Composition of biologically active substances of Vaccinium berries growing in the northwestern region of Russia

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Abstract. Using gas chromatography with preliminary derivatization of samples, the composition of the biologically active substances of wild-growing cranberries (Vaccinium oxyccocos L.) and lingonberries (Vaccinium vitis idaea L.) was studied. Biologically active substances contain phenolic compounds, tocopherols and phytosterols. Flavonoids prevail in the composition of phenolic compounds, the amount of which increases in berry press residues. Cranberries and lingonberries differ in the quantitative and qualitative composition of flavonoids and phenolic acids. In fresh cranberry berries, taxifolin and morin predominate among flavonoids; catechin and taxifolin predominate in lingonberries. In berry press residues, the number of flavonoids increases due to taxifolin and qercetin B, and in lingonberries, additionally due to catechin. Chlorogenic acid predominates in the composition of phenolic acids of fresh cranberries; 4-Oxybenzoic acid predominates in fresh lingonberries. Tocopherols quantitatively prevail in whole lingonberries, and phytosterols predominate in cranberries. In the berry press residues, their number increases.

1. Introduction

At the present time, the use of natural ingredients in the production of food products has become one of the main trends in nutrition science and food technology, and this explains the growing demand for them among consumers [1, 2]. Not only plant raw materials and their wastes, but also biologically active components extracted from them can be used as natural ingredients [3-5]. Modeling and optimization of technological processing of plant raw materials allows preserving their biologically active substances (BAS) to the maximum [6, 7]. As a result, the use of plant raw ingredients has stimulated production of foods, enriched with valuable sensory, antioxidant and / or antimicrobial properties [8-11].

Wild and cultivated berries are a popular source of antioxidant biologically active substances in human nutrition. Vaccinium berries — cranberries, lingonberries, and blueberries — are very popular among Russian consumers, especially the inhabitants of the northern regions. Consumption of fresh berries is limited by the short summer season, but in recent years berries have been actively used in the production of juices or other food products [12, 13]. Berry press residues that remain after pressing out the juice contain biologically active substances of phenolic nature, the amount of which is comparable to or even exceeds that found in whole berries [14-16]. Powders or pastes are usually obtained from
press residues, but recently they have been also used for extracting oil and wax [17-19]. Most often, berry press residues are used for obtaining biologically active substances of phenolic nature from water-alcohol extract solutions [20, 21].

The main corpus of research of Vaccinium berries has been aimed at studying composition of phenolic compounds as the main source of BAS; the analysis has been carried out by chromatographic methods [16, 17, 22, 23]. Studies of composition of BAS of lipid nature have been limited due to the low lipid content in berries, with the exception of seeds. Studies of carotenoids and tocopherols composition have been published [14, 24]. Gas chromatography with preliminary derivatization allows the simultaneous determination of substances with various properties, including fat-soluble compounds. Preliminary derivatization of samples blocks highly polar carboxyl, hydroxyl, thio and amino groups, replacing active hydrogen with nonpolar alkyl, acyl, trialkylsilyl residues, as a result of which strong intermolecular hydrogen bonds are broken, providing the necessary volatility of the obtained products without their thermal decomposition [25]. Derivatization reactions used in gas chromatography (GC) can be divided into three main types: alkylation, acylation and silylation. Silylation is the most common derivatization method, since it allows to easily vaporize the sample and is therefore very suitable for nonvolatile samples used for GC analysis, allowing the identification of water and fat soluble antioxidants [26, 27].

The purpose of this work has been to study composition of biologically active substances by GC with preliminary derivatization of Vaccinium berries (cranberries and lingonberries that grow in the northwestern region of Russia).

2. Materials and methods

2.1 Materials
Cranberries (Vaccinium oxycoccos L.) and lingonberries (Vaccinium vitis idaea L.) were picked up in the Leningrad Region, Russia. The berries were frozen at the temperature of minus 18°C and stored at the same temperature until the time of research. The juice was extracted from berries at EKM-3 electromechanical machine, at the Electrosila plant, St. Petersburg, Russia. For the purposes of this research, whole berries were used as well as press berry residues, obtained after pressing out the juice.

2.2 Methods
Composition of antioxidants was determined by gas chromatography at Agilent 6890 Series chromatograph, produced by Agilent Technologies, USA, at the analytical laboratory of AMT LLC, St. Petersburg, Russia.

Sample preparation: the 50% ethanol sample solution (1:15 weight / volume) was dried up in vacuum at 38 °C, and then treated in a mixture of 1 ml of pyridine and 1 ml of acetonitrile 1,1,1,3,3,3-hexamethyldisilazane in the presence of trifluoroacetic acid at 60°C for one hour. The resulting solution was placed in the chromatograph probe compartment.

Analysis conditions: SBP5-25 column (25 mx 0.25 mm x 0.2 μm); carrier gas N₂, 20 cm/s; temperature program — 1 min. 70°C, rise 4°C / min. up to 140°C and further up to 180°C / min., 5 min. 320°C; sample inlet temperature 240°C, flow divider 1:20, sample volume 2 μl; flame-ionization detector, temperature 325°C, hydrogen supply rate — 40 ml / min., nitrogen - 25 ml / min., oxygen — 250 ml / min.

Calculation of the components’ content by the average peak area was carried out without adjustments for their specific sensitivity. Peaks were assigned according to retention times after a series of calibration analyzes of model mixtures of certain compositions. Kovacs retention indices characterizing the retention of a substance in a column by the stationary phase at a given temperature relative to two n-alkanes with the number of carbon atoms n and (n + 1) were calculated by linear interpolation.
The measurement result is represented in the following form: $X \pm \Delta$ mg g$^{-1}$ ($X$ is the concentration of the component in the sample, mg 100 g$^{-1}$; $\Delta$ – the absolute error range in the identification, mg g$^{-1}$, with the confidential probability of $P \geq 0.90$). The arithmetic mean of the results from three parallel identifications was taken as the result of the test.

3. Results and discussion

In cranberries and lingonberries it was possible to identify 14 phenolic compounds in a free state (Table 1). Their qualitative composition differed slightly depending on the type of berries or berry press residues. Phenolic Acids — 2-Hydroxyphenylacetic, vanillic, caffeic, Rutin, and Salidroside glucoside — were not found in whole cranberry berries, but were found in lingonberry berries. At the same time, 3-Hydroxyphenylacetic acid, Chlorogenic acid, Morin, Kaempferol, and Arbutin glucoside, present in cranberry berries, were not found in whole lingonberry berries. Flavonoids predominated in phenolic compounds, regardless of the type of berries. In whole cranberries they amounted to 75.6%, and in lingonberries — to 67.3%. After the juice had been pressed out, their share in berry press residues increased and amounted to 88.2% for cranberries and 75.4% for lingonberries. The predominance of flavonoids in phenolic compounds was typical in cranberries and lingonberries growing in northern regions, for example, in Finland and Sweden [22, 28].

| Table 1. Composition of free BAS found in whole berries and berry press residues of cranberries and lingonberries, collected in the northwestern region of Russia, mg 100 g$^{-1}$ DM |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Compound**    | **RI, min**     | **Kovach Index**| **Cranberry**   | **Lingonberry** |
|                 |                 |                 | **berry**       | **berry press residues** |   |
|                 |                 |                 | **press residues** |   |   |
| Hydroquinone    | 22.07           | 1402            | 0.5             | 0.4             | 2.1 | 2.1 |
| Pyrogallol      | 26.55           | 1565            | 0.7             | 0.7             | 0.7 | 0.8 |
| **Sum**         | 1.2             | 1.1             | 2.8             | 2.9             |
| Phenolic Acids  |                 |                 |                 |                 |
| 2-hydroxyphenylacetic acid | 27.23 | 1589 | nd | nd | 0.5 | nd |
| 3-hydroxyphenylacetic acid | 28.30 | 1628 | 0.4 | nd | nd | nd |
| 4-Oxybenzoic acid | 28.86 | 1649 | 2.2 | 2.0 | 6.1 | 5.3 |
| Vanillic acid   | 32.70           | 1789            | nd              | nd              | 1.0 | 1.1 |
| 4-coumaric acid | 37.01           | 1941            | 3.5             | nd              | 2.9 | 4.7 |
| Gallic acid     | 37.81           | 1976            | 0.4             | 2.0             | 0.5 | 0.9 |
| Ferulic acid    | 40.53           | 2100            | 1.6             | 1.3             | 4.5 | 3.9 |
| Caffeic acid    | 41.64           | 2151            | nd              | nd              | 0.3 | 0.6 |
| Chlorogenic acid | 60.18 | 3168 | 4.6 | 3.2 | nd | nd |
| **Sum**         | 12.7            | 8.5             | 15.8            | 16.5            |
| Flavonoids      |                 |                 |                 |                 |
| Rutin           | 50.55           | 2604            | nd              | nd              | 3.5 | 5.1 |
| Catechin        | 56.32           | 2933            | 2.7             | 1.9             | 19.3 | 25.3 |
| Morin           | 57.28           | 2990            | 16.0            | 12.5            | nd | nd |
| Taxifolin       | 57.85           | 3024            | 29.4            | 74.0            | 15.3 | 18.3 |
| Kaempferol      | 59.38           | 3118            | 2.9             | nd              | nd | nd |
| Quercetin B     | 61.14           | 3228            | 1.6             | 5.4             | 3.2 | 14.5 |
| **Sum**         | 52.6            | 93.8            | 41.3            | 63.2            |
| Glycosides      |                 |                 |                 |                 |
| Arbutin         | 51.24           | 2642            | 3.1             | 2.9             | nd | nd |
| Salidroside     | 54.50           | 2826            | nd              | nd              | 1.4 | 1.3 |
| **Sum**         | 3.1             | 2.9             | 1.4             | 1.3             |

nd—substance not detected
The composition of flavonoids varied depending on the type of berries, with taxifolin and Morin prevailing in cranberries, and catechins and taxifolin — in lingonberries. An increase of flavonoids in berry press residues occurred due to taxifolin and Quercetin B, and in lingonberry also due to catechin. Unlike flavonoids, phenolic acids mostly passed into juice during processing of berries, which is proved by its decrease in berry press residues by 10.2% in cranberries and by 6% in lingonberries (as compared to whole berries). Although in lingonberries press residues they were contained in a greater amount than in berries. The qualitative and quantitative composition of free phenolic acids varied greatly depending on the type of berries. In whole cranberries 6 phenolic acids with a predominance of chlorogenic acid were identified, but they lacked 2-Hydroxyphenylactic acid, Vanillic, and caffeic acid, which were identified in lingonberries. In whole lingonberries, 7 phenolic acids with a predominance of 4-Oxybenzoic acid were identified, while chlorogenic and 3-Hydroxyphenylactic acid were not found. Not all phenolic acids passed into juice or were destroyed during berry processing. For example, the amount of free gallic acid increased in cranberry press residues, while lingonberry press residues showed the increase of 4-Coumaric, gallic and caffeic acids. Hydroquinone and Pyrogallol phenols were found in phenolic compounds of cranberries and lingonberries; their amount did not show any statistically significant differences between whole berries and berry press residues.

Lipid substances add to the biological activity of berries, despite the low content of lipids in the pulp and skin. In the berry pulp lipids are contained in the cytoplasm or can be associated with cell membranes. In the skin, they can be part of cuticular waxes. But most of lipids are contained in the seeds [18, 29, 30]. For the purposes of our study, free BAS of lipid nature were determined in skin and pulp of whole berries and berry press residues, taken without seeds (Table 2).

| Compound          | RI, min | Kovach Index | Cranberry berry | Cranberry berry press residues | Lingonberry berry | Lingonberry berry press residues |
|-------------------|---------|---------------|-----------------|-------------------------------|------------------|---------------------------------|
| Tocopherols       |         |               |                 |                               |                  |                                 |
| α-Tocopherol      | 60.08   | 3161          | 0.3             | 0.4                           | 0.6              | 0.9                             |
| γ-Tocopherol      | 57.39   | 2996          | nd              | nd                            | 0.3              | nd                              |
| δ-Tocopherol      | 55.79   | 2901          | 0.3             | 1.6                           | 1.3              | 2.4                             |
| Sum               |         |               | 0.6             | 2.0                           | 2.2              | 3.3                             |
| Phytosterols      |         |               |                 |                               |                  |                                 |
| Brassicasterol    | 61.32   | 3239          | nd              | 0.6                           | nd               | 0.8                             |
| Ergosterol        | 61.75   | 3265          | 0.9             | 0.6                           | nd               | nd                              |
| Stigmasterol      | 62.00   | 3282          | 0.3             | 0.8                           | nd               | nd                              |
| β-Sitosterol      | 62.81   | 3334          | nd              | 0.4                           | 0.3              | 0.8                             |
| Sum               |         |               | 1.2             | 2.4                           | 0.3              | 1.6                             |
| nd — substance not detected |

BAS of lipid nature of the studied whole berries and berry press residues contained tocopherols and phytosterols, with more phytosterols found in cranberries, and more tocopherols — in cranberries. Tocopherols were presented in α, γ, and δ forms. γ-tocopherol was identified only in whole lingonberries: it was absent in its berry press residues, as well as in whole cranberries and its press residue. Quantitatively, δ-tocopherol predominated, and in lingonberries it was 3.7 times higher than in cranberries. The prevalence of tocopherols in lingonberries as compared to cranberries was established by Clavins et al. [18]. At the same time, the variability of the fractional composition of tocopherols in Vaccinium berries can be associated with their varietal and geographical factors [14].
Cranberry and lingonberry press residues contained more δ-tocopherol than whole berries, which could be related to its content in cuticular waxes [30].

Phytosterols, including those present in berries, have antioxidant activity and are able to lower cholesterol in human bodies [29]. The most complete composition of phytosterols, both quantitative and qualitative, was found in cranberry press residues. It included 4 phytosterols: brassicasterol, ergosterol, stigmasterol and β-sitosterol. Only 2 phytosterols — ergosterol and stigmasterol — were identified in whole cranberries, which confirms their predominance in cuticular waxes [30]. In lingonberries brassicasterol was identified only in press residues, while β-sitosterol, although it was also found in whole berries, displayed the 5 times higher amount in press residues. In lingonberry, ergosterol and stigmasterol were not found. Clavins et al. [18] identified only β-sitosterol in fresh cranberries and lingonberries. As their studies have shown, the possibility of detecting phytosterols and tocopherols by GC is associated with extraction. The most effective method — to use Hexane or Diethyl ether. Its use in sample preparation (CH$_3$OH / CHCl$_3$ 2: 1) provides much better extraction, increasing the content of phytosterols and tocopherols 3-4 times.

4. Conclusion
Whole berries and press residues of cranberries and lingonberries, collected in the northwestern region of Russia, are the source of biologically active substances, phenolic compounds being the most valuable ones. Both in cranberries and lingonberries, flavonoids predominate in the composition of phenolic compounds, and their amount increases in berry press residues. Qualitative and quantitative differences in phenolic compounds composition of cranberries and lingonberries were established. In whole cranberries, taxifolin and morin predominate in flavonoids, while catechin and taxifolin predominate in whole lingonberries. In berry press residues, the amount of flavonoids increases due to taxifolin and quercetin B, and in lingonberry press residues — additionally due to catechin. In cranberries, 6 phenolic acids with a predominance of chlorogenic acid were identified. In lingonberries, 7 phenolic acids with a predominance of 4-Oxybenzoic acid were identified.

Berries contain biologically active substances of lipid nature, including tocopherols and phytosterols, which quantitatively prevail in berry press residues. Whole cranberries contain more phytosterols, while whole lingonberries contain more tocopherols. Tocopherols were represented by α-, γ- and δ-forms, with the predominance of the latter. In whole cranberries, only 2 phytosterols were identified — ergosterol and stigmasterol, while in lingonberries β-sitosterol was identified. The largest amount of phytosterols has been found in cranberry press residue.

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