Composition of Phenolic Acids in Sea Buckthorn (*Hippophae rhamnoides* L.) Berries

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ABSTRACT: The composition of phenolic acids in several varieties of sea buckthorn berries was determined by GC and MS. In six cultivars the total content of phenolic acids ranged from 3570 ± 282 to 4439 ± 405 mg per kg of berries, on a dry basis. Seventeen phenolic acids were tentatively identified in the berries. Salicylic acid was the principal phenolic acid in sea buckthorn berries, accounting for 55 to 74.3% of the total phenolic acids present. The phenolic acids liberated from esters and glycosidic bonds were the major fractions of phenolic acids in the berries, whereas free phenolic acids constituted only up to 2.3% of total phenolic acids present.

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KEY WORDS: Berries, composition, GC, phenolic acids, sea buckthorn.

Sea buckthorn (*Hippophae rhamnoides* L.) is a temperate, hardy bush that grows wild in Central Asia and Europe and produces nutritious and delicious berries (1). It has been domesticated in some parts of the world (2). Sea buckthorn berries can be processed into jams, juices, yellow pigments, and seed oils (2,3). The chemical composition of berries is affected by a number of factors including growing conditions, maturity stage, and time of harvest (2,4). The soft tissue of the berries contains 3–5% oil, whereas the oil content of the seeds is 12–13% (5,6).

Sea buckthorn berries are an excellent source of phytochemicals such as ascorbic acid, tocopherols, unsaturated FA, phenols, and carotenoids (2,3,5,6). Berries have been used for the treatment of radiation damage, burns, oral inflammation, and gastric ulcers (7). Other claimed positive health effects include reduction in plasma cholesterol level, inhibition of platelet aggregation, and regulation of immune function (8). Gao *et al.* (3) reported that phenolics were the major contributors to the antioxidant activity of sea buckthorn berries. Accordingly, there is a growing interest in the use of sea buckthorn berries for medicinal and cosmetic applications as well as in functional foods (2,4). A number of components of sea buckthorn berries such as vitamin C, organic acids, unsaturated FA, carotenoids, minerals, and phytosterols have been extensively studied (2,5,6,8,9), but there is still little information on their phenolics.

The aim of this study was to determine the composition of phenolic acids in whole berries of selected sea buckthorn varieties grown in Eastern Europe.

MATERIALS AND METHODS

Materials. Whole berries of six sea buckthorn (*H. rhamnoides* L.) cultivars, Nadbałtycka, Nevlejena, Otradnaja, Podarok Sadu, Trofimowskaja, and Hybrid 29-88, were used in this study. Berries of the Nadbałtycka cv. were collected near Olsztyn, Poland, whereas the other cultivars were obtained from the Byelorussian Horticulture Research Institute in Samochwałowicze (Byelorussia). Mature fruits were harvested by hand in the fall of 2002 at the stage of commercial maturity as judged by hand manipulation and juiciness. The berries were cleaned to remove diseased or pest-infested fruits, stems, and leaves and then stored in polyethylene bags at –20°C until analysis.

Preparation of crude phenolic extract. Soluble phenolics were extracted six times from 10 g of crushed berries into aqueous 80% (vol/vol) methanol (at a ratio of 1:1, wt/vol) at room temperature for 1 h using an orbital shaker operated at 250 rpm. The mixture was centrifuged at 1750 × *g* for 10 min, and the supernatants were collected, combined, and evaporated to near dryness under vacuum at ≤40°C, and then lyophilized.

Fractionation of phenolic acids. Phenolic acids present in the extract were fractionated into free and bound forms according to the procedure described by Kozłowska *et al.* (10) and Zadernowski (11). An aqueous solution of crude phenolic extract, prepared as described above, was acidified to pH = 2 using 6 N HCl, filtered to remove precipitated phospholipids, and then extracted five times with diethyl ether (1:1, vol/vol) at room temperature. The ether extracts of phenolic acids (referred to as free phenolic acids) were combined and evaporated to dryness under vacuum at ≤40°C. The water phase was adjusted to pH = 7 with 2 M NaOH and then evaporated almost to dryness under vacuum at ≤40°C. The residue

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was treated with 20 mL 4 N NaOH under nitrogen for 4 h at room temperature. The reaction mixture was then acidified with 6 N HCl to pH = 2 and extracted with diethyl ether as already described. The ether extracts of phenolic acids are referred to as phenolic acids liberated from ester bonds. Following this, the water phase was again adjusted to pH = 7 with 2 M NaOH and then evaporated almost to dryness under vacuum at ≤40°C. The residue was heated with 50 mL of 2 M HCl for 30 min at 95°C, cooled to room temperature, and extracted with diethyl ether as already described. These ether extracts of phenolic acids are referred to as phenolic acids liberated from glycosidic bonds.

Purification of phenolic acid fractions. Each of the residues of phenolic acid fractions, obtained as just described, was dissolved in 50 mL of 5% $NaHCO_3$ (pH = 8) and extracted five times with diethyl ether to remove residual fatty material. The water phase was then acidified with 6 N HCl to pH = 2 and extracted with diethyl ether as just described. The dry residues of phenolic acids were dissolved in 5 mL of 80% (vol/vol) methanol.

Formation of trimethylsilyl derivatives. To 0.5 mL methanolic solution of purified phenolic acids in the reaction vial was added 20–50 µL of *N*,*O*-bis(trimethylsilyl)acetamide (Sigma), the volume used depending on the phenolic acid concentrations. The vial was then tightly closed and left at room temperature for 24 h.

GC–MS identification of phenolic acids. The trimethylsilyl derivatives of phenolic acids were identified using GC–MS methodology as described by Zadernowski (11), Horman and Viani (12), Tian and White (13), and Xing and White (14). GC–MS analysis was carried out on a Hewlett-Packard 5890 Series II gas chromatograph interfaced with a Hewlett-Packard 5970 mass selective detector (Kennett Square, PA). Separations were performed using a 30 m × 0.25 mm (i.d.) SPB-1 fused-silica capillary column coated with a 0.25 µm film of poly(dimethylsiloxane) as the stationary phase (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at an average flow rate of 28 cm^3 per min. The injector and the transfer line temperatures were kept at 240°C. The oven temperature program used was 120–260°C at a rate of 20°C per min. Initial and final temperatures were held for 2 and 10 min, respectively. The injections were carried out in a split mode with a split ratio of 20:1. The mass spectrometer was operated with an ionization voltage of 235 eV and electron multiplier voltage of 1700 V and was scanned from 50 to 500 *m/z* at 0.8 s per scan. The volume of injected samples ranged from 1 to 2 µL, depending on the sample.

Quantification of phenolic acids. The phenolic acids were quantified as described by Zadernowski (11) using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an FID. Separations of trimethylsilyl derivatives of phenolic acids were performed as described in the previous paragraph. *N*-Tetracosane was used as an internal standard. The contents of the phenolic acids are expressed as mg/kg fruit on a dry weight basis.

Chemical analysis. The total contents of phenolic acids in crude extracts were estimated by the Folin-Ciocalteau assay (15) and were expressed in mg (+)-catechin equivalents per kg of berries on a dry matter (d.m.) basis.

Data treatment. The results presented in the tables are mean values of duplicate experiments (with at least three replicates per experiment). No statistically significant difference (*t*-test; $P > 0.05$) was found among experiments for each treatment.

RESULTS AND DISCUSSION

A methanol/water (80%; vol/vol) solvent system is commonly used for the extraction of phenolic acids and their derivatives from plant materials (15–17) and therefore was selected for the extraction of phenolics from sea buckthorn berries. The total content of phenolics in sea buckthorn berries ranged from 8.8 ± 0.9 to 14.4 ± 1.9 g of (+)-catechin equivalents per kg of berries on a d.m. basis (Table 1). These values are in good agreement with those reported by Gao *et al.* (3) for sea buckthorn berries harvested in southern Sweden.

The total content of phenolic acids in six cultivars of sea buckthorn berries ranged from 3570 ± 282 to 4439 ± 405 mg per kg berries, d.m. (Table 1). These values are up to 30 times higher than those reported for defatted evening primrose and borage seeds (18), as well as sesame and cottonseed flours

TABLE 1
Total Phenols, Total Phenolic and Related Acids, and Contributions of Phenolic Acid Fractions in Sea Buckthorn Berries

Sample	Total phenols (g/kg d.m.[a])	Total phenolic acids (mg/kg d.m.)	Total related acids (mg/kg d.m.)	Free phenolic acids (%)[b]	Phenolic acids liberated from Esters (%)[b]	Phenolic acids liberated from Glycosides (%)[b]
Nadbałtycka	8.82 ± 0.93	3746.2 ± 364.0[a]	818.9 ± 70.0	1.9 ± 0.2[a]	53.9 ± 7.5[a]	44.2 ± 5.2[a]
Nevlejena	12.65 ± 1.98[a]	4439.2 ± 405.0	313.4 ± 45.9[a]	2.2 ± 0.2[b]	55.7 ± 5.7[a]	42.1 ± 4.7[a]
Otradnaja	13.08 ± 1.12[a]	3883.4 ± 259.0[a,b]	16.9 ± 1.5	1.8 ± 0.2[a]	60.8 ± 4.7[b]	37.4 ± 4.6[b]
Podarok Sadu	12.51 ± 1.65[a]	3887.4 ± 208.0[a,b,c]	78.0 ± 4.6	1.3 ± 0.1	64.5 ± 4.2[b,c]	34.2 ± 5.5[c]
Trofimowskaja	13.45 ± 0.20[a]	3570.7 ± 282.5[a,b,c,d]	324.3 ± 22.4[a]	1.6 ± 0.2	66.6 ± 6.9[c]	31.8 ± 2.9[c]
Hybrid 29-88	14.42 ± 1.87[a]	3573.7 ± 204.0[a,b,d]	551.9 ± 50.7	2.3 ± 0.2[b]	61.7 ± 5.0[b,c]	36.0 ± 2.7[b,c]

[a]mg (+)-catechin equivalents per kg of dry matter (d.m.) of berries.
[b]Percentage of the total phenolic acids as determined by GC–MS methodology; values for the same column marked by the same letter are not significantly different ($n = 6$; *t*-test; $P > 0.05$).

TABLE 2
Free Phenolic Acids in Sea Buckthorn Berries (mg per kg of dry matter of berries)

Acid	Nadbałtycka	Nevlejena	Otradnaja	Podarok Sadu	Trofimowskaja	Hybrid 29-88
Hydroxybenzoic acid derivatives						
2,5-Dihydroxybenzoic	1.0 ± 0.1	6.1 ± 0.7	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	3.1 ± 0.4
Gallic	2.3 ± 0.3	1.2 ± 0.1	1.9 ± 0.2	1.0 ± 0.6	1.1 ± 0.2	4.6 ± 0.5
Pyrocatechuic	0.9 ± 0.1	2.5 ± 0.3	6.3 ± 0.1	0.8 ± 0.0	1.0 ± 0.1	2.4 ± 0.2
Protocatechuic	4.0 ± 0.2	1.4 ± 0.2	4.3 ± 0.5	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.1
Salicylic	38.7 ± 4.9	47.5 ± 5.9	21.0 ± 3.3	33.9 ± 1.4	25.0 ± 3.8	35.1 ± 4.5
Vanillic	0.8 ± 0.1	0.5 ± 0.1	1.8 ± 0.2	1.3 ± 0.1	0.5 ± 0.1	0.9 ± 0.1
Hydroxycinnamic acid derivatives						
Caffeic	0.9 ± 0.1	4.3 ± 0.5	6.6 ± 0.3	1.8 ± 0.1	6.7 ± 0.8	—[a]
m-Coumaric	0.8 ± 0.1	3.8 ± 0.4	6.1 ± 0.4	0.3 ± 0.1	5.6 ± 0.6	4.4 ± 0.5
o-Coumaric	2.2 ± 0.3	13.3 ± 1.0	8.1 ± 1.0	5.0 ± 0.3	3.1 ± 0.5	—
p-Coumaric	9.8 ± 1.0	5.8 ± 1.0	6.0 ± 0.4	1.4 ± 0.2	2.6 ± 0.2	7.8 ± 0.5
Other phenolic acids						
p-Hydroxyphenyl-lactic	9.5 ± 0.5	12.8 ± 2.5	9.1 ± 0.8	5.3 ± 0.6	8.8 ± 0.6	24.7 ± 2.2
Total phenolic acids	70.9 ± 5.0	99.2 ± 6.6	71.3 ± 3.6	51.9 ± 1.7	55.5 ± 4.0	83.7 ± 5.1

[a]—, Not detected.

(19), up to 5 times higher than those found in soybean flours (20,21), but up to 2–3 times lower than those reported for rapeseed and canola meals (21) and flaxseed (22).

Seventeen phenolic acids were tentatively identified in sea buckthorn berries as shown in Tables 2–4. Salicylic acid was the predominant phenolic acid in berries: It constituted between 55.0 (Otradnaja and Trofimowskaja cultivars) and 74.3% (Nevlejena cultivar) of the total phenolic acids present. Small quantities of *p*-hydroxybenzoic acid were detected only in berries of Nadbałtycka, Hybrid 29-88, and Podarok Sadu cultivars, whereas syringic acids and 3,4-dihydroxycinnamic acid were found only in phenolic acid fractions liberated from glycosides and esters, respectively. Other minor phenolic acids (below 50 mg per kg berries, d.m.) were caffeic, *o*-coumaric, 3,4-dihydroxycinnamic, hydroxycaffeic, pyrocatechuic, syringic, vanillic, and veratric acids. On the other hand, Häkkinen *et al.* (23) tentatively identified only four phenolic acids, namely *p*-coumaric, ferulic, *p*-hydroxybenzoic, and ellagic acids in sea buckthorn berries harvested in Finland. However, ellagic acid was not identified in this study. In addition, quinic acid, ranging from 3.5 (Podarok Sadu cultivar) to 193.9 (Trofimowskaja cultivar) mg per kg berries, d.m., and cinnamic acid ranging from 0.8 (Otradnaja cultivar) to 803.9 (Nadbałtycka cultivar) mg per kg berries, d.m., were also found in berries.

Free phenolic acids were a minor fraction of phenolic acids and constituted only 1.3–2.3% of the total phenolic acids present in sea buckthorn berries (Table 1). The total

TABLE 3
Phenolic and Related Acids Liberated from Esters in Sea Buckthorn Berries (mg per kg of dry matter of berries)

Acid	Nadbałtycka	Nevlejena	Otradnaja	Podarok Sadu	Trofimowskaja	Hybrid 29-88
Hydroxybenzoic acid derivatives						
25-Dihydroxybenzoic	30.8 ± 4.5	10.8 ± 1.9	—[a]	79.3 ± 8.6	61.1 ± 7.2	62.1 ± 3.2
Gallic	146.3 ± 19.0	504.3 ± 60.0	988.0 ± 60.0	485.0 ± 40.0	1008.0 ± 120.0	184.4 ± 20.5
p-Hydroxybenzoic	3.7 ± 0.5	—	—	14.5 ± 0.3	—	20.0 ± 2.5
Pyrocatechuic	0.2 ± 0.0	23.3 ± 3.0	32.1 ± 3.5	12.0 ± 1.9	0.6 ± 0.1	—
3,4-Dihydroxycinnamic	7.8 ± 0.8	26.4 ± 3.0	24.4 ± 3.6	27.3 ± 1.9	5.9 ± 0.6	15.0 ± 2.0
Protocatechuic	106.0 ± 10.9	90.5 ± 10.0	118.0 ± 20.9	54.6 ± 6.7	100.4 ± 10.5	125.0 ± 18.9
Salicylic	1558.0 ± 200.0	1726.4 ± 100.0	1004.1 ± 59.8	1348.5 ± 80.5	1050.0 ± 100.0	1522.2 ± 120.0
Vanillic	0.8 ± 0.1	16.4 ± 2.5	0.7 ± 0.1	—	—	14.3 ± 2.1
Veratric	3.3 ± 0.3	26.4 ± 3.5	63.0 ± 7.0	10.7 ± 1.8	15.1 ± 1.9	21.2 ± 2.0
Hydroxycinnamic acid derivatives						
Caffeic	6.3 ± 0.9	9.3 ± 1.0	—	10.8 ± 1.5	15.8 ± 2.1	15.7 ± 2.9
m-Coumaric	18.3 ± 2.2	18.1 ± 2.0	—	86.4 ± 4.8	23.3 ± 2.9	45.1 ± 5.5
o-Coumaric	12.5 ± 1.8	9.8 ± 1.0	—	10.0 ± 1.9	—	4.3 ± 0.6
p-Coumaric	101.9 ± 15.0	—	104.3 ± 10.9	290.8 ± 30.9	90.3 ± 10.0	133.0 ± 15.8
Ferulic	5.1 ± 0.6	—	13.0 ± 2.0	17.8 ± 3.0	—	11.0 ± 0.1
Hydroxycaffeic	19.8 ± 2.5	9.5 ± 0.6	14.5 ± 2.7	58.5 ± 7.0	9.1 ± 1.0	30.0 ± 7.8
Related acids						
Quinic	9.2 ± 0.4	13.0 ± 1.5	13.8 ± 1.5	—	190.7 ± 20.0	72.1 ± 5.3
Cinnamic	803.9 ± 70.0	300.4 ± 45.9	0.8 ± 0.1	74.5 ± 4.5	130.4 ± 15.0	474.5 ± 50.0
Total phenolic acids	2020.8 ± 201.8	2471.2 ± 117.2	2362.1 ± 88.4	2506.2 ± 96.2	2379.6 ± 157.1	2203.3 ± 124.7

[a]—, Not detected.

TABLE 4
Phenolic and Related Acids Liberated from Glycosides in Sea Buckthorn Berries (mg per kg of dry matter of berries)

Acid	Nadbałtycka	Nevlejena	Otradnaja	Podarok Sadu	Trofimowskaja	Hybrid 29-88
Hydroxybenzoic acid derivatives						
2,5-Dihydroxybenzoic	20.7 ± 2.5	1.0 ± 0.1	1.3 ± 0.1	—[a]	14.4 ± 1.9	0.3 ± 0.0
Gallic	113.7 ± 15.8	56.2 ± 6.8	78.0 ± 7.0	51.0 ± 3.7	62.6 ± 5.5	58.0 ± 6.0
Pyrocatechuic	—	—	5.2 ± 0.5	3.3 ± 0.4	3.8 ± 0.3	—
Protocatechuic	21.3 ± 2.8	9.7 ± 1.0	14.6 ± 1.9	7.9 ± 0.9	—	17.4 ± 0.7
Salicylic	899.5 ± 100.0	1524.1 ± 120.0	1112.5 ± 150.0	1066.0 ± 200.0	919.4 ± 51.0	997.4 ± 60.8
Syringic	6.7 ± 0.5	2.5 ± 0.1	4.2 ± 0.3	—	12.8 ± 1.6	3.0 ± 0.2
Vanillic	1.4 ± 0.1	—	8.4 ± 0.5	7.7 ± 0.8	—	8.0 ± 0.7
Veratric	0.3 ± 0.0	11.0 ± 0.1	—	—	20.0 ± 2.6	—
Hydroxycinnamic acid derivatives						
Caffeic	1.3 ± 0.1	9.5 ± 0.7	—	9.1 ± 1.0	10.0 ± 1.9	6.3 ± 0.7
m-Coumaric	9.3 ± 1.2	27.1 ± 3.6	5.7 ± 0.6	8.2 ± 0.5	16.7 ± 1.9	—
o-Coumaric	16.8 ± 2.0	26.6 ± 0.7	15.3 ± 1.0	—	6.3 ± 0.8	14.1 ± 1.9
p-Coumaric	328.4 ± 11.0	91.4 ± 10.0	85.0 ± 6.9	117.2 ± 21.9	43.9 ± 5.0	64.3 ± 8.1
Ferulic	201.3 ± 33.4	29.9 ± 3.0	97.5 ± 10.0	50.1 ± 5.5	11.4 ± 1.9	104.3 ± 12.9
Hydroxycaffeic	13.8 ± 1.6	9.8 ± 0.7	22.3 ± 3.0	1.3 ± 0.1	14.3 ± 1.8	11.3 ± 1.0
Other phenolic acids						
p-Hydroxyphenyl-lactic	20.0 ± 1.5	70.0 ± 0.8	—	7.5 ± 0.8	—	2.3 ± 0.2
Related acids						
Quinic	5.8 ± 0.6	—	2.3 ± 0.1	3.5 ± 0.6	3.2 ± 0.2	5.3 ± 0.4
Total phenolic acids	1645.5 ± 107.3	1868.8 ± 120.7	1450.0 ± 150.7	1329.3 ± 201.3	1135.6 ± 51.8	1286.7 ± 63.0

[a]—, Not detected.

contents of free phenolics found in berries were similar to those reported by Zadernowski *et al.* (18) for defatted borage and evening primrose seeds, but over 10 times lower than those found in defatted rapeseed meals (24). Eleven free phenolic acids were tentatively identified in this fraction (Table 2). Of these, salicylic acid was the predominant phenolic acid, constituting from 29.5 (Otradnaja cultivar) to 65.3% (Podarok Sadu cultivar) of the total free phenolic acids present. The level of free phenolic acids in sea buckthorn berries, however, did not exceed the taste thresholds reported in the literature (25). Thus, the fraction of free phenolic acids may not have any significant contribution to the flavor of sea buckthorn berries.

Phenolic acids liberated from soluble esters were the predominant phenolic acids in sea buckthorn berries. This fraction constituted from 53.9 to 66.6% of the total phenolic acids present in berries (Table 1). The total content of phenolic acids liberated from esters was up to five times lower than that found in rapeseed flours (24) and up to two times lower than that in flaxseed (22). Fifteen phenolic acids have tentatively been identified in this fraction (Table 3). Of these, salicylic acid constituted from 42.5 (Otradnaja cultivar) to 77.1% (Nadbałtycka cultivar) of total esterified phenolic acids present. Smaller quantities (below 50 mg per kg, d.m.) of caffeic, *m*- and *o*-coumaric, 3,4-dihydroxycinnamic, ferulic, *p*-hydroxybenzoic, pyrocatechuic, vanillic, and veratric acids were also found. In addition, *p*-coumaric, gallic, hydroxycaffeic, and protocatechuic acids were also detected in these berries.

Phenolic acids linked to sugars by glycosidic bonds constituted from 31.8 (Trofimowskaja cultivar) to 44.2% (Nadbałtycka cultivar) of total phenolic acids present in these berries (Table 1). The total content of phenolic acids liberated from glycosides was over 100 times higher than that reported for defatted evening primrose and borage seeds (18). Fourteen phenolic acids were tentatively identified in this fraction (Table 4). Sugar moieties of glycosides were not identified in this study. Salicylic acid was the principal phenolic acid present in this fraction, and 10 phenolic acids were found at concentrations below 30 mg per kg, d.m.

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