

Physico-chemical Parameters and Antioxidant Activity of Romanian Sea Buckthorn Berries

VIOLETA ALEXANDRA ION¹, OANA CRISTINA PARVULESCU^{2*}, DIANA VELCEA³, OANA POPA³, MIRELA AHMADI⁴

¹Research Center for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine Bucharest, 59 Marasti Blvd., 011464, Bucharest, Romania

²University Politehnica of Bucharest, Chemical and Biochemical Engineering Department, 1-3 Gheorghe Polizu, 011061, Bucharest, Romania

³SCIENT Research Center for Instrumental Analysis, 1 Petre Ispirescu Str., 077167, Tancabesti, Ilfov, Romania

⁴Banat's University of Agriculture Science and Veterinary Medicine "King Michael I of Romania" from Timisoara, Biochemistry and Molecular Biology Department, 119 Calea Aradului, 300645, Timisoara, Romania

Physico-chemical parameters of Romanian sea buckthorn fresh or dried berries, including titratable acidity (2.28±0.06 g malic acid/100 g FM), pH (2.60±0.08), contents of moisture (75.94±2.14 g/100 g FM), ash (2.83±0.11 g/100 g DM), ascorbic acid (1337.6±37.2 mg/100 g FM), oil (3.82±0.16 g/100 g FM), and macro elements, mainly K (11654 mg/kg DM), Mg (668 mg/kg DM), Ca (645 mg/kg DM), Na (401 mg/kg DM), and P (271 mg/kg DM), were evaluated in this paper. The most abundant volatile compounds found in the volatile fraction of berries were isopentyl isovalerate (30.8%), heptanal (11.7%), ethyl hexanoate (10.5%), ethyl isovalerate (8.1%), hexanal (7.5%), and ethyl 2-methylbutyrate (6.3%). Total phenolic content, total flavonoids content, and antioxidant activity (expressed as DPPH free radical scavenging activity by inhibition of DPPH) of extract solutions obtained from dried berries by MAE using ethanol/water mixture as a solvent were estimated at different ratios of solvent volume/vegetal material mass (15-30 mL/g) and ethanol/water (50/50, 60/40, 70/30, and 80/20 v/v). Inhibition of DPPH (22.61-48.60%) was well correlated with total phenolic content (4.42-10.04 mg caffeic acid equivalents/g DM) and total flavonoids content (5.37-11.14 mg quercetin equivalents/g DM).

Keywords: Sea buckthorn, ascorbic acid, titratable acidity, oil, volatiles, minerals, total phenols, total flavonoids, antioxidant activity

Hippophae rhamnoides L., commonly known as sea buckthorn (SB), is a plant widely studied for its nutritional and therapeutic benefits. *H. rhamnoides* species comprises nine subspecies which grow especially in temperate Europe and Asia, i.e., *carpatica*, *fluviatilis*, and *rhamnoides* in Europe, *caucasica*, *mongolica*, *sinensis*, *turkestanica*, *wolongensis*, and *yunnanensis* in Asia [1].

Although all parts of this plant (berry, leaf, root, twig, bark) are sources of bioactive compounds, the berry, containing *cca.* 200 components, is considered the most important part [2]. SB berries are rich in natural antioxidants, e.g., phenolic compounds (including flavonoids, phenolic acids, tocopherols), carotenoids (such as β -carotene, γ -carotene, lycopene, zeaxanthin, lutein), ascorbic acid (vitamin C), which exhibit anti-inflammatory, anticancer, and antimicrobial effects [2-18]. Moreover, the berries are important sources of sugars (mainly glucose and fructose), proteins, organic acids (predominantly malic acid), aminoacids, essential fatty acids (FAs), volatile compounds, phytosterols (mainly β -sitosterol), and mineral elements (especially K, Mg, Ca, Na, and P) [2-10,12-15,17-19]. The composition of SB berries varies depending on the subspecies, origin, climate, and harvest time.

The berries are usually pressed, resulting in juice and a pomace (containing seeds and a fraction of peel and pulp (PP)) SB juice is rich in flavonoids, carotenoids, sugars, organic acids, aminoacids, essential FAs, volatile compounds, phytosterols, vitamins, and minerals [4,5,13,18]. Oils extracted from berries, seeds, and PP fraction contain high amounts of unsaturated FAs, carotenoids, tocopherols, and phytosterols and can be used as ingredients in functional food, nutritional supplements, pharmaceutical and cosmetic products [2,3,9,10,13-15,20]. Oils from berries and seeds can be used for treatment of burns, wounds, gastrointestinal disorders, obesity, skin and liver diseases [2,3,10,13-15]. SB juice and oils have beneficial effects on the blood cholesterol level and cardiovascular system [2,9,10,13,15]. Antimutagenic, immunomodulatory, hepatoprotective, neuroprotective, radioprotective, anti-stress, and anti-aging effects of SB berries and related products were also reported [2,3,7,9,10,12-15,19].

This paper has aimed at evaluating the quality of Romanian SB. Contents of moisture, ash, ascorbic acid, oil, volatile compounds, and minerals as well as titratable acidity and pH of berries were determined by specific methods. Moreover, total phenolic content, total flavonoids content, and antioxidant activity of berry extracts were evaluated.

*email:oana.parvulescu@yahoo.com

Experimental part

Materials

SB wild berries (*Hippophae rhamnoides* L. ssp. *carpatica*) were collected in September 2017 in Prahova region of Romania. The fresh berries were manually cleaned to remove leaves, branches, and berries damaged by harvesting and then wind flow screened. Cleaned berries were dosed in samples of 0.5 kg and stored in a freezer at -20 °C until their analysis. Three replications of each analysis were conducted.

All reagents were purchased from Merck (Germany) and PerkinElmer (USA) and were analytical grade.

Methods

Determination of moisture content

Moisture content (wt% on a fresh matter (FM) basis) was measured using a Memmert Universal Oven UF 55. SB berries were dried at 60 °C for 50 h, until a constant mass was achieved.

Determination of ash content

Ash content (wt% on a dried matter (DM) basis) was determined using a Carbolite ELF Chamber Furnance. 25 g of SB dried sample was placed in a crucible and calcined at 550 °C, in 50 °C steps, until a clean white residue was obtained.

Determination of ascorbic acid content

Ascorbic acid content (mg/100 g FM) was measured by the iodometric method in the presence of starch as indicator. An amount of 10 g of fresh fruit sample was crushed and homogenized in a mortar with 2 g of quartz sand (prewashed with 2% HCl solution). Crushed fruits were added in 50 mL centrifuge tube and filed with 2% HCl solution, then centrifuged for 5 min at 5000 rpm. 10 mL of the fruit extract supernatant was transferred in an Erlenmeyer glass and 30 mL of ultrapure water, 5 mL of 2% KI solution, 5 mL of 37% HCl, and 1.5 mL of 2% starch solution were added. The mixture was titrated with a 0.002 M potassium iodate (KIO₃) solution.

Determination of oil content

Oil content (g/100 g FM) was determined by extraction with petroleum ether as solvent in a Soxhlet extractor. Prior to extraction, dried fruits were ground for 20 s in a laboratory mill (Merck IKA A11 basic). During petroleum ether extraction, the oil temperature was maintained at 80±1 °C.

Determination of titratable acidity and pH

10 g of fresh fruit was crushed and diluted with 10 mL ultrapure water. The mixture was homogenized, centrifuged, and the clear liquid was taken for analysis. Titratable acidity (g malic acid (MA)/100 g FM) was determined by titration with 0.1 N NaOH using phenolphthalein as indicator. pH was measured using a Mettler Toledo SevenExcellence pH-meter.

Determination of volatile compounds

Volatile compounds were determined by GC-MS analysis using a PerkinElmer Clarus 680 Gas Chromatograph (GC) coupled with a Clarus SQ 8 T Mass Spectrometer (MS) and a TurboMatrix 40 headspace (HS) accessory. Helium (1 mL/min flow rate, 20:1 split ratio) was used as a carrier gas. Initial temperature of GC column was 40 °C and then increased to 100 °C at a rate of 1 °C/min. Temperatures of the ion source (positive) and interface were 150 °C and 250 °C, respectively. 10 g of sample was crushed and put in a 20 mL clear HS vial. The vial containing sample was placed in the HS accessory and heated at 100 °C for 30 min under stirring.

Determination of minerals

Mineral analysis was conducted by atomic absorption spectrometry (AAS). 1 g of fresh fruit was added in a mineralization tube with 8 mL of ultrapure grade nitric acid and 2 mL of concentrated hydrochloric acid. A microwave oven (Anton Paar Microwave Reaction System Multiwave PRO) was used for samples mineralization using a power rap of 800 W for 15 min and hold for 30 min at 1100 W. The maximum temperature reached in the vessels was 191.7 °C. The mineralized samples were transferred in a 50 mL flask and completed with ultrapure water. 22 metals were analyzed by a quantitative method using a PerkinElmer ICP-OES Optima 8300DV apparatus (Al, B, Ca, Cu, Fe, Mg, Mn, Na, Ni, Zn, K) and a semiquantitative method in a Perkin Elmer ICP-MS NexION 300S apparatus (Br, Sr, Cd, Ba, Ce, Pb, Ti, P, V, Co, As).

Microwave assisted extraction (MAE) of antioxidants

Application of microwaves generally leads to enhanced antioxidant extraction yields compared to classical extraction [21-24]. MAE of antioxidants from ground dried SB berries using ethanol/water mixture as a solvent was

performed in an Anton Paar Multiwave PRO Microwave Reaction System equipped with a time controller, an integrated forced-air cooling system, power and temperature sensors. Extraction tests were performed at 60 °C and different values of SB mass/solvent volume (1/15, 1/20, 1/25, and 1/30 g/mL) and ethanol/water (50/50, 60/40, 70/30, 80/20 and v/v) ratios. Extract solutions obtained by MAE were further used for evaluating the active species content and antioxidant activity.

Determination of total phenolic content

Total phenolic content of SB extract solutions was evaluated based on Folin-Ciocalteu method [25-27]. 0.5 mL of extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 5 mL of 20% sodium carbonate (Na₂CO₃). The mixture was stirred for 10 min at room temperature in the dark and further filtered on a 0.45 µm filter. The absorbance was measured at 750 nm using a PerkinElmer LAMBDA 650 UV/Vis Spectrophotometer. A standard curve for caffeic acid (in the range 0.005-0.080 mg/mL) was used to determine total phenolic content (mg caffeic acid equivalents (CAE)/g DM).

Determination of total flavonoids content

Total flavonoids content of SB extract solutions was determined using a PerkinElmer LAMBDA 650 UV/Vis Spectrophotometer [25-27]. 0.250 mL of sample was added to 0.075 mL of 5% sodium nitrite (NaNO₂) solution, the mixture was stirred for 5 min, and then 0.150 mL of 5.5% aluminum chloride (AlCl₃) solution was added. After 6 min, 0.5 mL of 1 M sodium hydroxide (NaOH) solution was added, the volume was adjusted to 2.5 mL with distilled water, and the absorbance was measured at 510 nm. A standard curve for quercetin (in the range 0.050-0.300 mg/mL) was used to evaluate total flavonoids content (mg quercetin equivalents (QE)/g DM).

Determination of antioxidant activity

Antioxidant activity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method [25-27]. 0.1 mL of quercetin standard solution of different concentrations (0.010-0.200 mg/mL) was mixed with 3.9 mL of DPPH solution in methanol (0.035 mg/mL). The mixture was incubated for 40 min at room temperature in the dark and its absorbance was measured at 515 nm using methanol as blank. 0.100 mL of ethanol prepared similarly with the real sample was used as control sample. In order to perform the extract analysis, the same operations were conducted, with the exception that SB extract was used instead of standard solution. Inhibition of DPPH radical, *I* (%), was estimated by Eq. (1), where *A_c* is the absorbance of the control sample and *A_s* the absorbance of the test sample.

$$I = \left(1 - \frac{A_s}{A_c} \right) \times 100 \quad (1)$$

Results and discussions

Moisture, ash, ascorbic acid, and oil contents, titratable acidity, and pH

Physicochemical characteristics of SB berries in terms of moisture content (MC), ash content (AC), ascorbic acid content (AAC), oil content (OC), titratable acidity (TA), and pH are summarized in Table 1. Tabulated data highlight that the results obtained in this study are in the ranges reported in the related literature [2,3,9-11,13,15,16,18]. SB berries are known for their high content of vitamin C (up to 2500 mg/100 g FM), a natural antioxidant which is a scavenger of free radicals, protects the immune system, diminishes the severity of allergies, and has an essential role in bone formation, wound healing, and various metabolic functions [12,28].

Table 1
MOISTURE CONTENT (MC), ASH CONTENT (AC),
ASCORBIC ACID CONTENT (AAC), OIL CONTENT (OC),
TITRATABLE ACIDITY (TA), AND pH OF SB BERRIES

Parameter	Value
MC (%)	75.94±2.14
AC (%)	2.83±0.11
AAC (mg/100 g FM)	1337.6±37.2
OC (g/100 g FM)	3.82±0.16
TA (g MA/100 g FM)	2.28±0.06
pH	2.60±0.08

Volatile compounds

14 volatile compounds, including 5 aldehydes, 8 esters, and 1 alcohol (Table 2), were identified by GC-MS analysis of volatile fraction of SB berries. The most abundant compounds are as follows: isopentyl isovalerate (30.8%), heptanal (11.7%), ethyl hexanoate (10.5%), ethyl isovalerate (8.1%), hexanal (7.5%), and ethyl 2-methylbutyrate (6.3%). These results are in line with those found by other researchers [2,13].

Table 2
VOLATILE COMPOUNDS IDENTIFIED IN SB BERRIES

No.	Class	Compound	Percentage (%)
1	alcohols	3-methyl-2-buten-1-ol	3.8±0.12
2	aldehydes	pentanal	2.1±0.08
3		hexanal	7.5±0.25
4		heptanal	11.7±0.48
5		octanal	4.0±0.16
6		nonanal	5.1±0.21
7	esters	ethyl butyrate	4.0±0.14
8		ethyl 2-methylbutyrate	6.3±0.12
9		ethyl isovalerate	8.1±0.31
10		ethyl hexanoate	10.5±0.43
11		isoamyl acetate	1.5±0.06
12		isopentyl isovalerate	30.8±1.15
13		amyl isovalerate	1.9±0.07
14		3-methylbutyl octanoate	2.5±0.09

Mineral elements

Mineral concentrations summarized in Table 3 highlight high contents of essential elements such as K (11654 mg/kg DM), Mg (668 mg/kg DM), Ca (645 mg/kg DM), Na (401 mg/kg DM), and P (271 mg/kg DM), while the content of Cd, Pb, and As, which are potentially toxicological elements, was very low (≤ 0.7 mg/kg DM). These values are in line with those reported in the literature [2,18].

Table 3
MINERALS IDENTIFIED IN SB BERRIES

No.	Mineral	Concentration (mg/kg DM)
1	Al	47.2±1.63
2	B	14.5±0.41
3	Ca	644.6±21.4
4	Cu	6.9±0.08
5	Fe	37.5±1.57
6	Mg	667.8±21.6
7	Mn	9.0±0.14
8	Na	401.0±11.2
9	Ni	5.1±0.11
10	Zn	21.5±0.81
11	K	11654±417.5
12	Br	10.5±0.41
13	Sr	4.9±0.08
14	Cd	0.1±0.01
15	Ba	2.7±0.04
16	Ce	0.1±0.01
17	Pb	<0.05
18	Ti	6.2±0.12
19	P	270.7±11.3
20	V	0.3±0.01
21	Co	< 0.05
22	As	0.7±0.02

Total phenolic content, total flavonoids content, and antioxidant activity

Table 4 contains values of total phenolic content (C_{TP}), total flavonoids content (C_{TF}), and inhibition of DPPH (I) corresponding to MAE tests conducted at a SB mass/solvent volume ratio of 1/20 g/mL and 4 values of ethanol/water ratio, *i.e.*, 50/50, 60/40, 70/30, and 80/20 v/v. The best results (C_{TP} =8.01 mg/g DM, C_{TF} =8.44 mg/g DM, and I =35.06%) were obtained for an ethanol/water ratio of 60/40. Further, 3 MAE experiments were performed at an ethanol/water ratio of 60/40 and various levels of SB mass/solvent volume ratio, *i.e.*, 1/15, 1/25, 1/30 g/mL. Experimental data, which are presented in Table 5, highlight an increase in C_{TP} , C_{TF} , and I with an increase in the solvent volume.

Table 4

TOTAL PHENOLIC CONTENT, TOTAL FLAVONOIDS CONTENT, AND INHIBITION OF DPPH DEPENDING ON ETHANOL/WATER RATIO (SB MASS/SOLVENT VOLUME RATIO: 1/20 g/mL)

Ethanol/water ratio (v/v)	c_{TP} (mg CAE/g DM)	c_{TF} (mg QE/g DM)	I (%)		ε
Value	experimental	experimental	experimental	predicted	(%)
50/50	6.03	6.94	29.76	29.87	-0.35
60/40	7.65	8.58	35.06	37.95	0.55
70/30	5.82	6.77	28.82	29.19	-1.27
80/20	4.42	5.37	22.61	22.35	1.15

Table 5

TOTAL PHENOLIC CONTENT, TOTAL FLAVONOIDS CONTENT, AND INHIBITION OF DPPH DEPENDING ON SB MASS/SOLVENT VOLUME RATIO (ETHANOL/WATER RATIO: 60/40 v/v)

SB mass/solvent ratio (g/mL)	c_{TP} (mg CAE/g DM)	c_{TF} (mg QE/g DM)	I (%)		ε
Value	experimental	experimental	experimental	predicted	(%)
1/15	7.13	7.74	35.51	35.51	0.01
1/20	7.65	8.58	38.16	38.23	-0.18
1/25	8.94	9.97	44.02	43.88	0.32
1/30	10.04	11.14	48.60	48.67	-0.15

Experimental data listed in Tables 4 and 5 were processed by multiple regression analysis resulting in Eqs. 2 ($R^2=0.9979$, $R^2_{adjusted}=0.9937$) and 3 ($R^2=0.9997$, $R^2_{adjusted}=0.9991$), which correlate I with c_{TP} and c_{TF} . Tables 4 and 5 contain also values of I predicted based on Eqs. 2 and 3 as well as those of percent error, ε (%), defined by Eq. 4. Tabulated results ($-1.27\% \leq \varepsilon \leq 1.15\%$) highlight a very good agreement between experimental and predicted data, *i.e.*, I_{ex} and I_{pr} .

$$I = -3.759c_{TP} + 8.642c_{TF} - 7.442 \quad (2)$$

$$I = 2.664c_{TP} + 1.593c_{TF} + 4.181 \quad (3)$$

$$\varepsilon = \left(1 - \frac{I_{pr}}{I_{ex}} \right) \times 100 \quad (4)$$

Experimental values of c_{TP} , c_{TF} , and I , which are specified in Table 5, are plotted in Fig. 1 against solvent volume/SB mass ratio, R_{vm} (mL/g). All three parameters are well correlated with R_{vm} by straight lines described by Eqs. (5)-(7), where determination coefficients (R^2) corresponding to them are 0.9747, 0.9916, and 0.9813, respectively.

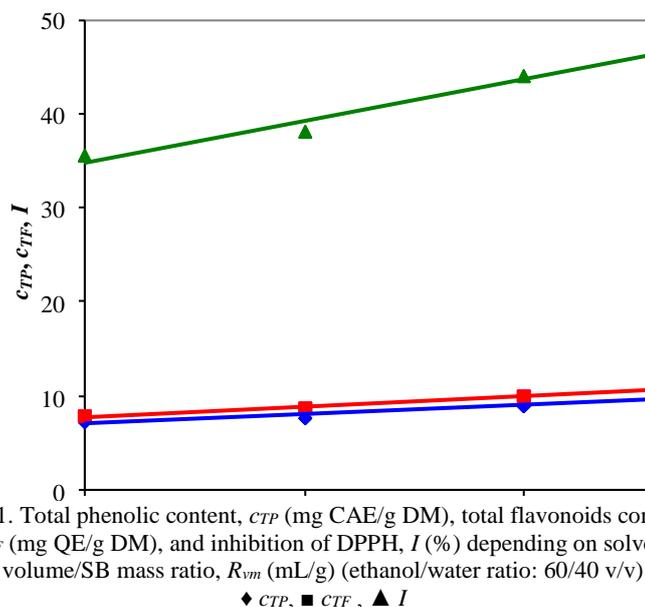


Fig. 1. Total phenolic content, c_{TP} (mg CAE/g DM), total flavonoids content, c_{TF} (mg QE/g DM), and inhibition of DPPH, I (%) depending on solvent volume/SB mass ratio, R_{vm} (mL/g) (ethanol/water ratio: 60/40 v/v):

◆ c_{TP} , ■ c_{TF} , ▲ I

$$c_{TP} = 0.200R + 3.931 \quad (5)$$

$$c_{TF} = 0.232R + 4.142 \quad (6)$$

$$I = 0.903R + 21.264 \quad (7)$$

Substituting Eq. (6) in Eq. (5) results in Eq. (8) ($R^2=0.9950$) and substituting Eq. (8) in Eq. (3) results in Eqs. (9) and (10) ($R^2=0.9989$ and $R^2=0.9979$), which highlight very good correlations between c_{TP} and c_{TF} , I and c_{TP} , and I and c_{TF} .

$$c_{TP} = 0.870c_{TF} + 0.301 \quad (8)$$

$$I = 4.486c_{TP} + 3.708 \quad (9)$$

$$I = 3.910c_{TF} + 4.983 \quad (10)$$

Conclusions

Some physico-chemical properties and antioxidant activity of Romanian seabuckthorn (SB) wild berries collected in Prahova region were evaluated. The results of physico-chemical analysis of fresh or dried berries were as follows: 75.94±2.14 g moisture/100 g FM, 2.83±0.11 g ash/100 g DM, 1337.6±37.2 mg ascorbic acid/100 g FM, 3.82±0.16 g oil/100 g FM, titratable acidity of 2.28±0.06 g malic acid/100 g FM, 2.60±0.08 pH, 11654 mg K/kg DM, 668 mg Mg/kg DM, 645 mg Ca/kg DM, 401 mg Na/kg DM, 271 mg P/kg DM. The most abundant volatile compounds determined by GC-MS analysis of volatile fraction of SB berries were isopentyl isovalerate (30.8%), heptanal (11.7%), ethyl hexanoate (10.5%), ethyl isovalerate (8.1%), hexanal (7.5%), and ethyl 2-methylbutyrate (6.3%). Total phenolic content (c_{TP}), total flavonoids content (c_{TF}), and antioxidant activity (expressed as DPPH free radical scavenging activity by inhibition of DPPH (I)) of extract solutions obtained from dried berries by MAE using ethanol/water mixture as a solvent were estimated at different ratios of solvent volume/SB mass ($R_{vm}=15-30$ mL/g) and ethanol/water (50/50, 60/40, 70/30, and 80/20 v/v). For an ethanol/water ratio of 60/40 v/v, c_{TP} (7.13-10.04 mg CAE/g DM), c_{TF} (7.74-11.14 mg QE/g DM), and I (35.51-48.60%) increased linearly with R_{vm} . Moreover, I was very well correlated with c_{TP} ($R^2=0.9989$) and c_{TF} ($R^2=0.9979$).

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