Characteristics of changes of the chemical composition of cranberry marsh in the process of obtaining puree

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Abstract

Introduction. Studies have been carried out to determine the impact of technological processing, in particular, on the processes of blanching and deaeration on the chemical composition of cranberry marsh puree.

Materials and methods. Puree of marsh cranberry was investigated for identification of bioflavonoid; methods of high-performance liquid chromatography, electron spectrometry, gas chromatography with mass detector of initial and hydrolyzed samples were used.

Results and discussion. It was established that as a result of technological reprocessing of cranberry into puree, the amount of ascorbic acid decreased by 13.5 times; the content of phenolic substances in puree from cranberry fruits is 983 mg%, anthocyanins – 160 mg%; the content of water-soluble pectin is increased to 3.0%, which is associated with partial hydrolysis of protopectin, fiber – 3.1% of the mass fraction of dry matter of puree. In puree from cranberries, 36.6% of sugars are contained in the mass fraction of dry matter of puree, of which 28.8% are reductive, namely glucose and fructose, the increase of which is due to partial acid hydrolysis of sucrose during the processing of berries in puree.

In puree, the presence of anthocyanin compounds found in the original sample is in a bound state with citric acid, as well as mono-oxycarboxylic acids. A number of organic acids have been identified: 3-hydroxybutyric acid; ferulic acid; amber, apple, citric acids. When processing cranberry into puree, it preserves natural preservatives contained in fresh berries. Thus, in cranberry puree there is benzoic acid in the amount of 122.2 mg%±15% and a small amount of sorbic acid is available up to 2.5 mg%. The positive effect of cranberry on growth retardation of the yeast of the genus *Candida* was investigated.

Conclusions. Cranberry puree is a natural source of biologically active substances and natural preservatives and is recommended for use in long-term storage functional foods.

Introduction

An analysis of the current world trends in the creation of a new range of foods with high nutritional value has shown the feasibility of using berry raw materials [1]. Such raw materials include berries of cranberry marsh (*Oxycoccus palustris Pers*). This is a high-yielding wild berry, rich in various groups of nutrients, which is dominated by a group of biologically active substances [2]. The value of wild berries is that the content of biologically active substances significantly exceeds the one that the cultivated berries contain [3].

In recent years, throughout the world, much attention has been paid to the study of the chemical composition and useful properties of this berry [3,4,6]. It is characterized by low demanding conditions of cultivation, high yields, rapid recoupment of costs, considerable nutritional value and rich chemical composition [1].

The chemical composition of the cranberry is unique. The cranberry contains mono -, di – and polysaccharides to 4.8–8.1% per 100 g of fruit pulp, of which mono- and disaccharides are up to 3.8%, and pectin substances – up to 2.8% [2].

The cranberries are rich in organic acids (benzoin, citric, apple, oxalic, quinic), according to data [6], the total amount of those is up to 3.5%, and high content of ursolic acid is noted in the pulp of berries [6]. By structure and genetic this ursolic acid is close to many physiologically important hormones, it has mineralocorticoid activity and is capable of delaying the development of aseptic inflammation [7].

The presence of mono- and disaccharides in combination with organic acids form the taste qualities of berries, and hence – the source product as well [1, 2]. High acidity of pulp of berries creates conditions for prolonged storage of raw materials in fresh state (8–10) months, in frozen – throughout the year [4]. The nutritional and medicinal quality of the berries depends on the degree of maturity of the cranberries. Unripe berries contain little benzoic acid, which is why they quickly deteriorate [7].

The content of vitamins, and especially vitamin C, varies significantly (12–35 mg%) depending on the season. In the berries of the autumn harvest, this vitamin breaks quickly, and in the snow gathering is almost absent [4]. Of vitamins, in addition to vitamin C, thiamine, riboflavin, nicotinic acid, there are routine (0.53–1.28 mg%), pantothenic acid, pyridoxine. Recently, the value of cranberries as an important source of phyllohitone (vitamin K1) has been proved, the deficit of which leads to processes of formation of prothrombin of blood, its share in cranberry berries makes 0,8-1,0 mg% [8].

There are a lot of flavonoids in berries of cranberry that own a powerful antioxidant effect and are useful in the fight against the major ailments of the present – cardiovascular diseases, malignant tumors, infections [9, 10]. Bioflavonoids have a wide range of pharmacological effects. Being powerful natural antioxidants, bioflavonoids protect the cells of our body from the destructive effects of free radicals [11].

In addition to physiological activity, phenolic compounds have important functional and technological properties – these are natural dyes, antioxidants, preservatives. Polyphenols also contain aromas that determine the taste in many foods.

Cranberries contain polyphenolic compounds: anthocyanins – up to 180 mg%, leucoanthocyanins – up to 160 mg%, catechins – up to 260 mg%, paints and tannins – up to 1200 mg% [1, 12]. Antioxidant, especially polyphenolic components of cranberries, inhibit the growth of cancerous and tumor cells. In addition, polyphenolic compounds cause the coloring properties of semi-finished cranberries, that is, they are natural dyes [13].

The colors of the cranberries are represented by chlorophyll, carotenoids and anthocyanins; when berries are ripening, the chlorophyll content is significantly reduced, and the anthocyanin content increases [4]. The average value of the content of anthocyanins in

cranberries in terms of cyanidin-3-glucoside is 80 mg of crude weight of berries [14]. Therefore, the use of puree from cranberries as a dye component, along with its other properties, is a promising solution.

Proanthocyanidins contained in the cranberry also act as antioxidants [12]. Due to the increased content of proanthocyanidins and antioxidants per 1 gram of berries (more than in any other fruit), cranberry strengthens the body's defenses in the fight against antiradicals, which are the cause of many chronic diseases [15, 16]. Therefore, the identification and quantification of phenolic compounds in cranberries must be accompanied by multilateral research.

From literary data it is known that cranberries contain natural antimicrobial components, including benzoic acid [17]. The first mentions of the presence of benzoic acid in the cranberry berries were brought to their articles by the American scientist G. F. Mason [17]. Later, a number of scientists, with the help of modern methods of analysis, determined the quantitative content of this natural preservative. It is known that a significant influence on the amount of benzoic acid is made by conditions of growth, weather characteristics of the growing season, etc. [2, 4]. Thanks to its antiseptic properties benzoic acid in a cranberry, provides long-term storage of fresh berries. The conducted studies [18, 19] of antimicrobial action of cranberry juice have shown that the concentration of juice in the amount of 5.33% is sufficient to stop the growth of fungi of the genus *Candida*.

The increase in interest in natural phytonutrients is due both to the rigid regulation of their use in food products, and to the desire of manufacturers to provide products with the status of natural ones [13].

The above information makes cranberry a promising raw material for use in food technology. To date, a large number of studies have been carried out on the study of the chemical composition of cranberry, which confirms the content of a wide range of biologically active substances [20, 21]. Many studies have been carried out on the chemical composition of cranberry, depending on climatic conditions, degree of ripeness, duration and storage conditions [3, 4, 24, 25]. But the berries undergo a certain technological treatment and are used in the form of puree, the chemical composition of which can significantly differ from the initial chemical composition of fresh berries [22].

The purpose of the research is to determine the influence of technological treatment of blanching and deaeration on the chemical composition of cranberry puree.

Materials and methods

Materials that are studied

Investigated cranberry marsh puree, collected in the Volyn region of Ukraine. The production of cranberry puree was carried out by blasting the berries with sharp steam for 5–6 minutes, their rubbing and deaeration. Blanching reduced microbial contamination, contributed to the destruction of the membrane, which prevents the penetration of steam into berries, partial denaturation of skin proteins and increase the penetration of tissue. Blanched fruits were rubbed and sent to deaeration. The deaeration process was carried out under vacuum to remove the residual moisture and air to prevent the oxidation of biologically active substances and preserve the color of puree [13].

Description of techniques

The **mass fraction of dry substances** was determined by the refractometric method, the essence of which is to determine the mass fraction of dry matter by the refractive index of its solution [1].

The **mass fraction of total sugars and reducing agents** was determined by hot titration [2].

Actual acidity was determined by potentiometric pH method by Lur'ye [3].

Pectin substances were determined by the titrimetric method, which is based on the titration of the alkaline pre-selected and prepared pectin substances before and after hydrolysis. The titration results are proportional to the number of free and esterified carboxyl groups [2, 3, 30].

The **content of food fibers** was investigated by the method of hydrolysis of readily soluble carbohydrates with a mixture of concentrated acetic and nitric acids [1, 3].

The **content of vitamin thiamine (B_1)** was determined by the method based on oxidation of thiamine in thiohrum, its extraction in an organic solvent and measuring the intensity of fluorescence.

The **method of determining vitamin riboflavin (B_2)** is based on fluorescence measurement spectrophotometrically in hydrolyzate with 4M KH_2RO_4 and the addition of standard riboflavin.

The **determination of vitamin niacin (PP)** is based on a reaction that takes place in two stages. At the first stage, the interaction of the peridine ring of nicotinic acid with the bromide rodanum occurs. At the second stage, the coloring of the derivative glutacone aldehyde is formed, which is directly proportional to the mass fraction of vitamin and is measured colorimetrically [3].

The **amount of ascorbic acid (C)** was determined by spectrophotometrically extracted and centrifuged sample with citrate-acetate buffer and 2, 6-dichlorophenylphenol solution, read adsorption at a wavelength of 520 nm [26].

The **mass concentration of phenolic substances** was determined by the colorimetric method [26].

To determine the **content of bioflavanoid**, the following methods were used:

- Ultrasonic high-performance liquid chromatography (UPLC) with diode-matrix detection (PDA), which simultaneously records the electronic absorption spectrum of compounds. The results are obtained on the device of brand WATERS (USA). The analysis was carried out in the gradient mode of changing the composition of the mobile phase (acetonitrile-water). Column ACQUITY UPLC®BEHC$_{18}$ 1.7 μm, 50 * 2.1 mm;
- Electronic spectroscopy. The results were obtained on the device of brand Specord 210 Plus (Germany);
- Gas chromatography with mass-selective detection and a library of mass spectra before and after acid hydrolysis of source and modified (TMS derivatives) forms. The results were obtained on the Agilent GC/MSD 7890A/5975C with a capillary column of HP-5MS [28, 29].

High-performance liquid chromatography (UPLC) [26] was used in this work. To identify compounds with mobile atoms, the method of derivatization (getting derivatives) [26] was used to increase the molecular weight of the starting compound at a known value, to carry out a higher quality chromatography, and also to increase its initial molecular weight – reliable identification. In studies, N-methyl-N-trimethylsilyl-trifluoroacetamide (TMS) reagent was used for this purpose.

The production of ethanol concentrate (organic compounds) was carried out as follows

[26]: 3,087 g of cranberry puree are transferred to a 100 cm^3 flat bottom flask, filled with 60 cm^3 of 96% ethyl alcohol, and added to the reflux condenser and kept in a boiling water bath for 90 minutes. After that, the water bath is cooled, the condenser is washed with 5 cm^3 of ethyl alcohol, and the contents of the flask are transferred (filtered) into a volumetric flask of 100 cm^3. Then 35 cm^3 of ethanol was added to the flask and the procedure was repeated. The volume of ethanol concentrate was adjusted to 100 cm^3.

When conducting an acid hydrolysis [26], the weight of the raw material (approximately 0,992 g) weighed to the fourth mark to the nearest quarter is transferred to a 100 cm^3 flat bottom flask, 20 cm^3 of ethanol, 20 cm^3 of distilled water and 10 cm^3 of concentrated hydrochloric acid are added. After attaching the flask to the reflux condenser, the mixture is kept in a boiling water bath for 90 minutes. After that, the condenser is washed with 20 cm^3 of distilled water, the flask is cooled. The contents of the flask are transferred into a separating funnel through a paper filter of 100 cm^3, adding 25 g of sodium chloride, mixing thoroughly and removing the organic compounds with ethyl acetate (pre-adding water to it), two times for 30 cm^3. After drying the ethyl acetate extracts with anhydrous sodium sulfate, the organic solvent is distilled in vacuo. The residual after distillation is dissolved in 50 cm^3 of ethanol.

To obtain TMS derivatives, 5 cm^3 of ethanol concentrate of the sample is placed in a beaker and at 80 °C ethanol is removed. To the dry residue, 300 μg of anhydrous pyridine and 100 μg of N-methyl-N-trimethylsilyl-trifluoroacetamide reagent are added. Beaker is closed and put in UZB for 30 minutes. After this, 1 cm^3 of acetonitrite is added to the beaker, mixed and GC/MS is tested according to the procedure described.

The study of the content of natural preservatives (benzoic and sorbic acid) was carried out according to the method described in work [30] and using the high-performance liquid chromatograph Varian 920-LC, the spectrophotometric detector.

To study the **microbiological criteria** as well as the microbiological stability of the cranberries purée, a research by counting the number of colonies formed as a result of sowing on the nutrient medium was carried out [31]. To determine the diameters of zones of growth retardation of microorganisms, preparations using cranberry puree of different concentrations were used.

Results and discussion

Chemical composition of cranberries berry puree

Table 1 shows the main organoleptic characteristics of the obtained puree.

Table 1

Organoleptic characteristics of cranberry puree

Indicator	Characteristic of the indicator
Appearance and consistency	Homogeneous, puree-like, rubbed mass
Color	Homogeneous throughout the mass, dark red
Scent and taste	Inherent in cranberries, sour

Preparation of puree is accompanied by a short-term effect of high temperature during blasting of berries, which may lead to the destruction of biologically active compounds [32]. Therefore, it was advisable to conduct a study of the chemical composition of puree of cranberries, the results of which are given in Table 2.

It was found that vitamin C is most susceptible to destruction, in berries of cranberry its amount was 35 mg%, in puree it remained 2.6 mg%, that is decreased by 13.5 times. This is due to the high thermal stability of ascorbic acid and its degradation under the influence of heat, which accompanies the process of blanching berries with cranes when processed in puree [32].

The content of water-soluble pectin in puree has increased and makes up almost 3.0% of the mass fraction of dry matter of puree; it is probable [32] during the heat treatment process under the influence of organic acids there was a partial hydrolysis of the protopetyte of plant tissues, as a result of this process, the amount of water-soluble pectin has increased. The increased amount of pectin in puree should have a positive effect on the formation of the composition of the produced and foamy-gelatanious like structures and to prevent the intensive removal of moisture from the product, which will extend the shelf life [23, 33].

The fiber content was 3.1% to the mass fraction of dry matter with cranberry puree, the total content of dietary fiber in puree exceeded 6.1%. Although they are not absorbed by the body [34], however, they contribute to the implementation of many positive functions: remove toxic metals and radionuclides from the body, inhibit the development of rotting microorganisms, prevent excessive boiling of carbs, and promote the binding of endogenous and exogenous toxins [35].

Table 2

Chemical composition of cranberry puree

Indicator	Content of substances per 100 g of puree	Content of substances to mass fractions of dry matter of puree
Total mass fraction of dry substances,%	24,0±1,5	
Mass fraction of water-soluble dry substances,%	12,5±1,5	
Actual acidity, pH	4,37±0,1	
Organic acid content,%	3,6±0,5	15,0±0,5
Total sugar content,%	8,8±0,5	36,6±0,5
The content of reductive sugars,%	6,9±0,5	28,8±0,5
Pectin content, г/100 г	0,72±0,1	3,0±0,1
Fiber content, г/100 г	0,75±0,1	3,1±0,1
Total content of phenolic substances, mg%	235±9,9	983±9,9
Incl. mass concentration of anthocyanins, mg%	38,4±0,25	160±0,25
Vitamin content, mg%		
Vitamin C	2,64±0,3	11±0,3
Thiamine (B_1)	0,035±0,3	0,14±0,3
Riboflavin (B_2)	0,018±0,3	0,43±0,3
Niacin (PP)	0,08±0,3	0,43±0,3
Ash,%	0,32	1,3±0,3

That is, it is possible to predict [30] that the adding cranberry puree in the production of food products may partially increase their nutritional value by adding to the product of useful nutritious fibers.

It was determined that in cranberry, 36.6% of sugars are contained in the mass fraction of dry matter of puree, of which 28.8% are reductive, namely glucose and fructose, the increase of which is due to partial acid hydrolysis of sucrose during processing berries in puree.

The most common class of organic compounds in plants are acids. Lemon and apple cranberry juice is preferred in puree. The total content of organic acids is 15% to the mass fraction of dry matter of puree.

Also vitamins – thiamine (B1), riboflavin (B2), niacin (PP) were identified in puree. The amount of ash elements was 1.3% of the mass fraction of dry matter of cranberry puree.

Identification of bioflavonoids in cranberry puree

Special attention to the cranberries has recently been crocheted due to the presence of a significant amount of bioflavonoid in it. Therefore, it was advisable to investigate the content of this class of compounds in the investigated berry.

The separation of the ethanol concentrate of cranberry purée using UPLC-PDA method confirms the presence of phenolcarboxylic acids in the sample (the mixture, since the chromatographic peak is highly blurred) – 4.38 minutes (Figure 1, A); Fennel compounds - 5.74; 6.23 min (Figure 1, B); as well as the mixture of anthocyanins -5.29 min (Figure 1, C).

The quantitative correlation between these compounds is determined (Table 3).

Table 3

Quantitative correlation between compounds according to Figure 1 a, b, c

Duration of detention	Mass fraction of the amount,%	Duration of detention	Mass fraction of the amount,%	Duration of detention	Mass fraction of the amount,%
PDA 335.0 nm		**PDA 350.0 nm**		**PDA 315.0 nm**	
4,384	88,39	5,738	39,38	5,287	100
5,193	11,61	6,229	37,19		
		6,639	23,43		

After acid hydrolysis, the chromatogram (Figure 2) is characterized by the presence of three anthocyanins with the same nature of the electron spectra, which indicates one nature of the aglucone [12]. It should be noted about the increase in the time of anthocyanins' coming out. This is due to the fact that in the original sample they were glycosylated, that is, they are connected with carbohydrates. Flavonoids, in addition to catechins and leukoanthocyanins, are relatively rare in the free state. Most of them are presented in the form of various O- and C-glycosides. The diversity of flavonoid glycosides is due to a significant amount of sugars (glucose, arabinose, xylose, etc.) and the ability to attach them to a number of positions of aglycones, as well as the fact that sugars may have different configuration of glycoside bonds and the order of the connections between them [12].

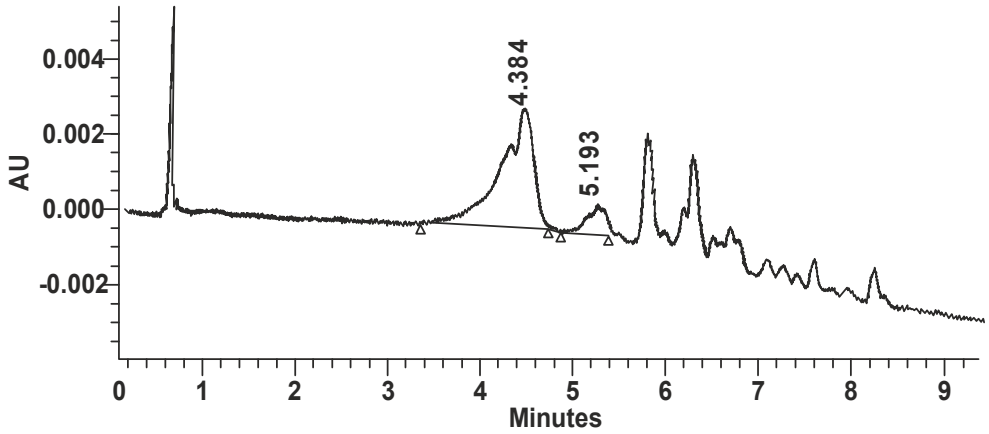

Figure 1a. Chromatogram of the source ethanol concentrate (PDA 335.0 nm)

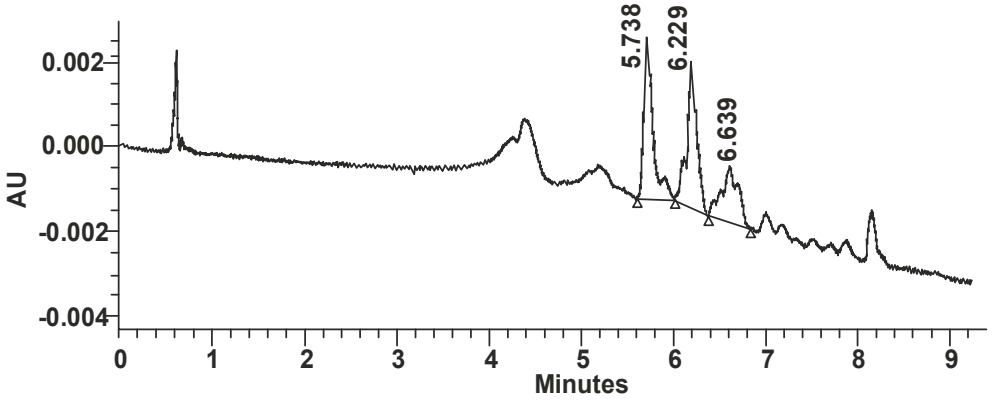

Figure 1b. Chromatogram of the source ethanol concentrate (PDA 350.0 nm)

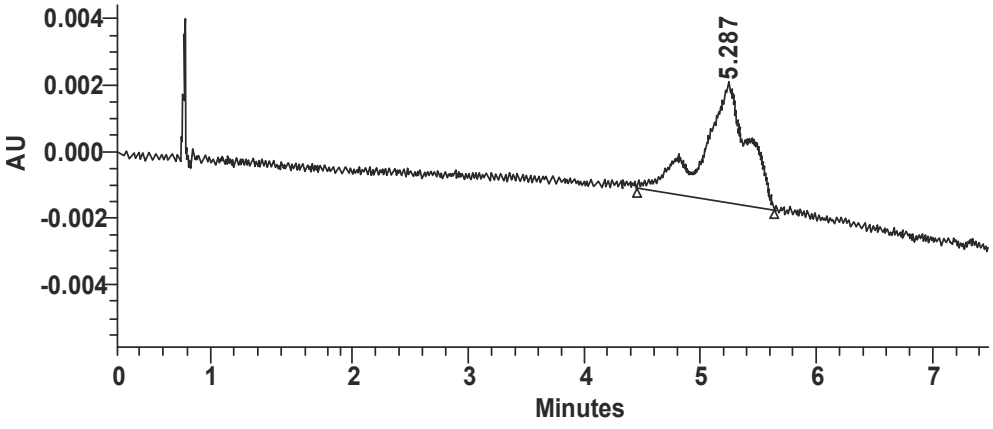

Figure 1c. Chromatogram of the source ethanol concentrate (PDA 315.0 nm)

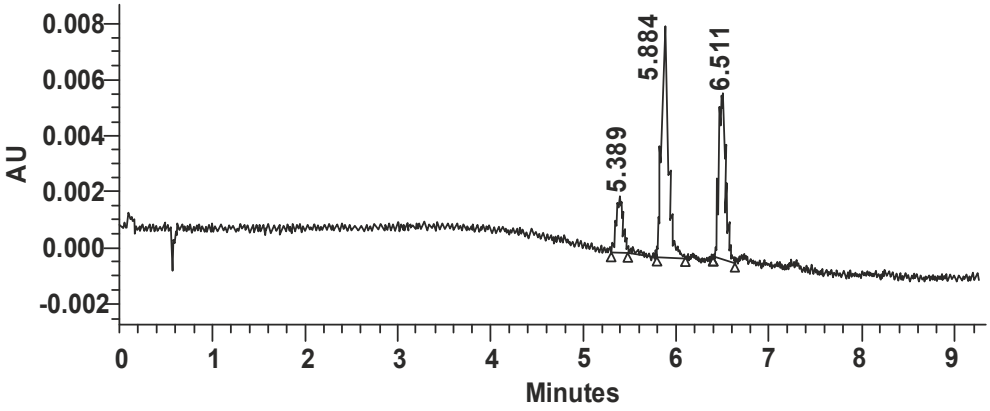

Figure 2. Acid hydrolyzate chromatogram (PDA 350.0 nm)

The quantitative correlation between these compounds is determined (Table 4).

Table 4

Quantative correlation of compounds in accordance with Figure 2 (PDA 350.0 nm)

№	Volume of sample, cm^3	Duration of detention, min	Mass fraction of the amount,%
1	1,0	5,389	10,81
2	1,0	5,884	52,39
3	1,0	6,511	36,60

Conducting the TMS derivatization reaction allowed to identify many more compounds: formaldehyde; 3-hydroxybutyric acid; fumaric acid; citric acid. In addition, a number of carbohydrates have been identified: sorbosis, glucose, butanic acid derivatives; dictone propanoic acid; malonic acid; ethyl ether and free citric acid. The total content of phenolic substances in puree of cranberries is up to 235 mg%, therefore, cranberries may be recommended for use in the creation of food products for health purposes [36].

The data of scientific literature [27] indicates the expressed antimicrobial action of cranberries isolated from the fruits of biologically active substances.

Antimicrobial action of benzoic acid and its salts is based on the ability to suppress the activity of enzymes [5]. Specific antibacterial and antifungal efficacy against Escherichia coli and Candida is active 24 hours after use [19]. Benzoic acid is able to block succinate dehydrogenase and lipase, the enzymes that break down fats and starch [19]. It suppresses the growth of yeast and bacteria of butyric fermentation, weakly acts on bacteria of vinegar fermentation and quite slightly – on lactic acid flora and mold [19].

Since berry purees have the optimal composition of nutrients, they are a good environment for the development of microorganisms of damage that can come from the surface of the skin of berries into pulp [37]. Particularly dangerous is the development of some species of fungi of the genus *Penicillium*, which are capable of secretion of mycotoxin patulin, which has a carcinogenic and mutagenic effect [37]. But in the sources [1, 3, 5, 17], there are discrepancies regarding the data on the quantitative content of preservative in wild

berries. From a scientific point of view, it was of interest to determine the amount of natural preservatives in puree, which was made from cranberries.

In cranberry puree, benzoic acid was identified in the amount of 122.2 mg%±15% and a small amount of sorbic acid – up to 2.5 mg%. Preservation of these natural preservatives in cranberry puree after the technological processing of berries, confirms preliminary studies of the preservation of the antimicrobial capacity of cranberry juice after autoclaving, are given in [32].

An experiment was conducted to investigate the effect of cranberries on puree yeast of the genus *Candida*. The genus *Candida* – the shape of cells is spherical, oval, cylindrical, elongated. Propagated by multilateral budding, as well as by agamous way – blastospores. It forms a pseudo-mycelium, and sometimes a true mycelium. On the surface of liquid substrates forms films: young – white, smooth, old – wrinkled. Assimilates glucose, sucrose, maltose, lactose. After growing in the thermostat, yeast growth retardation zones were observed, which proves the positive effect of cranberries on the growth retardation of the yeast of the genus *Candida*. Cranberries may be used as a source of natural preservatives, which will help lengthen the shelf life of food products, the dominant factor for which is microbiological damage in the process of storage [35, 36].

On the content of nutrients, cranberry puree is a promising raw material for use in creating a wide range of nutritional products for health and functional purposes of extended shelf-life.

Conclusions

1. Technological reprocessing of cranberry into puree leads to a decrease in the content of ascorbic acid by 13.5 times and to an increase of the content of water-soluble pectin up to 3.0% to the mass fraction of dry matter of puree.
2. The content of phenolic substances in puree of cranberries is 983 mg%, anthocyanins – 160 mg%. The presence of anthocyanin compounds found in the sample is in bound state with citric acid, as well as mono-oxycarboxylic acids.
3. During the processing of cranberry into puree, it preserves natural preservatives contained in fresh berries. Thus, in cranberry puree there is benzoic acid in the amount of 122.2 mg%±15% and a small amount of sorbic acid is available up to 2.5 mg%. The positive effect of cranberry on growth retardation of the yeast of the genus *Candida* was investigated.

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