної солі втрати аскорбінової кислоти становлять 5 %, ізотіоціанатів – 28,2 %. Вміст β -каротину порівняно з вихідною сировиною не змінюється, а частка хлорофілу збільшується на 6,3 %. Змін співвідношення форм хлорофілу не відбувається. Причинами стабілізації вмісту аскорбінової кислоти є відсутність високотемпературної обробки та часткова інактивація аскорбінат– та поліфенолоксидази, що пояснюється здатністю хлоридів витіснити іони міді з мідьвмісних сполук, до яких належать ці ферменти.

Незначні втрати вітаміну С пояснюються водорозчинністю аскорбінової кислоти. Попередня обробка у розчині кухонної солі індукує підвищення вмісту *a*-хлорофілу та *b*-хлорофілу, що зумовлено компенсаторною реакцією

Ключові слова: капуста броколі, біологічно активні речовини, вітамін С, ізотіоціанати, хлорофіл, попередня обробка

1. Introduction

Providing consumers with a sufficient amount of fruit and vegetable produce, the level of consumption of which, according to WHO recommendations, is 400 g/per person/per day, is directly dependent on the agro-climatic conditions of cultivation [1]. Almost all countries of the world face a deficit of vitamins, minerals, full proteins, and other biologically active substances in the nutrition of people. The essential source of the above-mentioned substances is fresh fruits and vegetables, as well as products of their processing [2, 3]. According to data from the European Council on Food Information [4], in Europe, in recent decades, the supply of fruit and vegetable produce (except for potato and legumes) has increased by 35 %. The stocks in Northern Eu-

rope are lower than those in the South. Thus, for example, in Finland, the average stocks of fruit and vegetable produce provide 195 g/per person/per day, corresponding to 71 kg/per person/per year, while Greece has an average stock of 756 g/per person/per day (276 kg/per person/per year). In Ukraine, this indicator is 553 g/per person/per day (202 kg/per person/per year) [5]. Despite the described positive dynamics in terms of the increased stocks of fruits and vegetables, more than 40 % of people suffer from a so-called “latent hunger” due to the consumption of food products of low biological value. The deterioration of environmental situation, the intensification of agriculture, technological techniques for processing, do not contribute to the preservation of the content of biologically active substances in products.

Among the vegetables of cabbage family, broccoli deserves special attention. In the United States, Japan, Canada, Great Britain, France, broccoli, grown at large areas, is very popular and used in the treatment and prophylactic diet. The value of broccoli is predetermined by the content of ascorbic acid, vitamin K, chlorophyll, carotene, mineral elements, glucosinolates. In terms of the amino acid composition, the protein of broccoli is not inferior to the beef protein, while the presence of tryptophan, lysine and isoleucine brings it closer to the protein of the chicken egg.

A specific property of broccoli is its significant content of sulfur-containing substances – glucosinolates, which predetermine the specific flavor and taste of cabbage. Native glucosinolates in plant cells are low-active, but, when disrupting the integrity of cell structures, they are hydrolyzed into glucose and aglycones: isothiocyanates, thiocyanates or organic nitriles, oxazolidines and others [6]. The derivatives of glucosinolates, specifically sulforaphane, have antioxidant properties, they suppress the negative effect of free radicals on the human body and increase immunity [7, 8]. The above-mentioned facts confirm the expediency of the inclusion of broccoli to the diet regardless of the harvesting season.

However, it should be noted that broccoli is a seasonal vegetable and unsuitable for long-term storage. The optimal technique to preserve broccoli is the refrigeration. The issue on the feasibility of using other canning techniques is debatable because of significant losses of biologically active substances under the influence of light, high temperatures, pH of the medium. In order to stabilize the consumer properties of frozen fruit and vegetable products, the technology of low-temperature treatment employs various techniques for the pre-treatment of plant raw materials before freezing. The most common are blanching, aging in the solutions of acids and salts, osmotic dehydration, partial dehydration, the application of high pressure. The choice of the technique of pre-treatment is affected by the type of a plant raw material, patterns in the chemical composition, as well as the purpose of further utilization: direct consumption or processing.

Given the above, it is a relevant task to study the biological value of the varieties of broccoli cultivated in the regions of Ukraine. It is equally important to determine the optimal pre-treatment techniques before freezing, in order to ensure the stable quality of the frozen products during low-temperature storage.

2. Literature review and problem statement

Changes in the quality of fruit and vegetable raw materials during freezing and long-term low-temperature storage depend on a number of factors. The most influential are the varietal features of raw materials and technological parameters for preparing fruits and vegetables for freezing.

Scientists developed modern technologies and techniques for the pre-treatment (physical, chemical, and biochemical) of plant raw materials before freezing. Minimization of undesirable changes in the consumer properties of fruits and vegetables at low temperature preservation is mainly ensured by the inactivation of enzymes.

Effective techniques for the inactivation of plant tissue enzymes are blanching (with water or steam) and treatment with chemical reagents.

The benefits of the blanching of fruits and vegetables before freezing include the preservation of natural color, taste,

and flavor of frozen foods during long-term low-temperature storage [9, 10]. This is ensured by the inactivation of enzymes, specifically polyphenol oxidase, catalase, and peroxidase, predetermined by changing the native properties of proteins at thermal treatment. Reducing the enzymatic activity helps to minimize changes in the taste and flavor of fruits and vegetables by reducing the oxidation processes with the participation of molecular oxygen and the oxygen of organic peroxides. The most resistant to high temperatures are catalase and peroxidase. They are often used as indicators in the design of blanching modes. The least resistant to high temperatures is ascorbate- and polyphenol oxidase, the oxidation catalysts of vitamin C and polyphenolic compounds.

Scientists proved that the vegetative forms of microorganisms are partially destroyed during blanching. The content of nitrates and some pesticides is reduced because of their capability to dissolve in water [11]. The displacement of air from the intercellular space is accompanied by an increase in the intensity of the expression of color.

The addition of citric acid to water for blanching contributes to a decrease in the activity of ferments as there are changes to pH [12] and the threshold of thermal denaturation of enzymes [13].

At the same time, blanching and long-term high-temperature treatment are inappropriate for the chlorophyll-containing vegetables due to a change in the natural green color and the sensitivity of chlorophyll to the effect of high temperatures [14], pH, air oxygen, and light [15, 16]. Thus, in the acidic environment, following the replacement of complex-bound magnesium in the molecule of chlorophyll with hydrogen, there emerge the substances of brown coloration – pheophyta.

The scientific literature reveals that the most common techniques for stabilizing the chlorophyll content in chlorophyll-containing vegetables are the use of low temperatures; antioxidants, mainly fat-soluble [14, 15]; addition of salts of various metals (zinc, iron, copper) [16]. The principle of action of these stabilization techniques is based on a change in the spatial orientation of cell structures, coagulation, and denaturation of protein substances [17].

It was established in papers [18, 19] that the blanching of leafy vegetables (spinach, dill, parsley) in the presence of 30 mg of Zn^{2+} /100 g for 15 minutes contributes to the stabilization of the vegetable pigment complex. A decrease in the losses of water-soluble nutrients of spinach, carrot, and pepper, was found, which is ensured by blanching at a temperature of 95 °C using a pulse microwave oven [20].

Changes of color in the chlorophyll-containing vegetables are also related to the activity of chlorophyllase, which catalyzes the cleavage of phytol from chlorophyll and its magnesium-free derivatives, forming chlorophyllides and pheophorbides, respectively. The temperature of 60–76 °C is optimal for chlorophyllase. At temperatures above 80 °C the activity of chlorophyllase somewhat decreases, and at 100 °C, the enzyme is inactivated [16].

Along with the benefits of blanching as a technique for the pre-treatment of fruits and vegetables before freezing, certain shortcomings should be indicated as well. The main is the loss of valuable water-soluble and thermolabile substances, specifically ascorbic acid, the adsorption of water by the product. The hydrolysis of protopectin during blanching causes a loss of turgor by vegetative tissue, which significantly reduces the organoleptic properties of the product due to the softening of consistency.

Therefore, the issue of the feasibility of blanching as a technique for the pre-treatment is debatable.

There are commonly applied techniques for the pre-treatment of plant raw materials with solutions that have the antioxidant properties or contain substances that can form insoluble complex compounds. At present, aging of fruits and berries in the solutions of sodium and potassium metabisulfite is practically implemented; in the extracts from the bark of oak, birch, St.-John's-wort; in the infusion of green tea [21]. The addition of sodium and potassium chlorides during production of preserved green peas helps stabilize the content of natural pigments and biologically active substances [22].

However, the recommended techniques for the pre-treatment of plant raw materials with aqueous solutions of salts and acids do not reveal a comprehensive stabilizing effect: some of them contribute to the improvement of consistency (calcium chloride) while some act as antioxidants (preparations of sulfur, acid). At the same time, most of the above chemical compounds render strange flavors to the products, some are allergens [21].

Partial dehydration of fruits and vegetables at temperatures of 100 °C and above also ensures inactivation of the oxidation-reducing enzymes. In addition, the speed of the freezing process is accelerated.

Pre-treatment in the following solutions of osmotic-active substances has a positive effect on the consumer properties of fruits: sugar; a mixture of starch, glucose, fructose; a mixture of sugar and salt; a mixture of sugar and sorbitol; concentrated fruit juices [21]. At osmotic dehydration, a part of free moisture decreases while that of the colloid-bound moisture increases [23]. This contributes to the preservation of microstructure of tissues and stabilization of consistency [24]. The aging of apple, pumpkin, and carrot in a solution of sugar provides for the stabilization of quality during freezing and storing, though it changes the taste of fruits and vegetables [25]. Despite the benefits of osmotic dehydration, such as increasing the content of dry substances, preserving the vitamin value, color, and the tissue structure of fruits, the osmotic dehydration is a rather costly process from a technological point of view.

An analysis of information sources revealed that the pre-treatment of plant raw materials before freezing is accompanied by a number of complex physical-chemical processes. Strong factors that affect the rate of the course of the processes are the chemical composition of fruits and vegetables, and parameters of the pre-treatment. The use of certain technological techniques is economically impractical owing to the complicated technological processes. The scientific community pays sufficient attention to determining the optimal techniques and parameters for the pre-treatment.

This confirms our prospects for undertaking a research with respect to the anatomical-morphological attributes and patterns in the chemical composition of different kinds of fruits and vegetables.

3. The aim and objectives of the study

The aim of this work is to investigate the effect of blanching and aging in the solution of salt, as the techniques for the pre-treatment of broccoli before freezing, on the content of biologically active substances. That would make it possible to determine the optimal techniques and

parameters for the pre-treatment, in order to preserve the biological value of frozen broccoli.

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

- to determine the effect of blanching on the content of ascorbic acid, isothiocyanates, a- and b-chlorophyll, β -carotene in broccoli;

- to establish the optimal parameters for the pre-treatment of broccoli before freezing: the concentration of a food salt solution and the duration of aging, which ensure the stabilization of the content of ascorbic acid;

- to determine the effect of aging in a food salt solution on the content of ascorbic acid, isothiocyanates, a- and b-chlorophyll, β -carotene.

4. Materials and methods to study the biological value of broccoli

The research was conducted at the Department of Commodity Studies, Safety and Quality Management at Kyiv National Trade and Economic University (Ukraine).

The object of the study is broccoli of the variety Parthenon [26], grown at the fields of LLC ARTI (Ukraine, Kharkiv Oblast) (Fig. 1).

Broccoli heads (not larger than 20.0 cm from the cut of the flower stem to the top of the head) are washed, cut



Fig. 1. Broccoli of the variety Parthenon

into inflorescences with a leg (not larger than 2.0 cm).

The samples were prepared to be studied according to GOST 26313 [27].

Indicators were determined using the following methods:

- the content of ascorbic acid – by the iodometric method [28];

- the content of a- and b-chlorophyll, β -carotene – by the spectrophotometric method at the spectrophotometer Specord 210 [29] (Fig. 2);

- the content of isothiocyanates – by the photo-colorimetric method [30] (Fig. 2);

- the activity of ascorbate oxidase and polyphenol oxidase – using the method by H. N. Pochynok [31].

The above-mentioned indicators were determined for the freshly harvested broccoli after blanching in water at 85 °C for 3 minutes (experiment 1) and for the broccoli, which was aged in a 3-% food salt solution for 20 minutes (experiment 2).

Results of the research were mathematically and statistically processed [32, 33].



Fig. 2. Spectrophotometer Specord 210

5. Results of research into biological value of broccoli

Experimental studies confirmed that broccoli is a valuable source of biologically active substances. It was established that the pre-blanching of broccoli before freezing negatively affects the most important components of biological value – ascorbic acid, isothiocyanates, chlorophyll and β -carotene (Table 1).

Table 1

Effect of blanching on the content of biologically active substances in broccoli, mg/100 g ($n=2; P \geq 0.95; \epsilon \leq 5$)

| Indicator | Before blanching | After blanching |
|------------------------------------|------------------|-----------------|
| Mass fraction of ascorbic acid | 116.4 | 93.3 |
| Mass fraction of isothiocyanates | 0.59 | 0.25 |
| Mass fraction of chlorophyll | 49.0 | 43.9 |
| Mass fraction of β -carotene | 4.6 | 4.4 |

It was established that broccoli of the variety Parthenon contains 116.4 mg/100 g of ascorbic acid. After blanching, its content decreased to 93.3 mg/100 g. The losses amounted to 19.3 %. We also detected a significant reduction in the quantitative content of isothiocyanates, by 43 %, compared with the content in the original raw material.

The study of the pigment complex of broccoli that we conducted confirm the high enough content of chlorophyll in broccoli: 49 mg/100 g. After blanching, the content reduces to 43.9 mg/100 g. The losses of a -chlorophyll reach 10 %, of b -chlorophyll – 16 %. It is established that not only the absolute content of chlorophyll is changed, but also the ratio of a - and b -chlorophyll: from 3.05:1 to 2.75:1 (Fig. 3).

Chlorophyll in green vegetables is always accompanied by carotenoids. The results of determining β -carotene, which has a high biological value and is a natural antioxidant, confirm the content at the level of 4.6 mg/100 g. After blanching, the amount reduces only by 4 %.

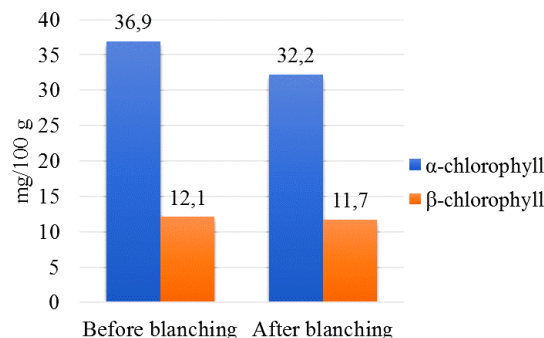


Fig. 3. Change in the chlorophyll content in broccoli

In order to determine the optimal parameters of broccoli aging in a food salt solution, a multifactorial experiment was conducted. To this end, a series of experiments were performed, in which variable parameters were the duration of aging of broccoli with a step of 5 units (from 5 to 25 min), the concentration of a food salt solution with a step of 0.5 units (from 1 to 3 %). The optimization criterion was the activity of enzymes (towards a minimum) and the content of vitamin C (towards a maximum), which has an antioxidant effect, contributes to the regeneration and healing of tissues, improves human body resistance to stresses, provides for a normal immunological and hematological status (Table 2).

Table 2

Effect of the treatment parameters on the activity of enzymes and the content of vitamin C

| Treatment parameters | | Enzyme activity, μ mol AA oxidized over 1 min | | Vitamin C content, mg/100 g |
|----------------------|------------------------------|---|--------------------|-----------------------------|
| τ , min | concentration of solution, % | ascorbic oxidase | polyphenol oxidase | |
| 0 | 0 | 1.57 | 16.73 | 116.43 |
| 5 | 1 | 1.53 | 16.67 | 115.94 |
| 5 | 2 | 1.51 | 16.63 | 115.85 |
| 5 | 3 | 1.49 | 16.59 | 115.71 |
| 10 | 1 | 1.47 | 16.52 | 115.52 |
| 10 | 2 | 1.43 | 16.48 | 115.34 |
| 10 | 3 | 1.41 | 16.32 | 115.26 |
| 15 | 1 | 1.37 | 16.27 | 115.14 |
| 15 | 2 | 1.35 | 16.22 | 115.02 |
| 15 | 3 | 1.36 | 16.15 | 114.89 |
| 20 | 1 | 1.32 | 16.03 | 114.76 |
| 20 | 2 | 1.27 | 15.94 | 114.97 |
| 20 | 3 | 1.24 | 15.88 | 115.15 |
| 25 | 1 | 1.34 | 15.94 | 115.08 |
| 25 | 2 | 1.37 | 16.02 | 115.01 |
| 25 | 3 | 1.39 | 16.13 | 114.93 |

It was established that the maximum decrease in the activity of polyphenol oxidase and ascorbate oxidase

under the condition of maximum preservation of C-vitamin value is observed during the preliminary aging of broccoli in a 3-% solution of food salt over 20 min. These are the pre-treatment parameters that are applied in the course of further research.

The aging of broccoli in a food salt solution before freezing makes it possible to maximally retain its biological value, as evidenced by the research results presented below.

Experimental data confirm that the pre-treatment of broccoli in a food salt solution does not significantly affect the change of biologically active substances (Table 3).

Table 3

Effect of aging in a food salt solution on the content of biologically active substances in broccoli, mg/100 g ($n=2$; $P \geq 0.95$; $\varepsilon \leq 5$)

| Indicator | Before aging | After aging |
|------------------------------------|----------------------------|-------------|
| | in a solution of food salt | |
| Mass fraction of ascorbic acid | 116.4 | 110.58 |
| Mass fraction of isothiocyanates | 0.59 | 0.42 |
| Mass fraction of chlorophyll | 49.0 | 52.1 |
| Mass fraction of β -carotene | 4.6 | 4.6 |

A reduction of the ascorbic acid content by 5 %, and isothiocyanates by 28.2 %, was established. The content of β -carotene, when compared with the original raw material, does not change, and the proportion of chlorophyll increases by 6.3 %. There are no changes in the ratio of chlorophyll forms (Table 4).

Table 4

Effect of aging in a solution of food salt on the chlorophyll content, mg/100 g ($n=2$; $P \geq 0.95$; $\varepsilon \leq 5$)

| Variety | <i>a</i> -chlorophyll | <i>b</i> -chlorophyll | Ratio <i>a</i> -chlorophyll/ <i>b</i> -chlorophyll |
|-----------|---|-----------------------|--|
| Parthenon | Before aging in a solution of food salt | | |
| | 36.9 \pm 1.8 | 12.1 \pm 0.6 | 3.05:1 |
| | After aging in a solution of food salt | | |
| | 39.3 \pm 1.6 | 12.8 \pm 0.6 | 3.07:1 |

The results obtained allow us to conclude that the pre-treatment of broccoli before freezing by aging it in a solution of food salt, when compared with blanching, ensures the stabilization of the content of biologically active substances.

The results derived from the experimental research create the basis for developing a model for forecasting the content of ascorbic acid. Among the set of factors, the most significant ones are chosen, those that have the highest correlation coefficients: the duration of aging in a solution of food salt, min ($r=0.95$), the concentration of a food salt solution, % ($r=0.42$), and the activity of AO, μ mol of ascorbic acid oxidized over 1 min ($r=-0.83$).

The developed model takes the following form:

$$y = 140.78 - 0.34x_1 + 0.03x_2 - 1.25x_3 \quad (R^2 = 0.936),$$

where y is the mass fraction of vitamin C, mg/100 g, x_1 is the duration of treatment, min., x_2 is the concentration of the solution of food salt, %, x_3 is the AO activity, μ mol of ascorbic acid, oxidized over 1 min.

The adequacy of the obtained model is confirmed by comparing the estimated and the tabular value of the Fisher's criterion ($F_{est}=7.348$, $F_{table}=5.318$).

6. Discussion of results of studying the biological value of broccoli

The study that we conducted (Table 1) confirmed that broccoli is a natural source of biologically active substances.

The results showed that the classic broccoli freezing technology, which implies preliminary blanching, significantly reduces the biological value of broccoli.

The proposed technique for the pre-treatment by aging in a solution of food salt with a 3-% concentration for 20 minutes contributes to the preservation of the content of biologically active substances.

The causes for stabilizing the ascorbic acid content are the lack of high-temperature treatment and partial inactivation of ascorbate- and polyphenol oxidase. The thermally labile vitamin C starts to degrade at a temperature of 40 °C. The proposed technique for the pre-treatment does not imply the application of high temperatures, which contributes to the preservation of ascorbic acid. It should be noted that we identified the oxidizing-reducing enzymes of ascorbic oxidase and polyphenol oxidase in broccoli. Ascorbic oxidase and, partially, polyphenol oxidase are involved in the transformation of ascorbic acid into dehydroascorbic acid. The dehydroascorbic acid, although it has a C-vitamin value, it is very unstable. Further transformation of dehydroascorbic acid into diketigulonic acid is accompanied by a decrease in the vitamin value. Partial inactivation of enzymes in the course of the proposed pre-treatment process, as an alternative to blanching, is explained by the capability of chlorides to remove copper ions from copper-containing compounds, which include both ascorbate oxidase and polyphenol oxidase.

At the same time, we should note that the aging in a food salt solution results in a 5-% loss of vitamin C. This is due to the water solubility of ascorbic acid. In order to ensure maximum preservation of ascorbic acid, it is recommended to adhere to the specified optimum duration of broccoli aging.

We have proved the positive effect of the proposed pre-treatment technique on the quantitative content of isothiocyanates; their loss during blanching is 43 %; when using the alternative method of treatment, it is 28 % only.

Results of the effect of different techniques of pre-treatment have confirmed the effectiveness of broccoli aging in a solution of food salt in terms of the content of chlorophyll, which has the antitumor effect, enhances immunity, and normalizes metabolism. Pre-treatment in a solution of food salt induces an increase in the content of *a*-chlorophyll and *b*-chlorophyll due to the compensatory response. In this case, the ratio of chlorophyll forms does not change.

The reason for reducing the chlorophyll content in the blanched broccoli is an increase in the activity of

chlorophyllase, which is observed under condition of high blanching temperatures.

The stability of β -carotene against short-term high-temperature thermal treatment was confirmed.

Based on the research results, it was proposed to use the preliminary aging of broccoli in a solution of food salt as an alternative to blanching. The industrial implementation of the proposed solution would make it possible to stabilize the biological value of broccoli during its freezing and long-term low-temperature storage.

The research results we report here are only part of the comprehensive work aimed at stabilizing the biological value of frozen broccoli. The prospects for the further research relate to the identification of the effect of pre-treatment techniques on the elemental and amino acid composition of broccoli as the components of biological value. Similar studies into other varieties and hybrids of broccoli are planned in the future.

7. Conclusions

1. The research results confirmed the negative effect of blanching on the biological value of broccoli. The content of vitamin C after blanching reduces by 19.3 %, isothiocyanates by 43 %, chlorophyll by 26 %, β -carotene by 4 %.

2. The rational parameters for the pre-treatment of broccoli are defined. The maximum preservation of vitamin C is ensured when broccoli ages in a 3-% solution of food salt for 20 min. We have developed a model for predicting the preservation of the content of vitamin C.

3. It is proved that the preliminary aging of broccoli in a food salt solution minimizes the loss of biologically active substances. At the same time, an increase in the content of chlorophyll is registered, which is explained by the compensatory reaction of the plant organism. This confirms the effectiveness of the developed method.

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