

Research of Biochemical Composition and Antioxidant Activity of Freeze-dried Cranberry Powder Obtained on the Basis of Enzymatically Processed Berry Pulp

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Abstract

Aim: An important indicator of the quality of fruit and berry powder products, which determines the suitability of their use in the preparation of food products, is the chemical composition. Complex studies were conducted to study the chemical composition and antioxidant activity (AOA) of the freeze-dried cranberry berries obtained from the pulp of berries pre-treated with a piece of enzymatic preparations of pectolytic and cellulolytic action (Rapidase CR and Laminex BG2). The profile of flavonoids, qualitative composition of organic including phenolic acids, catechins, and minerals, has been studied. **Results and Discussion:** In the flavonoids, quercetin and its glycosides have been identified: Quercitrin, hyperoside, and avicularin. Among the catechin compounds, epicatechin (more than 65%) is detected in the most considerable amounts; the epigallocatechin (about 18%) is the second in the quantitative series of catechins. Gallates are present in smaller amounts: Epicatechin gallate, gallic acid gallate, and epigallocatechin gallate with a predominance of the latter (9%). In the composition of fruit acids, citric acid is present in the most significant amounts, and in the spectrum of hydroxycinnamic acids, chlorogenic acid (~45%) and 4-caffeoyl acid (~22%) predominate; ferulic, coffee, p-coumaric, and caftaric acids have also been identified. A diverse range of mineral substances has been defined: Potassium, phosphorus, and calcium are leading, substantially magnesium content. From trace elements in significant quantities, there are manganese, iron, aluminum, silicon, and zinc. The high AOA of the sublimed cranberry berries powder is established. **Conclusion:** The obtained data convincingly demonstrate that the berry powder contains a wide range of natural biologically active and minor components useful for human health: Sugars, organic acids including natural preservative - benzoic acid, dietary fibers, protein, minerals, and Vitamin C, and polyphenolic compounds including flavones, flavonols, anthocyanins, catechins, proanthocyanidins, and tannins, as well as natural dyes and preservatives, which opens the prospect of using a freeze-dried powder berries cranberries in the preparation of foods and functional foods and therapeutic and prophylactic purposes.

Key words: Antioxidant activity, biochemical composition, cranberry berries, enzymatic treatment, vacuum freeze drying

INTRODUCTION

In connection with the growing interest of the population in healthy food products, researchers and food manufacturers are increasingly turning to the modification of traditional formulations using products of processing of fruit and berry raw materials, which contain a wide range of healthy functional food ingredients, as well as natural dyes and flavors. From this point of view,

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the cranberry berries deserve particular attention thanks to the high content of vitamins, antioxidants, a wide range of flavonoid compounds, minerals, and organic acids including benzoic and salicylic, having antimicrobial properties.^[1-6] Particular attention to cranberries is due to the presence of proanthocyanidins in berries, which have a unique effect in preventing urinary tract infections, which is characteristic only of seeds belonging to the species of cranberry (blueberry and cranberry).^[7]

Analysis of modern trends in the development of technologies for processing fruits and berries shows that at the present stage the production of fruit and berry powders is developing dynamically as technological semi-finished products that are convenient in application, storage, and transportation.^[8-11] In recent years, there has been a noticeable increase in interest in vacuum freeze drying as a method of dehydration and canning of forest and garden berries. The use makes possible to obtain powder products with maximum preservation of the natural components of raw materials,^[12-17] which opens up prospects for their use in food for enrichment useful for human health biologically active substances of berries and partial replacement or in some cases. Complete elimination of synthetic food additives intended for the consumer properties of the product such as appearance, taste, aroma, and conditional on the relevant shelf life.^[18-21] The advantages of freeze-dried berry powders are small mass. Chemical and microbiological stability under prolonged storage, the unique properties in rapid rehydration, and restoration of the original quality characteristics of the berries, and above all, the attribute inherent ripe berries (saturation flavor, aroma, and color), and a high concentration biologically active compounds of the original natural raw materials. The latter factor is crucial in assessing the efficiency of processing berries and realizing their resource potential.

However, when receiving powder semi-finished products from natural berry raw materials, it should be borne in mind that a significant part of the berries components useful for human health is not able to assimilate the human body and displays its physiological activity due to the natural organization of plant tissue. Sorption of proteins and structural components of the cell wall, based on non-starch polysaccharides, significantly reduces the digestibility of biologically active substances. Only a fraction of them is dissolved in the cell sap and therefore bioavailable. Therefore, important and promising direction in the development of powder products technology is the use of biocatalytic methods preprocessing berries that enhance plant tissue extractive capacity by carrying out partial enzymatic hydrolysis of non-starch polysaccharides berries. It destroys the cell walls, and as a consequence, the enrichment juice fraction (soluble part of the powder product) additional quantities of natural components of berries that have biological the activity and forming a bouquet of color, taste, and aroma.^[22-26] Currently, in the industrial processing of fruit and berry raw materials, enzyme preparations are increasingly being involved as

technological auxiliaries, the use of which makes it possible to develop and introduce qualitatively new, environmentally safe technologies, to increase the yield of finished products, to improve its quality, and to reduce losses of biologically active substances processing of raw materials.^[27-31]

Currently, little attention is paid to the use of enzymes in the pre-treatment of raw materials in the production of berry powders and their characteristics from the position of nutritional value and the manifestation of antioxidant properties useful for human health. Based on experience, the successful application of enzymes in food technology rational combination of biocatalytic methods of pretreatment berries and modern methods of drying may be the key to a productive and successful implementation of intensive technologies of processing of vegetable biological resources, focused on preservation and the most complete use of the unique natural composition of the berries and obtaining powder semi-finished products increased nutritional value and antioxidant activity (AOA) for use in septum food.

The purpose of the present studies was to study the biochemical composition and AOA of the sublimed cranberry powder obtained by the enzyme.

METHODS OF RESEARCH

We used berries cranberries, crop 2013, growing in the Tver region of the Russian Federation, and enzyme preparations (FP) pectolytic and cellulolytic action Rapidash CR (strain producer - *Aspergillus niger*, the manufacturer - DSM-Food-Specialties, France) and Laminex BG2 (the producer strain is *Trichoderma reesei*, manufacturer, Danisco, Denmark).

ENZYMATIC TREATMENT OF PULP OF THE BERRIES CRANBERRIES

For hydrolysis, the berries were crushed, the resulting pulp was introduced FP in the composition in concentrations: 3.4 units. Polygalacturonic acid (PGA)/g pectin for pectolytic drug Rapidase CM 21.3 units. Congenital adrenal hyperplasia/g cellulose for glucanolytic drug Laminex BG2 and hydrolysis conducted at a temperature of 45°C for 1.5 h. After hydrolysis, enzymes were inactivated by heating. Dosages of FP and the duration of hydrolysis were chosen based on the analysis of the obtained dependences illustrating the dynamics of the juice fraction yield in the berry pulp under the influence of individual FP [Figures 1 and 2].^[32] The output of the cell SAP was an indirect measure by which to judge the degree of degradation of the structural polysaccharides under the influence of FP.

The production of freeze-dried cranberry powder on the basis of enzymatically processed pulp was carried out at

the experimental stand of SVP-0.36 (a stand of vacuum processes).^[33] Drying parameters: The temperature at the stage of moisture removal by the “ice-steam” phase transition was -20 – -22°C ; the maximum temperature at the drying stage is 36 – 38°C (with radiation energy supply); the average pressure in the chamber is 100 Pa; the total drying time is 12 h; the moisture content in the final product is 5%. The dried product was a fairly dense layer with a brittle consistency; when unloading from the baking tray, it was easily crushed. After unloading, the resulting product was further crushed with a mixer designed to work with V-Type Kewei Mechanical Manufacturing Co., Ltd., powder products, model VH-0.1.^[34] The freeze-dried product was a finely dispersed crumbly powder of characteristic cranberry color with a pronounced aroma [Figure 3].

METHODS FOR DETERMINING THE ACTIVITY OF ENZYME PREPARATIONS

Endoglucanase activity was established by the viscometric method based on a determination of the rate of viscosity decrease of 0.3% Na-CMC solution after the action of cellulase.^[35]

The pectinase PGA of the enzyme preparation was determined by the colorimetric method by the procedure described in Polygalina *et al.*^[35]

Methods for studying the chemical composition

The mass fraction of moisture was determined by the Karl Fischer method by the procedure^[36] in the “701-KF Titrino” installation of the firm “Metrohm” (Switzerland).

The content of total sugar (the full content of sucrose and reducing sugars) was determined by a photocolometric method based on the oxidation of total sugar by potassium dichromate in a strongly acid medium. Colorimetry was carried out on a KFK-3 photoelectric colorimeter at a wavelength $\lambda = 670$ nm and a cell thickness of 5 cm.^[37]

Reducing sugars were determined by the accelerated iodometric method by the procedure.^[38]

Pectic substances were determined by the carbazole method, based on obtaining specific violet-pink staining of uronic acids with carbazole in a sulfuric medium. Colorimetry was carried out on an SF-26 spectrophotometer at $\lambda = 535$ nm and a cell layer thickness of 10 mm. The number of uronic acids was calculated from the calibration chart plotted for galacturonic acid.^[39]

The determination of cellulose was carried out according to the method of Kushner and Ganek, based on the oxidation, degradation, and dissolution of various chemical compounds

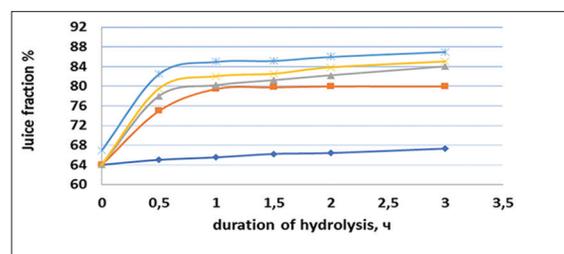


Figure 1: Change in the share of the juice fraction in the mash of berries cranberries under the action of the OP Rapidase CR at various concentrations of 6.8–27.2 units (polygalacturonic acid/g pectin)

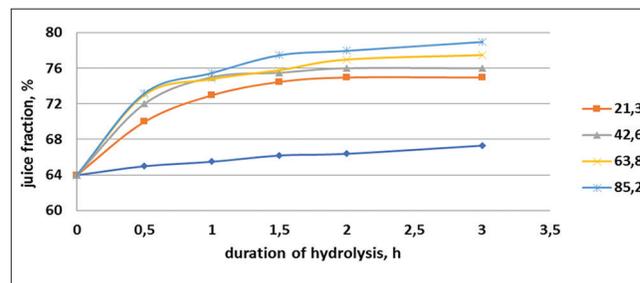


Figure 2: Change in the proportion of juice fraction in the mash of berries cranberries under the action of OP Laminex BG2 at various dosages of 21.3–85.2 units (CAA/g cellulose)



Figure 3: Sublimated cranberry powder obtained by fermented mashed berries in an experimental sublimation unit SVP-0.36, after grinding

that make up plants, with a mixture of acetic and nitric acids. At the same time, cellulose (cellulose) practically does not dissolve, is filtered out and weighed.^[39]

Determination of dietary fibers was carried out in accordance with the methodology described in Russian Ministry of Health.^[40]

The content of total protein was determined by the Kjeldahl method.^[38]

The content of soluble protein was determined by the colorimetric method (according to Lowry), based on the

reaction of proteins with Folin reagent, giving blue staining. Colorimetry was carried out on an SF-26 spectrophotometer at $\lambda = 750$ nm and a cell layer thickness of 10 mm. The protein content of the product was calculated using a preconstructed calibration curve (albumin).^[38]

The determination of the content of titrated acids was carried out by the potentiometric method by GOST ISO 750-2013 on the pH meter-ionomer “Agilent 3200P.”

The qualitative composition of organic acids was investigated by high-performance liquid chromatography (HPLC).^[41] Chromatographic analysis conditions: Kromasil C18 column 5 mm, 250 mm \times 4.6 mm; mobile phase 0.2 M phosphate buffer pH 2.6; speed 1.0 ml/min; and UV, $\lambda = 254$ nm. The volume of the injected sample: 5 μ l.

The determination of benzoic acid was carried out by RP-HPLC with spectrophotometric detection.^[41] Chromatographic analysis conditions: column: octadecylsilica gel 250 mm \times 4.6 mm, 5 μ m (Phenomenex Luna 5 μ m C18 (2)); mobile phase: 0.025 M CH₃COONa, pH 4, 5 - 82%: acetonitrile - 18%; mobile phase feeding rate: 1.0 ml/min, and detection: UV, $\lambda = 272$ nm. The volume of the sample is 10 μ l.

The determination of hydroxycinnamic acids was carried out using the HPLC method.^[41]

Chromatography conditions: Octadecylsilica-silica column 5 m km, 250 \times 4.6 (Hypersil Gold C18); mobile phase feeding rate: 0.9 ml/min; column temperature: 25°C; and detection: UV, $\lambda = 330$ nm. The volume of the sample is 10 μ l. Mobile phase: (A) 0.05% trifluoroacetic acid and (B) acetonitrile.

Time, min	0	18	30	35
A, %	95	77	70	50
B, %	5	23	30	50

The total content of polyphenolic compounds was determined by the modified Folin–Ciocalteu method. The absorption of the solution at 750 nm is proportional to the content of phenolic compounds. The value of the mass concentration of phenolic substances in mg/l was determined from a graduated graph plotted against gallic acid.^[40]

The total content of anthocyanins was determined by differential spectrophotometry.^[40]

The content of proanthocyanidins was determined by the Beit-Smith method followed by a photometric determination.^[41] The measurement was carried out on an SF-26 spectrophotometer at $\lambda = 550$ nm in cuvettes with a layer thickness of 0.5 cm. The content of proanthocyanidins was calculated using a preconstructed calibration curve (using a standardized extract). The solution of the comparison is a solution based on the grape seed extract.

Determination of the total content of flavones and flavonols (in terms of routine) was performed by spectrophotometric method.^[40] Optical density measurements were carried out at a wavelength of 415 nm. For quantitative calculations, a preconstructed calibration curve was prepared using a standard solution of rutin (Routine Trihydrate, 95%, (Sigma), CAS: 207671-50-9).

The flavonoid profile was analyzed by HPLC with the sequential use of a diode-array detector (DMD) and a mass spectrophotometric detector (MS).^[34] The studies were carried out on an Agilent 1200 chromatograph. As an extractant, a 50% methanol solution was used. Chromatography conditions: ProteCol C18 HPH125 column 250 \times 4.6 mm, 5 μ m; mobile phase: A - 0.1% solution of formic acid, B - acetonitrile (040 min, 10–60% B). Column temperature 30° C, mobile phase speed of 0.5 ml/min, sample volume 10 μ l, analytical wavelengths: 360, 330, and 290 nm. Mass scanning - in the mode of registration of positive ions in the range of m/z 150–1000. Operating parameters of the ionization source: the voltage on the capillary is 3500 V, the gas-dryer stream (nitrogen) is 9 L/min, the temperature is 325°C, and the pressure on the nebulizer is 0.27 MPa.

The group composition and quantitative content of catechins and gallic acid were determined by RP-HPLC with spectrophotometric detection at $\lambda = 275$ nm.^[41] Chromatographic analysis conditions: Octadecylsilica-column column 250 \times 4.6 mm, 5 μ m (Phenomenex Luna 5 μ m C18 (2)); the mobile phase feeding rate is 1.0 ml/min; and the volume of the injected sample is 10 μ l. Mobile phase: (A) - trichloroacetic acid, pH = 2.5 and (B) -acetonitrile;

Time, min	A, %	B, %
0	96	4
25	80	20
30	80	20
50	50	50

The content of tannins and Vitamin C was determined in accordance with the methods described in Russian Ministry of Health.^[40]

Determination of the ash content was carried out in accordance with the procedure.^[39] The determination of the content of macro- and micro-elements was carried out using atomic emission spectroscopy with inductively coupled plasma on the iCAP 6300 Duo (Thermo Fisher Scientific, USA).^[42,43] The samples were preliminarily cleared on the tile by the “wet ashing” method, then burned in a muffle furnace at 600°C, the ash was dissolved in 50 cm³ of a 2% solution of H₂SO₄ with heating. For analysis, a solution was prepared with a mass fraction of the elements corresponding to the calibration curve.

METHOD FOR DETERMINATION OF AOA

AOA was determined with respect to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.^[44] The kinetics of optical density loss at $\lambda = 517$ nm of the DPPH solution was recorded in the presence of antioxidants of the test sample and the standard solution (Trolox analog of Vitamin E) after preliminary incubation.

The optical density was measured on a Shimadzu spectrophotometer Model UV 1800.

STATISTICAL PROCESSING OF EXPERIMENTAL DATA

To process the results of the studies, a statistical method for processing experimental data was used, during which the mean values of the determined value were determined from 3 to 6 replicas, the standard deviation and the confidence interval.^[45]

RESULTS AND ITS DISCUSSION

An important indicator of the quality of fruit and berry powder products, which determines the expediency of their use in the preparation of food products, is the chemical composition. The results of studies carried out to study the effect of enzymatic processing of the cranberries pulp on the juice fraction composition, and its antioxidant properties made it possible to evaluate the effectiveness of the use of OP in the stage of preliminary treatment of cranberry berries in the preparation of a powder product. An increase in the extractive capacity of plant tissue by biologically active substances, natural dyes, and preservatives and AOA of the juice fraction by 40% has been established (by 1.2–2 times).^[46,47] Qualitative transformations of the composition of the juice fraction of enzymatically processed pulp of berries have been identified. Epigallocatechin has been recognized with high antiradical properties, the presence of which has not been found in juice obtained without the use of PTs.^[47] The obtained results give the basis for assuming an increase in the nutritional value of

the soluble (juice) part of the cranberry powder obtained by fermented mashed berries by additional extraction of natural berry components into the juice. Moreover, the use of the vacuum freeze-drying method, according to the literature data, will preserve biologically active, minor, and technologically significant elements of berries.

The results of studies of the biochemical composition of the freeze-dried cranberry berries powder obtained by fermented mashed berries are presented in Table 1.

As shown by the data presented in Table 1, the sublimed cranberry powder is characterized by the high content of sugars and organic acids. In their powder, their content is 39.2 ± 0.9 g/100 g and 24.4 ± 0.7 g/100 g [Table 1].

As shown by chromatographic data, the dominant role in the spectrum of organic acids belongs to citric acid; its content is 16.22 g/100 g [Table 2].^[48] The citric acid in the food industry is used as a regulator of acidity, to protect against the flow of destructive processes. The most typical of fruits and berries is malic acid, which is present in the cranberry powder in the amount of 1.86 g/100 g. Of the others, a quinic acid in the amount of 5.61 g/100 g is determined [Table 2].

The spectrum of hydroxycinnamic acids is dominated by chlorogenic acid ($\approx 45\%$ of the total number of acids detected) and its structural isomer 4-caffeolic acid ($\approx 22\%$) [Table 3 and Figure 4].

Ferulic and p-coumaric acids are found in one concentration range (89.04 and 94.29 mg/kg); in almost half the quantity, coffee acid (48.32 mg/kg) is present and the content of kaftar acid (7.77 mg/kg) is insignificant [Table 3].

Identified acids have high antioxidant properties. It has been established that the AOA of chlorogenic acid is 27 times higher than the AOA of naringenin (the main grapefruit flavonoid). Moreover, for AOA, they can be arranged in a row: Coffee > ferulic > chlorogenic >> naringenin.^[49] For chlorogenic and caffeic acids, hypoglycemic action is shown.^[49] In the literature, data are given that chlorogenic, coffee, and ferulic acids have a mild hypocholesterolemic

Table 1: Biochemical composition of freeze-dried cranberry powder

Component name	Content, in 100 g	Component name	Content, in 100 g
Water, g	5.0	Polyphenol compounds, mg	3330±270
Total ash, g	1.1	Anthocyanins, mg	1237±28
Total sugar, g	39.2±0.9	Proanthocyanidins, mg	28.5±1.7
Protein, g	4.2±0.3	Tannins, mg	1208 90
Organic acids (titrated) in terms of apple), g	24.4±0.7	Flavones and flavonols (in terms of rutin), mg	711.3±8.5
Alimentary fiber	20.3±1.5	Vitamin C, mg	139.6±2.5

effect, reduce the risk of developing cholelithiasis.^[50,51] Data are reported that ferulic and p-coumaric acids prevent the development of colorectal cancer.^[52]

The unique natural acid contained in cranberry berries is benzoic acid. Berry berries occupy a leading position in the content of benzoic acid among other fruits and berries (up to 0.2%).^[1,2] The antimicrobial action of benzoic acid is widely used in the food industry.^[48] The preservative effect, which is achieved by the use of benzoic acid (salts) in the preparation of food products, is due to the manifestation of the antimicrobial properties of benzoic acid, which are based on the inhibition of enzyme activity in the cells of the infecting microflora.^[48] Using reversed-phase-HPLC, the content of benzoic acid in the freeze-dried cranberry powder was determined, which was 238 mg/100 g.

Table 2: Content of some organic acids in freeze-dried cranberry powder

Name of acid	Content, μ /100 μ
Quinic acid	5.61
Malic acid	1.86
Citric acid	16.22

Table 3: Composition and content of hydroxycinnamic acids in freeze-dried cranberry powder

Name of acid	Content, mg/kg
Caftaric acid	7.77
Chlorogenic acid	333.27
4-Caffeolic acid	160.23
Caffeic acid	48.32
p-coumaric acid	94.29
Ferulic acid	89.04
Sum of hydroxycinnamic acids	732.92

Sublimated cranberry powder is a carrier of components of non-alimentary nature of natural origin - dietary fiber, which is given great importance in the prevention of a number of diseases of the gastrointestinal tract, diabetes, metabolic disorders, etc.^[53,54] The content of dietary fiber in the powder product is significant and amounts to 20.3 ± 1.5 g/100 g, which indicates the prospects of its use for the enrichment of food products [Table 1].

A large group of biologically active substances are compounds of polyphenolic nature, which are represented quite extensively in the sublimated cranberry powder. The total content of phenolic compounds is 3330 ± 270 mg/100 g; flavones and flavonols (in terms of routine) - 711.3 ± 8.5 mg/100 g [Table 1].

The high content (1237 ± 28 mg/100 g) of natural pigments - anthocyanins, which determine the characteristic cranberry color of the powder product [Table 1] is revealed, which allows to view cranberry powder as a source of natural dyes in the production of food products. The prophylactic effect of anthocyanins is shown: When present in the diet, they reduce the risk of developing cardiovascular and cancer diseases.^[55-57]

Of the unique flavonoid compounds inherent in cranberry berries, proanthocyanidins are defined in the powder product. Their content is 28.5 ± 1.7 mg/100 g [Table 1]. For proanthocyanidins, onco- and cardio-protective effects have been identified.^[58,59]

The most famous group of flavonoids is catechins. The content of catechins in sublimated cranberry powder is 556.5 mg/kg [Table 4 and Figure 5].

Analysis of chromatographic data shows that more than 65% of all detected catechins account for the share of epicatechin [Table 4]. The second in the quantitative

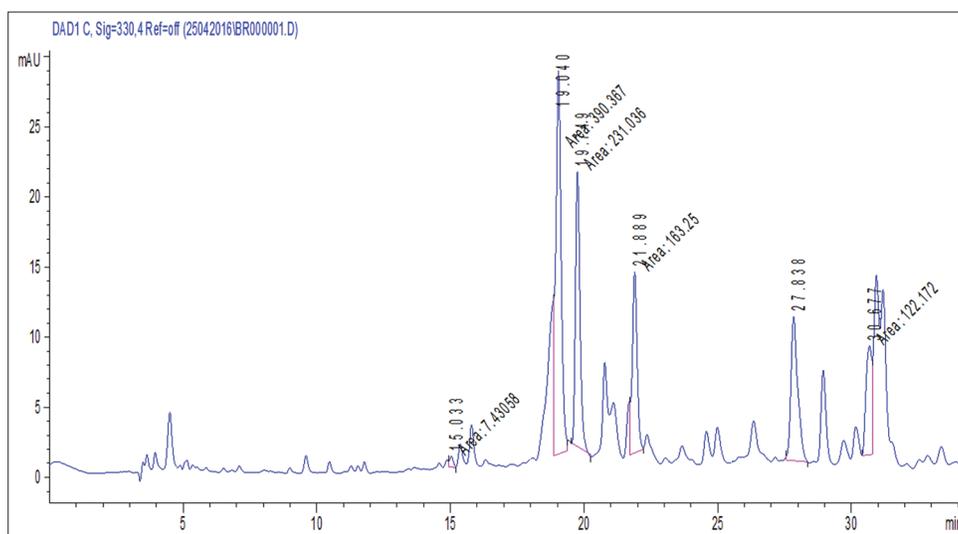


Figure 4: Chromatogram of hydroxycinnamic acids of freeze-dried cranberry powder

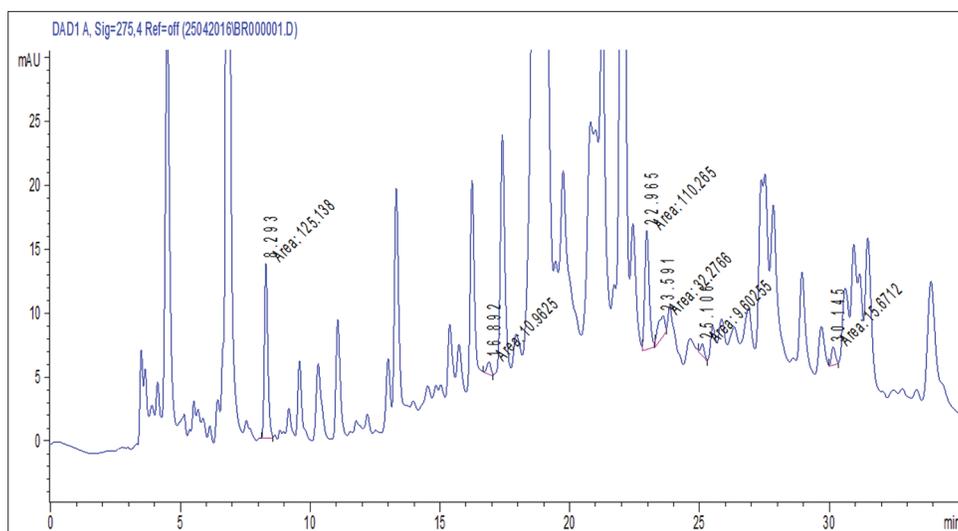


Figure 5: Chromatogram of catechins and gallic acid of freeze-dried cranberry powder

Table 4: Composition and content of catechins and gallic acid in freeze-dried cranberry powder

Name	Content, mg/kg
Gallic acid	61.11
Epigallocatechin	99.12
Epicatechin	366.87
Epigallocatechin gallate	49.56
Gallocatechin gallate	25.41
Epicatechin gallate	15.54
Sum of catechins (excluding gallic acid)	556.50

series of catechins is epigallocatechin - 99.12 mg/kg ($\approx 18\%$). As a second quantitative significance among catechin compounds, lingonberry berries, namely epigallocatechin could be identified as part of the pulp fruit juice fraction cranberries after enzymatic pretreatment.^[47] Complement set catechin compounds esters with gallic acid, which have a higher antiradical activity compared to the baseline catechins - epigallocatechin gallate - 49.56 mg/kg, gallocatechin gallate - 25.41 mg/kg, and epicatechin gallate - 15.54 mg/kg [Table 5]. The resulting chromatograms identify gallic acid. Its content in the freeze-dried cranberry powder is 61.11 mg/kg [Table 4]. Gallic acid has antibacterial, antiviral, hypoglycemic, and antioxidant action accelerates the healing of wounds and burns.^[60,61]

A characteristic astringent, astringent taste of cranberry berries and products of its processing are given by tannins (tannins) belonging to the group of phenolic compounds. By their nature, tannins are phenolic acid esters, preferably ellagic and gallic acids or formed by condensing phenolic compounds - flavonols, mainly of flavan-3-ols (catechins), or flavan-3,4-diols (leucoanthocyanins).^[6] Representatives of both classes of tannins are present in vegetable raw materials including cranberries in berries.^[1,2,6] As shown

by the data of the conducted studies, the content of tannins in cranberry powder was 1208 ± 90 mg/100 g [Table 1]. In the food industry, tannin-containing raw materials are used to impart to the products a tart taste, a specific color, and aroma (brewing and winemaking).^[48,62] Anti-inflammatory properties of tannins have found application in medicine.^[61]

Using the HPLC-DMD-MS, the profile of flavonoids in the freeze-dried cranberry powder was studied and the content of their individual representatives was determined [Table 5, Figures 6 and 7].

According to the results obtained, quercetin is present in cranberry powder (15.6% of the flavonoid compounds detected), but in its overwhelming amounts, its glycosides: Quercitrin, avicularin, and hyperoside (65.3%). Two more representatives of quercetin glycosides, acylated for carbohydrate residues, were not identified [Table 5].

Of vitamins, the content of Vitamin C was determined, which was 139.6 ± 2.5 mg/100 g [Table 1].

Supplementing the nutritional value of freeze-dried cranberry powder is available in a wide range of macro- and micro-elements [Table 6]. Mineral substances are the most important group of natural compounds, which are a necessary component of the normal life.

In the sublimed powder of cranberry, potassium, phosphorus, and calcium are leading (4363.8 mg/kg, 2134.4 mg/kg, and 1058.8 mg/kg, respectively) [Table 6]. Significant amounts of magnesium are 568.9 mg/kg. In comparison with other macro elements, the sodium content is insignificant (72.8 mg/kg) [Table 6].

From trace elements in significant quantities revealed manganese, iron, aluminum, silicon, and zinc. The presence

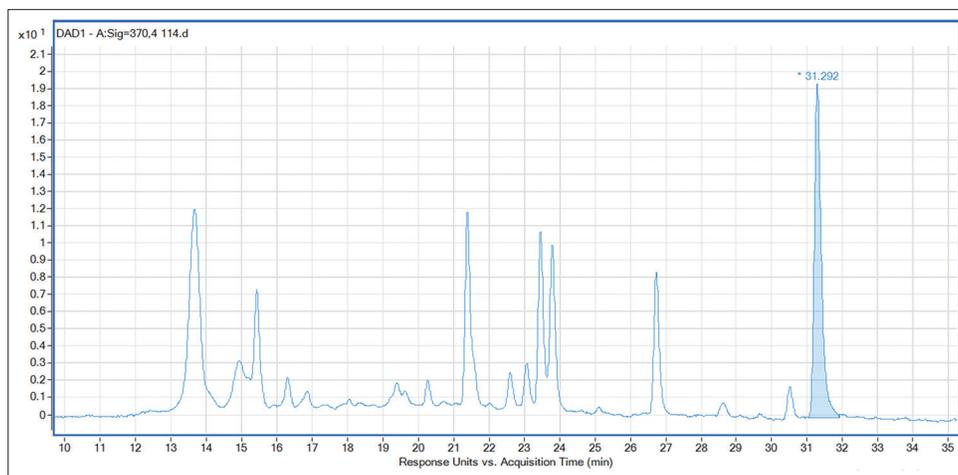


Figure 6: Chromatogram of flavonoids of sublimated cranberry powder, $\lambda = 370$ nm (quercetin)

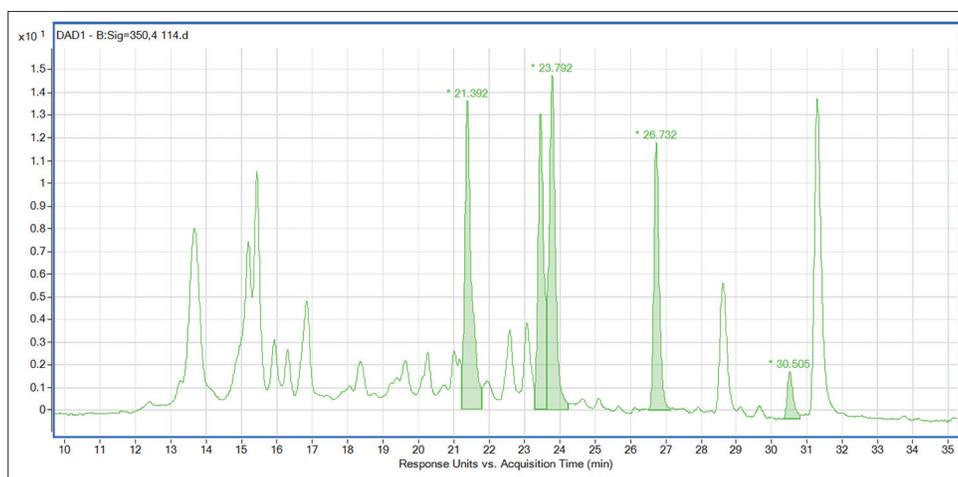


Figure 7: Chromatogram of flavonoids of sublimated cranberry powder, $\lambda = 350$ nm (quercetin glycosides)

Table 5: Content of individual flavonoids in freeze-dried cranberry powder, mg/100 g

Flavonoid	Content	Flavonoid	Content
Hyperoside	20.8	Quercetin	14.0
Avicularin	16.9	Unidentified acylated glycoside of quercetin	14.4
Quercitrine	21.2	Unidentified acylated glycoside of quercetin	2.8

Table 6: The content of some minerals in the freeze-dried cranberry powder

Name of the element	Content, mg/kg	Name of the element	Content, Мг/кг
Potassium	4363.8	Phosphorus	2134.4
Calcium	1058.8	Magnesium	568.9
Aluminum	31.5	Manganese	211.1
Zinc	12.1	Sodium	72.8
Iron	40.9	Nickel	0.83
Silicon	23.3	Lithium	0.79
Silver	0.11	Chromium	0.65

of lithium, nickel, chromium, and silver supplements and the range of mineral substances of cranberry powder are shown in Table 6.

The final stage of the study was the determination of the AOA of the sublimated cranberry powder about the DPRN radical, which was 5370 mg/100 g (in TEAS units).

CONCLUSION

The results of the conducted studies indicate that the freeze-dried cranberry powder obtained by fermented mashed berries contains a wide range of natural berries compounds, functional food ingredients, minor, and biologically active substances and is a source of natural dyes and preservatives. The combination of elements with antioxidant properties in freeze-dried cranberry powder causes its manifestation of a high AOA, which is the result of a synergistic effect due to the joint action of the complex of recognized antioxidants present in the berry powder. All this in aggregate gives grounds to talk about the prospects of using freeze-dried cranberry powder in obtaining traditional food products to increase nutritional value for the content of essential and minor micronutrients, as well as products of functional and therapeutic and prophylactic purposes.

REFERENCES

1. Lyutikova MN. The Study of the Composition of Biologically Active Components of Wild Berries *Vaccinium vitis-idaea* and *Oxycoccus palustris*, Depending on the Degree of their Maturity and Storage Conditions: Author's Abstract. Dis... Candidate of Technical Sciences. Chernogolovka; 2013. p. 26.
2. Lyutikova MN, Kh BE. Chemical composition and practical application of berries of cranberries and cranberries. *Chem Plant Raw Mater* 2015;2:5-27.
3. Su Z. Anthocyanins and flavonoids of *Vaccinium L.* *Pharm Crops* 2012;3:7-37.
4. Tsybukova TN, Petrova EV, Rabtsevich ES, Zeil LA, Tikhonova OK, Agasheva EA. Elemental composition of fruits of cranberry and cranberry marsh. *Chem Plant Raw Mater* 2017;4:229-33.
5. Afanasyeva LV. The content of microelements in the berries of *Vaccinium vitis-idaea* in the Southern Baikal region. *Chem Plant Raw Mater* 2016;3:103-8.
6. Kurkina AV. Flavonoids of Pharmacopeia Plants: Monograph. Samara: Ofort; 2012. p. 290.
7. Tempera G, Corsello S, Genovese C, Caruso FE, Nicolosi D. Inhibitory activity of cranberry extract on the bacterial adhesiveness in the urine of women: An *ex-vivo* study. *Int J Immunopathol Pharmacol* 2010;23:611-8.
8. Loskutova EV, Prikhodko OL, Bibik IV, Babi NV. Obtaining dry powders from fruit and berry raw materials of the far east. *Technol Prod Proc Agric Prod Blagoveshchensk* 2010;9:93-6.
9. Yarliyeva ZA. Perfection of the Technology of Cryoporous Powders from Fruits and Berries Grown in the Foothills of Dagestan: Dis Cand. Tech. Sciences: 05.18.01/Z.A. Yarliyeva. Krasnodar; 2017. p. 141.
10. Gribova NA, Berketov LV, Akimova NA, Prospects for the development of the processing industry in the processing of fruit and berry raw materials. *Sci Rev* 2015;24:107-10.
11. Antipov ST, Kazartsev DA, Zhuravlev AV, Ryazhskikh EV, Vinichenko SA. Development of a line for the processing of black currant berries and powders from them. *Technol Food Proc Ind AIC Prod Healthy Food* 2014;1:110-3.
12. Semenov GV. Vacuum Freeze Drying. Moscow: DeLi Plus; 2013. p. 264.
13. Aleksashina SA, Makarova NV. Influence of Freeze-drying on the Content of Biologically Active Substances of Vegetable Raw Materials of the Samara Region. *Mat. IX Vseros. Scientific-Practical. Conference Students, Graduate Students and Young Scientists with International Participation*; 2016. p. 523-5.
14. Semenov G, Budantsev EV, Krasnova IS. Antioxidant activity of plant raw materials in the processes of freezing and vacuum dehydration. *Sci J NIU ITMO* 2014;4:210-3.
15. Ermolaev VA, Yakovenko MA. Analysis of the processes of freeze drying of raspberries. *Herald Agrar Ind Complex Up Volga Reg* 2017;4:55-8.
16. Barbashin DI, Gribova NA, Baranov BA. Freeze drying of berry raw materials. *Prog Modern Sci* 2017;3:67-70.
17. Korotky IA, Raschepkin AN, Fedorov DE. Research of the process of vacuum drying of crushed red bilberries. *Tech Technol Food Prod* 2014;5:183-7.
18. Suslova AA, Krasnova IS, Posokhov ND, Suvorov OA. Sublimated berries in confectionery technology. *Innov Commodity Sci Public Cater Long Term Storage Foodstuffs*, 2015;2:234-9.
19. Nilova LP. Antioxidant activity of bakery products enriched with blueberry powder. *Vestnik UYGU* 2014;4:57-63.
20. Luneva ON. Enrichment of Curd Dessert with Vegetable Components. Priorities and Scientific Support for the Implementation of the State Policy of Healthy Nutrition in Russia: Proceedings of the V International Scientific and Practical Internet Conference. Orel; 2017. p. 115-119.
21. Tipsina NN. The use of the sea-buckthorn powder in the production of confectionery products. *Vestn Krasnoyarsk State Agrarian Univ* 2013;5:112-7.
22. Buchert J, Koponen JM, Suutarinen M, Mustranta A, Lille M, Törrönen R, *et al.* Effect of enzyme-aided pressing on anthocyanin yield and profiles in cranberry and blackcurrant juice. *J Sci Food Agric* 2005;85:2548-56.
23. Kapasakalidis PG, Rastall RA, Gordon MH. Effect of a cellulose treatment on extraction of antioxidant phenols from black currant (*Ribes nigrum L.*) pomace. *J Agric Food Chem* 2009;57:4342-51.
24. Sun T, Powers JR, Tang J. Effect of enzymatic macerate treatment on rutin content, antioxidant activity, yield and physical properties of asparagus juice. *J Food Sci* 2007;72:267-71.
25. Alekseenko EV, Dikareva YM, Ostashenkova NV, Trautenberg SE. Influence of the conditions of biocatalysis of sea-buckthorn berries on the yield in juice and physiologically functional ingredients. *Storage Proc Agric Raw Mater* 2012;9:38-40.

26. Shichkina ES, Bolotov VM. Influence of enzymatic preparations on the yield of coloring substances in the production of natural dyes. *Storage Proc Agric Raw Mater* 2013;1:46-7.
27. Volchok AA. New Multi-enzyme Complexes for Destroying Polysaccharides of Fruit Raw Material in Conditions of Winemaking: Dis. Candidate of Technical Sciences: 03.01.06. Moscow: Volchok Anastasia Alexandrovna; 2016. p. 138.
28. Ivanova LA, Voino LI, Ivanova IS. Food biotechnology. In: Gracheva IM, editor. Book. 2. Processing of Vegetable Raw Materials. Moscow: Kolos S; 2008. p. 472.
29. Matveeva IV, Martynov VY. Enzyme preparations: Safety, innovative applications, environmental protection. *Food Ingredients Raw Mater Addit* 2010;2:24-8.
30. Naumenko NV. Possibilities of using biotechnologies in food production. *Actual Biotechnol* 2013;2:14-7.
31. Rimareva LV, Serba EM, Sokolova NV, Borshcheva YA, Ignatova NI. Enzyme preparations and biocatalytic processes in the food industry. *Nutr Issues* 2017;5:63-74.
32. Alekseenko EV, Bystrova EA. Monitoring of the effectiveness of the use of enzyme preparations for processing berries cranberries in the production of juice. *Proc VSUET* 2015;3:203-7.
33. Alekseenko EV, Bystrova EA, Semenov GV, Chernykh VY. Technology of obtaining and evaluation of the quality of freeze powder from berries cranberries. *Food Ind* 2017;11:70-3.
34. Zhogova AA, Perova IB, Samylina IA, Ramenskaya GV. Identification and quantitative determination of the main biologically active substances of the motherwort grass using HPLC-mass spectrometry. *Chem Pharm J* 2014;754-9.
35. Polygalina GV, Cherednichenko VS, Rimareva LV. Determination of enzyme activity. *Directory*. Moscow: De Li Print; 2003. p. 375.
36. Hydranal-Manual. Eugen Schoolz Reagents for K. Fischer Titration. Seelze, Germany: Hydranal-Manual; 1990. p. 126.
37. Alekseenko EV. Modern Methods of Analysis in the Technochemical Control of Raw Materials and Food Products: Textbook. Moscow: CNTB Food Industry; 2015. p. 120.
38. Nechaev AP, Trautenberg SE, Kochetkova AA, Kolpakova VV, Vitol IS, Kobeleva IB. Food Chemistry. Laboratory Practice. St. Petersburg: GIORD; 2006. p. 304.
39. Ermakov AI, Arasimovich VV, Smirnova-Ikonnikova MI, Yarosh NP, Lukovnikova G. A Methods of Biochemical Research of Plants. Leningrad: Agropromizdat; 1987. p. 429c.
40. Russian Ministry of Health. Management P. 4.1. 1672-03 Manual on methods for quality control and safety of biologically active food additives. Moscow: The Federal Center for Sanitary and Epidemiological Supervision of the Russian Ministry of Health; 2004. p. 240.
41. Tutelian VA, Eller KI, Aristarkhova TV. Methods for the Analysis of Minor Biologically Active Food Substances. Moscow: Publishing House (Dynasty); 2010. p. 180.
42. Less VR. In: Zenkevich IG, Shurduby NA, Boldyreva IV, editors. Practical Guidance for the Laboratory. Special Methods: Translation from German. 2nd ed. St. Petersburg: Profession; 2011. p. 472.
43. Din GP. Inductively Coupled Plasma: A Practical Guide. St. Petersburg: Profession; 2017. p. 200.
44. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. *LWT Food Sci Technol* 1997;6:609-15.
45. Yu PG, Plaksin YM. Mathematical methods of experiment planning. Moscow: Delhi Print Publishing House; 2005. p. 296.
46. Alekseenko EV, Bystrova EA, Dikareva YM. Investigation of the influence of preliminary processing of berries with red cranberry using the composition of enzyme preparations on the chemical composition of juice. *Proc VSUET* 2017;1:282-9.
47. Bystrova EA, Alekseenko EV. Investigation of the component composition of phenolic compounds and antioxidant activity of cranberry juice. *News of universities. Appl Chem Biotechnol* 2017;3:19-26.
48. Vitol IS, Gorbatyuk VI, Gorenkov ES, Gorelikova AN, Kutina OI, Shlenskaya TV. In: Nechaev AP, editor. Introduction to Food Technology. Moscow: Deli Plus; 2013. p. 720.
49. Jung UJ, Lee MK, Park YB, Jeon SM, Choi MS. Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice. *J Pharmacol Exp Ther* 2006;318:476-83.
50. Levitsky AP, Vertikova EK, Selivanskaya IA. Chlorogenic acid: Biochemistry and physiology. *Microbiol Biotechnol* 2010;2:6-20.
51. Leitzmann MF, Stampfer MJ, Willett WC, Spiegelman D, Colditz GA, Giovannucci EL, *et al.* Coffee intake is associated with lower risk of symptomatic gallstone disease in women. *Gastroenterology* 2002;123:1823-30.
52. Roy N, Narayanankutty A, Nazeem PA, Valsalan R, Babu TD, Mathew D, *et al.* Plant phenolics ferulic acid and P-coumaric acid inhibit colorectal cancer cell proliferation through EGFR down-regulation. *Asian Pac J Cancer Prev* 2016;17:4019-23.
53. Nechaev AP, Trautenberg SE, Kochetkova AA, Kolpakova VV, Vitol IS, Kobeleva IB. Food Chemistry. In: Nechaev AP, editor. 6th ed. Corrected and Additional. St. Petersburg: GIORD; 2015. p. 672.
54. Tutelyan VA, Pogozhevoy AV, Vysotsky VG, editors. The Role of Dietary Fiber in Human Nutrition. Moscow: The New Millennium Foundation; 2008. p. 320.
55. Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. *Cancer Lett* 2008;2:281-90.
56. Novotniy GA. Anthocyanins, flavonoids and cardiovascular diseases. *Quest Diet* 2014;3:28-31.
57. Wang S, Melnyk JP, Tsao R, Marcone MF. How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. *Food Res Int* 2011;44:14-22.

58. Kim YJ, Park HJ, Yoon SH, Kim MJ, Leem KH, Chung JH, *et al.* Anticancer effects of oligomeric proanthocyanidins on human colorectal cancer cell line, SNU-C4. *World J Gastroenterol* 2005;11:4674-8.
59. Sato M, Maulik G, Ray PS, Bagchi D, Das DK. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. *J Mol Cell Cardiol* 1999;31:1289-97.
60. Tutelyan VA, Laschneva NV. Biologically active substances of plant origin. Phenolic acids: Prevalence, food sources, bioavailability. *Nutr Issues* 2008;1:4-19.
61. Shabrov AV, Dadali VA, Makarov VG. In: Dadali VA, editor. *Biochemical Bases of Action of Microcomponents of Food*. Moscow: Avvalon; 2003. p. 184.
62. Pomozova VA. *Manufacture of Kvass and Non-alcoholic Beverages: Textbook*. Kemerovo: Stereotip; 2006. p. 148.

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