

Note

The nutritional value of leaves of selected berry species

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ABSTRACT: Although the medicinal properties of berry fruit are well known, there is relatively little information available concerning the applications of other parts of berry plants. Thus, in this study we determined the nutritional value of the leaves of selected berry species and their possible application in health promoting diets. The levels of nutrients, and macro- and microelements in the leaves of four species collected from allotment gardens in the city of Szczecin, Poland (53°26'17" N, 14°32'32" E; altitude 7 m a.s.l.) were identified: raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), chokeberry (*Aronia melanocarpa* L.), and sea buckthorn (*Hippophae rhamnoides* L.). Sea buckthorn leaves were the richest source of protein, raspberry leaves had the highest levels of lipids, and the leaves of all four species studied were a rich source of crude fibre and dietary fibre fractions. Desirable Ca:P and Na:K ratios indicated their potential as a good source of minerals essential to bone formation and the treatment of hypertension. Sea buckthorn leaves contained high but also safe Fe levels (within recommended WHO limits) and, therefore, may become an alternative rich source of this element.

Keywords: nutrients, berry plant leaves, macroelements, microelements

Introduction

The constant increase in the incidence of diet-related chronic diseases has revealed the disadvantages of the popular nutrition model, and growing awareness in this regard has resulted in increased interest in health foods. These products – containing bioactive substances that may help prevent or treat metabolic diseases – include berries, most commonly found in the temperate zones in the northern hemisphere. Known to be a rich source of minerals, vitamins, pectins, fats, simple sugars and organic acids (including phenols and essential oils), the fruit of many berry species have been valued for their therapeutic properties since ancient times (Hummer, 2010). However, relatively little is known about the other parts of these plants. One notable exception is raspberry leaves (*Rubus idaeus* L.), which are used as a rich source of tannins in diuretic and choleric herbal mixtures (Zhang et al., 2011). Raspberry leaves have also been shown to reduce the duration of labor and, thus, reduce the need for medical intervention during delivery (Parsons et al., 1999; Simpson et al., 2001). Red raspberry leaves also contain biologically active polyphenols which can be used to supplement the daily intake of valuable natural antioxidants (Durgo et al., 2012). In order to confirm their potentially beneficial role in human and animal nutrition, the aim of the present study was to assess the nutritional values of the leaves of selected berry species in the context of their application in health promoting diets.

Materials and Methods

The leaves of four berry species: raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), chokeberry

(*Aronia melanocarpa* L.) and sea buckthorn (*Hippophae rhamnoides* L.) were collected from allotment gardens in the city of Szczecin, Poland (53°26'17" N, 14°32'32" E; altitude 7 m a.s.l.), in 2014 and 2015. The leaves were collected during harvesting, from shoots without fruit. Leaves were collected from five plants (one plant was treated as a replication) from the external sides, at half the plant height. The leaves were taken from one year old shoots, without any signs of ageing or mechanical damage.

The chemical composition of samples was determined according to procedures established by the Association of Official Analytical Chemists (AOAC, 2012): dry matter, by drying at 105 °C to constant weight; crude fat, by Soxhlet extraction with diethyl ether; crude ash, by incineration in a muffle furnace at 580 °C for 8 h; crude protein (N × 6.25) by the Kjeldahl method; crude fibre was determined using the Hennenberg-Stohmann method; total carbohydrates were calculated as: total carbohydrates (%) = 100 – % (moisture + crude protein + crude fat + ash + crude fibre). The fibre components were determined by using the detergent method according to Van Soest et al. (1991), performed with a fibre analyzer, ANKOM 220. Determination of neutral detergent fibre (NDF) was conducted on an ash-free basis and included sodium dodecyl sulphate (NaC₁₂H₂₅SO₄) (Merc 822050). Determination of acid detergent fibre (ADF) included hexadecyl-trimethyl-ammonium bromide (CH₃(CH₂)₁₅N(CH₃)₃Br) (Merc 102342), while acid detergent lignin (ADL) was determined by hydrolysis of an ADF sample in 72 % sulphuric acid (H₂SO₄). Hemicellulose was calculated as the difference between NDF and ADF, while cellulose as the difference between ADF and ADL. All the tests were conducted on dry matter (DM). The material for the macro-element concentration

analyses was subjected to digestion in concentrated sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$), whilst the material for micro-component concentration analyses was subjected to digestion in a nitric acid (HNO_3), perchloric acid ($HClO_4$) mixture. The concentration of phosphorus (P) was determined by colorimetric method using a Specol 221. An Atomic Absorption Spectrometer was used to determine potassium (K), sodium (Na) and calcium (Ca) – by means of emulsion flame spectroscopy, as well as magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), lead (Pb), chromium (Cr), molybdenum (Mo), cadmium (Cd), cobalt (Co), nickel (Ni) and copper (Cu), which were subjected to absorption flame spectroscopy.

Statistical analysis was carried out using Statistica 10 software. In order to determine the significance of differences in chemical composition of the analysed plant samples, a one way analysis of variance (ANOVA) was conducted. For the purpose of determining homogenous subsets of means, Duncan tests were carried out at $p \leq 0.05$.

Results and Discussion

Mean dry mass in fresh material was the highest in chokeberry leaves (366.4 g). Drying increased the dry mass percentage in the material studied to 89-95 % (Table 1). The highest dry mass level was found in dried sea buckthorn leaves, 6 % more than in chokeberry.

In this study, crude protein levels in the examined leaves ranged from 112.2 to 249.7 g per kg of dry mass (DM). The highest total of protein was found in sea buckthorn leaves, while the lowest was in chokeberry leaves. Protein levels similar to those found in sea buckthorn were reported by Singh et al. (2001) in mint leaves (*Mentha spicata* L.). Kashif and Ullah (2013) reported protein levels in sea buckthorn leaves about two times lower than in our study (120.3 g kg^{-1} DM), yet they were still significantly higher than in other species they had analysed, such as neem (*Azadirachta indica* L.), pomegranate (*Punica granatum* L.) and basil (*Ocimum tenuiflorum* L.) (99.6, 36.9 and 51 g kg^{-1} DM, respectively).

Raspberry had the highest crude fat content in dried leaves (70.4 g kg^{-1} DM), while the lowest fat content was found in chokeberry leaves (55.2 g kg^{-1} DM). In comparison, utazi (*Gongronema latifolium* L.), a herb popular in the US as a constituent of tisane blends for the maintenance of healthy glycemic control, had lipids of 61.3 g kg^{-1} DM (Atangwho et al., 2009). Fan et al.

(2010) showed that fat in raspberry leaves helps reduce blood sugar levels (glucose), thereby having a possible beneficial effect on diabetes, similar to utazi tisane (Okolie et al., 2008).

The main component of dry mass is total carbohydrates, which perform numerous essential roles in living organisms; monosaccharides being the major source of energy for human metabolism, and polysaccharides serving as the storer of energy and structural components. In our study, the highest carbohydrate levels were found in chokeberry (668.0 g kg^{-1} DM) and the lowest in blackberry (515.6 g kg^{-1} DM).

The highest amount of crude ash, representing the mineral content, was found in blackberry leaves and the lowest in sea buckthorn leaves.

The functional properties of food are associated with the content of fibre in the form of non-digestible carbohydrates, on which symbiotic bacteria feed in the large intestine (Dhingra et al., 2011; Fuller et al., 2016). In people, dietary fibre is of great importance to the prevention and treatment of diabetes, obesity, coronary heart disease, as well as colon and large intestine cancers (Ferguson, 2005; Mann and Cummings, 2009; Brownlee, 2011). The function of dietary fibre in the human organism is associated with the amount in the diet as well as with the fractional composition, which can vary depending on the species of the plant, development level, anatomical part of a plant, and the technological procedure used (McDougall et al., 1996).

In our study, the highest level of crude fibre was found in blackberry (189.8 g kg^{-1} DM) and the lowest in chokeberry (89.8 g kg^{-1} DM); the same regularity was observed for dietary fibre fractions: neutral detergent fibres NDF, ADF, ADL, HCEL and CEL (Table 2). Blackberry leaf levels of neutral detergent fibre (NDF), consisting of acid detergent fibre (ADF) fractions plus hemicellulose (HCEL), were more than two times higher than in chokeberry leaves (298.9 g kg^{-1} DM vs. 175.7 g kg^{-1} DM), although not statistically significantly higher than in raspberry leaves. Acid detergent fibre (ADF), consisting of insoluble cellulose and lignin, significant in the digestibility of food (as ADF increases, the ability to digest the food decreases), was over 60 % higher in blackberry than in chokeberry leaves. Hemicelluloses (HCELS), accounting for up to one third of total dry plant biomass, are important components of dietary fibre, which (apart from pectins) exhibit strong sorptive properties for heavy metals, thereby increasing the health-promoting value of

Table 1 – Chemical composition of berry plants leaves (g kg^{-1} DM).

Specification	Moisture (in fresh material)	Dry mass (after drying, g kg^{-1})	Crude ash	Crude protein	Crude fat	Crude fibre	Total carbohydrates
Raspberry	700.6 ^{a*}	947.3 ^b	70.0 ^c	148.0 ^b	70.4 ^a	140.4 ^b	572.1 ^b
Blackberry	658.5 ^b	935.9 ^c	77.1 ^a	151.4 ^b	66.0 ^b	189.8 ^a	515.6 ^c
Chokeberry	633.6 ^c	893.1 ^d	74.7 ^b	112.2 ^c	55.2 ^d	89.8 ^c	668.0 ^a
Sea buckthorn	700.6 ^a	952.3 ^a	48.3 ^d	249.7 ^a	61.3 ^c	122.0 ^b	579.8 ^b

*Mean values with the same letter in each column are not significantly different at $p \leq 0.05$.

food. High HCEL concentrations have a beneficial effect as they expand and absorb water in the human alimentary canal, and positively influence physiology (Nawirska, 2005). Schädel et al. (2010) examined four herb species and reported HCEL content between 6 % and 22 % in dry mass. Although, as mentioned, HCEL levels were the highest in blackberry leaves, they were not statistically significantly higher than in raspberry or sea buckthorn leaves. Although CEL fibres are virtually not digested in the alimentary canal (Kahlon et al., 2007), they support intestinal peristalsis. CEL and acid detergent lignin (ADL) are also important in heavy metal binding, although not as much as HCELS. In our study, raspberry and blackberry leaves contained statistically significantly more cellulose (CEL) than sea buckthorn or chokeberry leaves.

Mean levels of macroelements identified in the leaves of berry plants are presented in Table 3. The analysis of the results obtained indicates a variation in mineral content between the berry plant species studied. Nitrogen (N), a component of amino acids, nucleic acids, vitamins, enzymes, and chlorophyll, was 23.5 g N kg⁻¹ DM on average. The highest N content was found in sea buckthorn leaves and the lowest in chokeberry.

Calcium (Ca) deficiency can affect the formation of bones and teeth, but an excess retention can cause kidney stones (Ştef et al., 2010). The mean concentration of Ca in berry plant leaves was 8.2 g kg⁻¹ DM. In medicinal plants the Ca content is in the range from 4.40 to 37.6 g kg⁻¹ DM, and Ştef et al. (2010) found the highest Ca levels in *Urtica dioica* L. and *Plantago major* L.

Mean phosphorus (P) levels in the leaves stood at 3.2 g kg⁻¹ DM. The least P was found in sea buckthorn leaves (2.47 g P kg⁻¹ DM). P levels in sea buckthorn leaves cultivated in a different location (Ladakh, India) ranged from 0.3 to 0.4 g kg⁻¹ DM, depending on the exact habitat (Sharma et al., 2014).

Table 2 – Fibre fractions in berry plant leaves (g kg⁻¹ DM).

Specification	NDF	ADF	ADL	HCEL	CEL
Raspberry	298.9 ^{ab*}	196.0 ^b	31.2 ^c	103.0 ^{ab}	164.8 ^a
Blackberry	371.6 ^a	253.7 ^a	70.9 ^a	117.9 ^a	182.8 ^a
Chokeberry	175.7 ^c	151.7 ^c	29.6 ^c	61.6 ^b	122.1 ^b
Sea buckthorn	284.4 ^b	180.6 ^b	58.0 ^b	79.8 ^{ab}	122.6 ^b

NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; CEL = cellulose; HCEL = hemicellulose; *Mean values with the same letter in each column are not significantly different at $p \leq 0.05$.

Table 3 – Content of macroelements in berry plant leaves (g kg⁻¹ DM).

Specification	N	Ca	P	K	Na	Mg	Na:K	Ca:P
Raspberry	22.2 ^{b*}	8.08 ^{ab}	3.90 ^a	16.6 ^a	3.87 ^a	5.43 ^{ab}	0.20	2.1
Blackberry	22.1 ^b	8.02 ^{ab}	3.32 ^b	17.2 ^a	0.42 ^b	5.76 ^a	0.02	2.4
Chokeberry	18.5 ^c	9.23 ^a	3.32 ^b	15.8 ^a	0.35 ^b	4.69 ^c	0.05	2.8
Sea buckthorn	31.4 ^a	7.38 ^b	2.47 ^c	9.12 ^b	0.11 ^c	3.88 ^c	0.01	1.6

*Mean values with the same letter in each column are not significantly different at $p \leq 0.05$.

Calcium and phosphorus are the minerals present in the largest quantity in the structure of the body and bones (Ihedioha and Okoye, 2011). A Ca:P ratio < 0.5 was first introduced by Shills and Young (1988) as an indicator of a diet rich in animal protein and phosphorus, which enhances Ca loss through the excretion of urine, resulting in a lower content in bones (Ihedioha and Okoye, 2011). The Ca:P ratio in all of the examined species reached ca. one, which indicates that all of them may be good sources of minerals essential to bone creation.

Potassium (K) is a very important macroelement due to its role in muscle contraction, lipid metabolism, protein synthesis, fluid and electrolyte balance in the body and nerve impulse transmission (Ştef et al., 2010). In our study, the lowest K level was found in sea buckthorn (9.1 g kg⁻¹ DM) and the highest in blackberry (17.2 g kg⁻¹ DM), which is consistent with research by Jabeen et al. (2010), who reported *Achyranthes aspera* L plants K levels to be 17.8 g kg⁻¹ DM.

Sodium (Na) is present in extracellular fluids in animals and humans. It is responsible for the depolarization of cellular membranes and for water equilibrium in intra- and extracellular media (Ştef et al., 2010). In medicinal plants, the value of Na ranges from 765.1 to 813.8 mg kg⁻¹ DM (Subramanian et al., 2012). In this study, Na levels in leaves ranged between 0.1 and 3.9 g kg⁻¹ DM. The Na:K ratio, significant for blood pressure (Yusuf et al., 2007) and recommended to be less than one, was below this level in all the berry plants studied, and, consequently the leaves of raspberry, blackberry, chokeberry and sea buckthorn can be used in a dietary regime to lower blood pressure.

Magnesium (Mg), present in many enzymes involved in proteins, lipids and carbohydrates metabolism, can also be found in chlorophylls in plants (Ştef et al., 2010). The leaves of all the plants studied contained 4.9 g mg kg⁻¹ DM on average, which is several times more than in many medicinal plants, including *Mentha spicata* L. which contains the most Mg (532.7 mg kg⁻¹ DM) (Subramanian et al., 2012).

The levels of the micro-nutrients selected in the leaves of the berry plants are presented in Table 4. These are metals which, in adequate concentrations, are essential to the healthy functioning of the body, and also to the proper growth and development of plants, but which, at higher concentrations, are likely to act as toxins (Nkansah and Amoako, 2010; Hina et al., 2012). One problem is the deficiency of one essential element, for example iron (Fe) or zinc (Zn) deficiencies which are found in approximately 1.5 billion people worldwide (Assunção et al., 2003; Palmgren et al., 2008). As Fe plays an important part in the production of hemoglobin and red blood cells, deficiency thereof leads to anaemia (Sarpong et al., 2014).

In this study, the highest Fe level was found in sea buckthorn leaves (177.8 mg kg⁻¹ DM), more than 1.5 times higher than in raspberry or blackberry, and over six times higher than in chokeberry. Thus, sea buckthorn

Table 4 – Content of micronutrients in berry plant leaves (mg kg⁻¹ DM).

Specification	Fe	Zn	Cu	Co	Mn	Cr	Mo	Ni	Pb	Cd
Raspberry	64.1 ^{b*}	30.2 ^a	3.54 ^a	0.42 ^a	64.2 ^b	0.98 ^{ab}	21.2 ^c	5.47 ^a	5.76 ^b	2.91 ^c
Blackberry	61.6 ^b	20.1 ^a	5.23 ^a	0.62 ^a	52.9 ^b	0.91 ^b	20.3 ^c	3.88 ^b	9.18 ^a	3.12 ^a
Chokeberry	23.5 ^b	25.1 ^a	1.38 ^a	0.48 ^a	150.9 ^a	1.06 ^{ab}	24.5 ^a	3.85 ^b	9.28 ^a	3.05 ^b
Sea buckthorn	177.8 ^a	20.8 ^a	5.07 ^a	0.68 ^a	39.9 ^b	1.47 ^a	23.0 ^b	3.44 ^c	7.70 ^{ab}	3.11 ^a

*Mean values with the same letter in each column are not significantly different at $p \leq 0.05$.

leaves may be an alternative rich source of Fe; it can be considered safe as its Fe level is below the WHO limit (300 mg kg⁻¹) (Nkansah and Amoako, 2010). On average, Fe in the leaves of berry plants stood at 81.7 mg kg⁻¹ DM, compared to a range of 2.90 to 6.07 mg kg⁻¹ DM in medicinal herbs (Maobe et al., 2012).

Zinc (Zn) affects the immune system and wound healing, as well as the senses of taste and smell. Zn seems to support normal growth and development in pregnancy, childhood, and adolescence (Fraga, 2005). Copper (Cu) deficiency in humans is rare, but when it does occur it leads to normocytic, hypochromic anemia, leucopenia and neuropenia, and even osteoporosis in children. Cu deficiency can be caused by excessive dietary Zn although chronic Cu toxicity is rare in humans, and is mostly associated with liver damage (Kanumakala et al., 2002; Fraga, 2005). In this study, we found no significant differences between Zn and Cu levels in the leaves of the species studied, and both were below the WHO limit (24.1 mg Zn kg⁻¹ DM and 3.8 mg Cu kg⁻¹ DM., on average). In comparison, bay leaves contain 59 mg kg⁻¹ DM of Zn, while garlic and ginger leaves contain 9 mg kg⁻¹ DM of Cu (Nkansah and Amoako, 2010).

Cobalt (Co), in the form of cobalamin is a part of the vitamin B12 molecule, and has no other known function in humans. Cobalt deficiency is ultimately a deficiency in vitamin B12, resulting in anaemia, anorexia and depression (Dutta and Mukta, 2012). There are no regulatory limits by WHO/FAO for Co content in herbal plants and their derivatives (Moses et al., 2012). In the present study Co levels in the leaves ranged, on average, from 0.4 to 0.7 mg kg⁻¹ DM, 0.55 mg kg⁻¹ DM.

The role of manganese (Mn) in preventing certain diseases is still undetermined and, therefore, little is known about the influence of Mn on the human organism (Zabłocka-Słowińska and Grajeta, 2012). We found the highest Mn levels in chokeberry (150.9 mg kg⁻¹ DM) and the lowest in sea buckthorn (39.9 mg kg⁻¹ DM). In comparison, Mn levels in the leaves of sea buckthorn cultivated in the North-West Himalayas ranged from 0.20 to 0.56 mg kg⁻¹ DM (Sharma et al., 2014). Significant differences in Mn content between our and other studies (Sharma et al., 2014) are probably attributable to differences in habitat and confirm that the environment significantly influences the chemical composition of sea buckthorn (Zeb and Malook, 2009).

The primary function of chromium (Cr) in the human body is to enhance the interaction of insulin with its receptor on the cell surface. Cr deficiency in humans

has only been described in long-term total parenteral nutrition (TPN) patients receiving insufficient chromium (Mason, 2008). In our study, sea buckthorn leaves had the highest concentration of Cr, while all of the remaining studied samples had a mean concentration of 1.12 mg kg⁻¹ DM. Concentration of Cr varied from 0.67 µg g⁻¹ DM in *Papaver somniferum* L. to 7.15 µg g⁻¹ DM in *Capsicum annum* L. (Soylak et al., 2004).

Molybdenum (Mo) is a cofactor in several enzymes, most prominently xanthine oxidase and sulphite oxidase. Its toxicity has not been well described in humans, although in high doses it may interfere with copper metabolism (2 mg) (Mason, 2008). Its content in food products, including plants, is mainly influenced by the properties of the soil and the plant species. Berry plant leaves contained Mo 22.3 mg kg⁻¹ DM on average, and the highest content was found in chokeberry.

Herbs are an important food source both in the diet regime and medicines for human beings. However, environmental pollution and contamination during processing may result in the presence of toxic elements in herb products and a toxic effect on the human body. Thus, the determination of trace elements in herbs as an aid to measuring toxic element levels is important. Heavy metal contents in plants varied depending on the country of origin, environmental pollution or plant part (Başgel and Erdemoğlu, 2006).

Based on studies of nickel (Ni) in workers and laboratory animals, all nickel compounds, except for metallic nickel, have been classified as carcinogenic for humans by the International Agency for Research on Cancer (IARC) (Das, 2008). Plants contain relatively low amounts of nickel and its absorption greatly depends on soil type and fertilisation. None of the berry plant samples exceeded the WHO limit for nickel (50 mg kg⁻¹) (Nkansah and Amoako, 2010).

Under normal conditions plants contain low levels of lead (Pb), varying from trace amounts to a few which, rarely, may run to several mg kg⁻¹ DM. The increase in content of this element in both the soil and the atmosphere also raises its content in plants (Lin and Jiang, 2013). Pb exposure has been shown to cause severe anaemia, permanent brain damage, neurological disorders, reproductive problems, diminished intelligence and a host of other diseases. Pb concentrations in the leaves of the berry plant species examined was below the WHO limits for food (100 mg kg⁻¹) (Nkansah and Amoako, 2010). The highest Pb content was found in the leaves of blackberry and chokeberry. Mean Pb concentration in all of the sam-

ples tested stood at 8 mg kg⁻¹ DM, higher than for spices and herbs and were obtained from markets in Poland (0.25-0.79 mg kg⁻¹ DM) (Krejpcio et al., 2007).

In the studied berry plants studied, cadmium (Cd) levels, a highly toxic metal which can cause many severe diseases (Batool and Khan, 2014), exceeded the limits set by the FAO/WHO (Ziyaina et al., 2014). This may reflect environmental pollution in Poland, resulting in Cd accumulation in plant tissues. Mean Cd content in the leaves of the berry plants (3.0 mg kg⁻¹ DM) was similar to that in medicinal plants and spices from India and the Kirov region (between 0.68 and 2.75 mg kg⁻¹ DM) as reported by Subramanian et al. (2012) and Luginina and Egoshina (2013).

Conclusions

The results of the present study show the basic chemical composition, fibre fractions and selected mineral levels in the leaves of selected berry plants, filling a known gap in the data in the literature. The leaves of sea buckthorn were the richest source of protein compared to the remaining species tested. The majority of lipids were found in raspberry leaves. Berry plant leaves were a rich source of crude fibre and dietary fibre fractions, the highest being found in blackberry leaves. Correct Ca:P and Na:K ratios indicated that the leaves of raspberry, blackberry, chokeberry and sea buckthorn may become a good source of minerals essential to bone formation, and may successfully contribute to the treatment of hypertension. The possibility of using the leaves of the berry plants studied in the human diet regime are also indicated by high Ca, Mg and K levels. Sea buckthorn leaves contain a lot of Fe and, therefore, may become an alternative source of this element which is both rich and safe and the application of berry plant leaves may also provide a source of enrichment of the human diet regime and herbal agents in terms of essential minerals. However, as cadmium levels exceeded WHO limits, the leaves of these berry plants should be constantly monitored if used for consumption.

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