A study of the chemical composition and antioxidant properties of products of wild berries processing

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Abstract. The article presents the results of studies of the chemical composition and composition of individual antioxidants of bilberries and lingonberries and their marc, which are waste products after squeezing the juice from the berries. The yield of juice from berries averaged 64%. Dry substances, acidity, composition of sugars, pectin substances and individual antioxidants - total phenolic compounds, total flavonoids, total anthocyanins were determined in berries and in the marc. During squeezing of the juice, a redistribution of chemical components was occurred with a predominance of total phenolic compounds, total flavonoids and total anthocyanins in the marc. As a result, the marc showed higher antioxidant properties than juices, which were investigated using the DPPH and FRAP methods. The antioxidant properties of the marc are due to the transition in them of anthocyanins, which make up 64% and 59%, respectively, of their total amount in bilberries and lingonberries. The correlation between DPPH and FRAP tests for anthocyanins was more than 0.952.

1. Introduction

Wild berries growing in northern latitudes can be an additional source of biologically active substances in human nutrition. Bilberry, lingonberry, blueberry, cranberry, and cloudberry grow in the forests of Russia, Finland, Norway, and Sweden. Recent studies have shown that these berries contain significant amounts of polyphenols, anthocyanins, hydroxycinnamic and hydroxybenzoic acids, which are natural antioxidants [1-5]. Their qualitative and quantitative composition varies widely depending on the type of berries, place of growth, cropping period, storage time, and processing - freezing and thermal heating. Wild berries contain more biologically active substances than their cultivated forms [6-8]. The quantitative composition of phenolic compounds between freshly harvested berries and berries sold in retail can vary by about 10 times [2, 4, 6, 9]. Thus, in wild berries sold in the consumer market in Sweden, the content of total phenolic compounds is 253.6 mg/100 g DW in bilberry, and 219.7 mg 100 g DW in lingonberry, while in freshly picked berries it can reach 3050 and 1350 mg/100 g DW, respectively [2, 4, 10, 11]. Processing berries in contact with oxygen leads to the loss of total phenolic compounds and flavonoids, while the anthocyanins are more stable [10, 12, 13]. Heat treatment of food using wild berries and their derivative products can lead to an increase in the antioxidant properties of the product being developed due to the synergism of biologically active substances and melanoidins [14]. To preserve the biologically active substances of extracts of berries, innovative encapsulation systems using natural whey proteins have been proposed [15]. A high content of biologically active substances is characteristic not only of berries, but also of leaves, stems, and sprouts, which are used medicinally [4, 11, 16].
It has been established that consumption in the diet of wild berries with a high content of fats contributes to their better metabolism by gut microflora, and prevents lipid peroxidation [17]. Due to the presence of natural antioxidants, especially anthocyanins, the consumption of fresh berries or dry preparations from them contributes to the prevention of alimentary dependent diseases, the main of which being cardiovascular and oncological ones [3, 18-20]. The pharmacological action of bilberry is the most widely studied. To obtain pharmacological preparations, bilberry extracts are used [13]. However, the usual extraction does not allow for the complete transfer of biologically active substances contained in the peel and seeds into the extract. For this purpose, cavitation, fermentation, and production of \( \text{CO}_2 \)-extracts are used for extraction [3, 21-23]. Fermentation of berries with saccharomycete allows obtaining additives from them with a high content of sugars while enhancing antimicrobial properties [9, 21]. It has been established that phenolic compounds and ellagotannin extracts of wild berries inhibit the development of Staphylococcus aureus and Bacillus cereus [6].

Seasonality of berry production has led to the growing need to obtain food from them with a long shelf life. In addition to traditional jams, confitures, fruit drinks, juices are obtained from wild berries, which are used as one of the components in fermented dairy products and mixed juices. The marc, remaining expression of juices is not used in food production.

The aim of the research was to study the chemical composition and antioxidant properties of the wild berries marc that remained after expressing the juice.

2. Materials and methods

2.1. Research objects
The most common wild berries of the northwestern federal district of Russia of the heather bloodline of the Vaccinium genus — blueberry and lingonberry — were chosen as objects of research. Berries were collected in the territory of the Vyborg district of the Leningrad region. The juice was obtained from the berries using an electromechanical-fy machine for expressing juice EKM-3, Electrosila JSC, St. Petersburg, Russia. The obtained juice was filtered to separate the pulp. Berries and marc after expressing the juice were used for research.

2.2. Research methods

2.2.1. Chemical composition research methods
The mass fraction of moisture was determined by the complete desiccation method at a temperature of 60 °C; titrated acids - by a titration method after preliminary extraction on a water bath; pectic substances - by the Metlitz method, the mass fraction of fiber - by the gravimetric method after preliminary hydrolysis [24].

The mass fraction of the sugars composition was determined on an Agilent 1260 Infinity II liquid chromatograph (USA). Capillary column - Bio-Rad Carbohydrates 300 mm x 7.8 mm. The mobile phase is bidistilled water, the flow rate of the mobile phase was 0.6 ml/min; the column temperature was 80 °C; the detection was performed using a refractometric detector; isocratic mode., the volume of the injected sample is 1 ml. Solutions of sucrose, glucose, and fructose in bidistilled water were internal standard. The results were processed using the Agilent 1260 Infinity II tool software.

2.2.2. Research methods for individual antioxidants
The determination of ascorbic acid was performed by a titrimetric method with a solution of sodium 2,6-dichlorophenolindophenolate, after preliminary extraction of ascorbic acid with a 2% hydrochloric acid solution [24].

Total phenols assay by Folin–Ciocalteau reagent. Ethanol extracts of the marc were kept in the dark with Folin – Ciocalteau reagent for 30 minutes at room temperature. Optical density was measured on a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 735 nm. The results obtained are expressed in mg of gallic acid (GA) [4].
Total flavonoids assay was determined spectrophotometrically by reaction with aluminum chloride. Extraction of flavonoids was carried out with 60% ethyl alcohol. Optical density measurement was carried out after 30 minutes on a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 420 nm. The results obtained were expressed in mg of rutin [24].

Total anthocyanins assay expressed in terms of cyanidine was determined by pH-differential spectrophotometry of samples at pH 1.0 and 4.5 at a wavelength of 700 nm and maximum values in the visible area on a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan).

2.2.3. Antioxidant properties research methods
Antiradical activity was determined by Glewind [24]. Extraction was carried out with a 50% ethanol solution. AOA was determined using a diphenylpyrlyhydrayl (DPPH) stable free radical, which is recovered in the reaction with the antioxidants of the product for 30 minutes in the dark at room temperature. The light absorption of the original solution of DPPH is 0.5 relative units. Optical density was measured at a wavelength of 517 nm on a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan). The calibration curve was built by ascorbic acid (AC).

Antioxidant activity (chelating capacity) was determined by the FRAP method with ferric chloride, α-phenanthroline and triton X 100 on a SHIMADZU 1240 spectrophotometer at a wavelength of 505 nm [24]. The calibration curve was built by AC.

3. Results and discussion
The yield of juice from bilberry and lingonberry was almost the same and was about 64%. The share of marc accounted for about 20%, while the rest was pulp (Figure 1). Bilberry and lingonberry are real berries, so the juice with pulp contained seeds with a more noticeable amount of them in the bilberry pulp.

Further research was carried out in whole berries and marc after juice expression. The obtained marc contained 2.6-2.7 times dry solids than the juice (Table 1). Dry solids of the marc predominantly contained cellulose, reducing sugars and titrated acids. Pectic substances passed into the juice with pulp, while the marc contained their trace quantities.

The total reducing sugars assay in bilberry was 1.8 times higher than in lingonberry. In berries, regardless of their type, fructose and glucose prevailed. The amount of sucrose was not material and did not exceed 1.0% of dry solids. When processing berries, the distribution of sugars in the constituent parts depended on their type. In bilberry marc, as well as in berries, fructose and glucose prevailed. In lingonberry marc, the content of glucose and sucrose increased, and fructose decreased quantitatively, which indicates its transition to juice and pulp. As a result, glucose was the dominant sugar. The distribution of titrated acids was also uneven. In the marc of bilberry and lingonberry, the titrated acidity decreased by almost 2 times. At the same time, titrated acidity was 1.5 times higher in the lingonberry marc than in bilberry marc.
Table 1. The chemical composition of berries and marc after juice expression

| Indicators                      | Bilberry |                  | Lingonberry |                  |
|--------------------------------|----------|------------------|-------------|------------------|
|                                | whole    | marc             | whole       | marc             |
| Dry matter, %                  | 18.2±0.4 | 49.7±0.6         | 18.8±0.5    | 48.9±0.5         |
| Sucrose, % DM                  | 1.00±0.02| 0.5±0.02         | 0.87±0.01   | 1.29±0.02        |
| Glucose, % DM                  | 11.50±0.10| 6.9±0.20       | 5.67±0.20   | 10.77±0.30       |
| Fructose, % DM                 | 13.00±0.42| 10.0±0.30      | 7.5±0.32    | 6.96±0.30        |
| Acidity, % on citric acid      | 1.50±0.01| 0.76±0.01       | 2.1±0.01    | 1.1±0.03         |
| Pectic substances, % DM        | 3.74±0.12| 0.01±0.01       | 3.35±0.10   | 0.01±0.01        |
| Cellulose, % DM               | 8.62±0.28| 34.9±0.45       | 8.99±0.23   | 36.9±0.52        |

Wild berries are considered as sources of antioxidants containing a complex of compounds of phenolic nature [2, 4, 10, 11]. The study of the composition of individual antioxidants showed that bilberry and lingonberry contain total phenolic compounds, common flavonoids, total anthocyanins and vitamin C in their composition, with their predominance in bilberry (Table 2). And while the amount of total phenolic compounds and flavonoids in bilberry is 1.4 and 1.2 times more than in lingonberry, respectively, the amount of anthocyanins is more than 2 times greater in bilberry, causing its dark purple color. But the berries of lingonberry contained more vitamin C.

Table 2. Individual antioxidants assay

| Indicators                      | Bilberry |                  | Lingonberry |                  |
|--------------------------------|----------|------------------|-------------|------------------|
|                                | whole    | marc             | whole       | marc             |
| Total phenolic compounds, mg GA/ 100 g | 588.9±12.6| 682.4±10.9      | 425.5±14.5  | 508.1±10.1       |
| Total flavonoids, mg rutin / 100 g | 465.0±11.4| 510.2±10.5      | 371.2±11.8  | 450.4±7.3        |
| Total anthocyanins, mg cyanidin / 100 g | 313.0±8.8 | 514.8±8.5       | 182.5±9.0   | 207.4±5.3        |
| Vitamin C, mg / 100 g           | 18.34±0.62| 6.88±0.53       | 22.10±0.60  | 7.25±0.55        |

When expressing juices, a significant part of the individual antioxidants was transferred into the marc. Thus, the amount of total phenolic compounds in marc increased by 15.9 and 19.4%, respectively, of their content in bilberry and lingonberry. Marc contained less total flavonoids than total phenolic compounds, especially the bilberry marc, which was only 9.7% more. Total anthocyanins, which are glycosides, often associated with the matrix of plant materials, have passed into the marc most of all. In the bilberry marc, their number was 64.5% more than in berries, which was more than 2.6 times than in the lingonberry marc. The content of vitamin C both in the berries and marc was insignificant, regardless of the type of berries.

The presence of individual antioxidants determined their antioxidant properties, which were determined by their antiradical capacity with respect to the DPPH-radical and antioxidant capacity by their chelating capacity using the FRAP method (Figure 2).
Bilberry and lingonberry marc had greater antioxidant properties compared with berries. The antiradical activity of bilberry marc, determined by the DPRH-test and the chelation capacity by the FRAP method, was 1.7-1.8 times higher than in berries. In lingonberry, the differences between berries and the marc were less significant. Antiradical activity differed only by 14%, and chelating capacity - by 33.6%.

The greatest influence on the antioxidant properties of berries and marc was made by common anthocyanins. The correlation between the DPRH-test and the content of anthocyanins approached to 1, amounting to 0.977 with an acceptance probability of 0.95. The number of anthocyanins influenced the chelating capacity with a high degree of probability (Table 3). A similar dependence of the transition of anthocyanins and their effect on antioxidant properties was also obtained by other authors [7, 8], both with whole berries from different regions and with different fractions of berries obtained by extraction.

| Indicators | Total phenolic compounds | Total flavonoids | Total anthocyanins | P-value |
|------------|--------------------------|------------------|--------------------|---------|
| DPPH       | 0.829                    | 0.682            | 0.977              | < 0.05  |
| FRAP       | 0.869                    | 0.777            | 0.952              | < 0.05  |

The effect of total phenolic compounds on the antioxidant properties of berries and marc also turned out to be great and ranged from 0.829 to 0.869, but was more associated with chelating capacity. Common flavonoids had the least effect on antioxidant properties, especially on antiradical activity. The correlation coefficient, in this case, was the lowest - 0.682.

4. Conclusion
Wild bilberry and lingonberry are natural sources of antioxidants that can be used to prevent oxidative stress when used fresh or processed. In the process of juice production, about 20% of marc is formed, into which antioxidants transfer, forming their antiradical activity and chelating capacity. The total phenolic compounds, total flavonoids and total anthocyanins assay in the marc is higher than in whole berries. Total anthocyanins transfer to the marc most of all, the amount of them in marc is 64.5% more than in the berries causing dark purple color. Lindonberry marc contains 2.6 times less common anthocyanins than bilberry marc. The more bilberry and lingonberry and the products of their processing contained anthocyanins, the higher their antioxidant properties were. The correlation between DPPH and FRAP- tests for anthocyanins was 0.977 and 0.952, respectively.

References
[1] Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskanen J, Törrönen R 1999 *Food Research International* 32 pp 345-53
[2] Hajazimi E, Landberg R, Zamaratskaia G 2016 *LWT - Food Science and Technology* 74 pp 128-34
[3] Fan Z-L, Wang Z-Y, Liu J-R 2011 *Food Chemistry* 129 pp 402-7
[4] Bujor O-C, Ginies Ch, Popa V I, Dufour C 2018 *Food Chemistry* 252 pp 356-65
[5] Ancillotti C, Ciofi L, Pucci D, Sagona E, Giordani E, Birciocolt S, Gori M, Petrucci W A, Giardi F, Bartoletti R, Chiuminatto U, Orlandini S, Mosti S, Del Bubba M 2016 *Food Chemistry* 204 pp 176–84
[6] Tian Ye, Puganen A, Alakomi H-L, Uusitupa A, Saarela M 2018 *Food Research International* 106 pp 291–303
[7] Colak N, Torun H, Gruz J, Strnad M, Hermosin-Gutierrez I, Hayirlioglu-Ayaz S, Ayaz F A 2016 *Food Chemistry* 201 pp 339–49
[8] Feng Ch, Su Sh, Wang L, Wu J, Tang Z, Xu Ya, Shu Q, Wang L 2016 *Food Chemistry* 204 pp 150-8
[9] Puišo Ju, Jonkuvienė D, Mačionienė I, Šalomskienė J 2014 Colloids and Surfaces B Biointerfaces 121 pp 214–21
[10] Bornšek Š M, Polak T, Skrt M, Demšar L, Ulrih N P, Abram V 2015 Food Chemistry 173 pp 61–9
[11] Bujor O-C, Le Bourvellec C, Volf I, Popa V I, Dufour C 2016 Food Chemistry 213 pp 58–68
[12] Nilova L, Malyutenkova S 2017 Agronomy Research 16 pp 1444-56
[13] Šaponjac V T, Čanadanović-Brunet Ja, Ćetkovic G, Djilas S, Cetojević-Simin D 2015 LWT - Food Science and Technology 61 pp 615-21
[14] Nilova L P, Pilipenko T V 2016 Problems of Nutrition 85 (6) pp 39-47
[15] Betz M, Steiner B, Schantz M, Oidtmann J, Mader K, Richling E, Kulozik U 2012 Food Research International 47 pp 51–7
[16] Feng Ch-Yo, Wang W-W, Ye J-F, Li Sh-Sh, Wu Q, Yin D-D, Li B, Xu Ya-Ju, Wang L-Sh 2017 Food Chemistry 219 pp 490–5
[17] Lehtonen H M, Lindstedt A, Jarvinen R, Sinkkonen Ja, Graca G, Viitanen M, Kallio H, Gil A M 2013 Food Chemistry 138 pp 982-90
[18] Wang S, Meckling K A, Marcone M F, Kakuda Yu, Tsao R 2011 Food Research International 44 pp 2545–54
[19] Kivima A S, Ehlers P I, Turpeinen A M, Vapaatalo H, Korpela R 2011 J. of Functional Foods 3 pp 267-74
[20] Leon-Gonzalez A J, Sharif T, Auger C, Abbas M, Fuhrmann G, Schini-Kerth V B 2018 J. of Functional Foods 44 pp 227–34
[21] Viljanen K, Heinio R-L, Juvonen R, Kosso T, Puupponen-Pimia R 2014 Food Chemistry 157 pp 148-56
[22] Fatkullin R, Popova N, Kalinina I, Botvinnikova V 2017 Agronomy Research 16 pp 1295-303
[23] Babova O, Occhipinti A, Capuzzo A, Maffei M E 2016 J. of Supercritical Fluids 107 pp 358–63
[24] Rogozhin V V, Rogozhina T V 2015 Workshop on biochemistry of agricultural production (St. Petersburg: GIORD)
[25] Salishcheva O V, Donya D V 2013 Foods and Raw Materials 1 (2) pp 76–84