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Original Research Article

Evaluation of the inorganic content of six underused wild berries from Portugal: Potential new sources of essential minerals

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ABSTRACT

The mineral content and levels of trace elements in six wild underused berries (*Elaeagnus umbellata*, *Myrica faya*, *Rubus grandifolius*, *Sambucus lanceolata*, *Vaccinium padifolium* and *Vaccinium cylindraceum*) have been determined by inductively coupled plasma-mass spectrometry after microwave digestion. The inorganic content of these foodstuffs has not been previously described in scientific literature (except for *E. umbellata*). Hence, this information is of high interest concerning the introduction of these non-commercial berries in the mainstream market. The analytical method has been validated analyzing a certified reference material and performing recovery experiments. The results obtained have been discussed using the Recommended Daily Allowance for minerals provided by the Commission of the European Community, and a comparison between the composition of the analyzed berries and different commercialised berries has been carried out. Data obtained in this study support the consumption of these less-known berries, in particular *R. grandifolius* and *S. lanceolata,* as potential novel sources of essential minerals.

1. Introduction

Berries are small fleshy fruits with delicious flavour and taste, of wild or cultivated origin, that are commonly consumed in fresh and in processed forms (Skrovankova et al., 2015). In recent years, there has been a constant increase in the popularity of berries as important dietary sources of bioactive compounds with antioxidant activity (polyphenols, carotenoids, vitamins and minerals) (Brauch et al., 2016; Jimenez-Garcia et al., 2013; Nile and Park, 2014).

Berries are rich in both macro- and micronutrients such as phosphorus, potassium, calcium, magnesium, iron, manganese, copper, sodium, and aluminium (Nile and Park, 2014). Many elements are required in small amounts to maintain human health due to their nutritious value, as they play important roles in various important physiological and biochemical processes in the human body. These elements must be regularly provided through the diet (Hua et al., 2014; Khattak, 2012). Some minerals (Cu, Fe, Mn and Se) have also been recognized to act as antioxidants, contributing to the beneficial properties of foodstuffs (Fraga, 2005; Tavares et al., 2010). In contrast, toxic metals may turn a potential foodstuff unfit for human consumption, hence the importance of elemental composition determination. Inductively coupled plasma-mass spectrometry (ICP-MS) is one of the most important techniques used for the simultaneous determination of essential and toxic elements in fruit and vegetables (Brauch et al., 2016; Ekholm et al., 2007; Hua et al., 2014; Llorent-Martínez et al., 2013; Tavares et al., 2010)

The vascular flora of Madeira archipelago (Portugal) includes several endemic species, including berry producing-plants like *Myrica faya* (candleberry-myrtle), *Rubus grandifolius* (wild blackberry), *Sambucus lanceolata* (Madeiran elderberry) and *Vaccinium padifolium* (Madeiran blueberry). These wild berries are edible and collected in late Summer (August-September), being also used as folk medicines for diverse applications (diuretic, emmolient, astrigent, cough, diabetic and opthalmic problems) (Rivera and Obón, 1995). However, the collection and eventual cultivation of these species for commercial purposes is undermined by the lack of scientific information required to promote market entry.

The main goal of this study was to determine the inorganic content (essential and toxic elements) of six wild berry species: four endemic to Madeira Island (*M. faya*, *R. grandifolius*, *S. lanceolata* and *V. padifolium*), one native to Azores archipelago

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(*V. cylindraceum*) and one non-native species from Madeira Island (*Elaeagnus umbellata*). Except for *E. umbellata*, this is the first study carried out with this purpose. The results obtained by ICP-MS after microwave digestion were critically discussed, using Recommended Daily Allowance (RDA) values for minerals. A comparison between the composition of the berries analyzed in this study and other berries of known composition has been carried out too.

2. Experimental

2.1. Instrumentation

The quadrupole ICP-MS used in this work was an Agilent 7500a (Agilent Technologies, CA, USA) equipped with a Babington nebuliser, a Peltier-cooled quartz spray chamber and a standard torch (2.5 mm i.d.). The instrument was tuned using an aqueous multi-element standard solution (Agilent, Madrid, Spain) of 10 ng mL^{-1} each of Li, Y, Ce and Tl for consistent sensitivity (^{7}Li, ^{89}Y and ^{205}Tl) and minimum doubly charged and oxide species levels (^{140}Ce). Operating conditions are shown in Table 1.

The samples were digested using a MARSXpress (CEM) ultra-high throughput microwave digestion system (obtained from Gilson, Madrid, Spain), with 50 mL PFA vessels designed for temperatures up to 260 °C. The power control was automatically adjusted by the system (maximum 1000 W).

2.2. Reagents

A 100 μg mL^{-1} multi-element standard solution (SCP Science, Paris, France) containing Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Ti, Tl, V, and Zn; a 10 μg mL^{-1} Hg solution (Agilent, Madrid, Spain); and 1000 μg mL^{-1} solutions of In, P and Sn from Sigma-Aldrich (Madrid, Spain) were used. Suitable dilutions were performed with sub-boiling HNO_3 in ultrapure deionized water when required.

Solutions of H_2O_2 for ultratrace analysis were purchased from Sigma. The certified reference material (CRM), cranberry fruit SRM-3281, was obtained from the National Institute of Standards and Technology (NIST).

Analytical reagent grade HNO_3 65% (Sigma-Aldrich, Madrid, Spain) was additionally cleaned by sub-boiling distillation. The sub-boiling still was built up with components from Savillex (www.savillex.com). Sub-boiling HNO_3 was used in all experiments. All plastic containers were soaked in 10% v/v sub-boiling HNO_3 for at least 24 h, and then thoroughly rinsed with Milli-Q water prior to use.

Ultrapure deionized water (18.2 MΩ cm) was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Sample preparation and digestion

Berries were collected from the following plants grown in the wild in different locations of Madeira (Madeira archipelago, Portugal) and Terceira Island (Azores archipelago, Portugal): *E. umbellata*, *M. faya*, *R. grandifolius*, *S. lanceolata*, *V. cylindraceum*, and *V. padifolium*. A total of 16 samples were collected during 2013 and 2014 (see Table 2 for more details). Each of the samples reported in Table 2 was collected in the following way: berries were manually picked from different plant specimens of the same collection area, choosing the ripe ones, and they were combined (pooled) into one sample (approximately 2 kg of each sample). In this way, each sample was representative of the species collected at each location. Possible variations among different specimens of a given species at each collection site were not considered, as the berries were combined into single batches.

For analysis, ripe berries were washed with distilled water, dried with paper, and stored at −80 °C. Then, berries were lyophilized to dryness for 72 h (Alpha 1–2 LD plus freeze dryer, CHRIST), ground to powder in a mechanic grinder, and stored at −20 °C in plastic sealed bags (Spínola et al., 2014). The percentage of humidity was calculated weighing samples before and after lyophilisation.

For the digestion of berries, aliquots of 0.25 g of sample (previously homogenized), 6 mL HNO_3 and 1 mL H_2O_2 were added to a digestion vessel (Llorent-Martínez et al., 2013). The vessels were kept at room temperature for 30 min. Then, they were placed in the microwave system and the temperature was increased to 200 °C in 15 min, and maintained at 200 °C for 15 min. The power of the microwave oven was set at 1000 W and was automatically adjusted by the equipment when required.

After cooling at room temperature, digestion liquors were quantitatively transferred into plastic containers and diluted to 60 mL with ultrapure water. The internal standard, In, was added to yield a concentration of 5 μg L^{-1}. After each analytical batch, the vessels were cleaned (8 mL HNO_3 + 1 mL H_2O_2) using the same microwave operating program; after cooling at room temperature, all vessels were thoroughly rinsed with Milli-Q water.

Three different aliquots of each sample (Table 2) were independently digested and analyzed. ICP-MS measurements were performed in triplicate for each aliquot.

Table 1
ICP-MS operating conditions and selected isotopes.

Plasma conditions	
RF power	1.2 kW
Plasma Ar flow rate	15 L min^{-1}
Auxiliary Ar flow rate	0.89 L min^{-1}
Carrier Ar flow rate	0.95–1.0 L min^{-1}
Torch horizontal alignment	– (0.5–1.0) mm
Torch vertical alignment	0.2–0.5 mm
Sampling depth	6.0–8.0 mm
Instrument	
Sampler cone	Nickel, 1.0 mm orifice diameter
Skimmer cone	Nickel, 0.4 mm orifice diameter
Isotopes	$^{107,109}Ag$, ^{27}Al, ^{75}As, ^{137}Ba, $^{43,44}Ca$, $^{111,114}Cd$, ^{59}Co, ^{53}Cr, $^{63,65}Cu$, ^{57}Fe, $^{199,202}Hg$, ^{115}In, ^{39}K, $^{24,25,26}Mg$, ^{55}Mn, $^{95,98}Mo$, ^{23}Na, $^{60,62}Ni$, ^{31}P, $^{206,208}Pb$, $^{121,123}Sb$, ^{82}Se, $^{116,118,120}Sn$, ^{47}Ti, $^{203,205}Tl$, ^{51}V, $^{66,68}Zn$

Table 2
Description of the collected berry species.

Species	Common-name	Collection area	Collection date
E. umbellata[a]	Autumn olive	Funchal, Madeira Island	September 2013
		Funchal, Madeira Island	September 2014
M. faya	Candleberry-myrtle	Machico, Madeira Island	July 2013
		Machico, Madeira Island	July 2014
		Faial, Madeira Island	July 2014
		Arco S. Jorge, Madeira Island	July 2014
		Boaventura, Madeira Island	July 2014
		Seixal, Madeira Island	July 2014
		Ribeira da Janela, Madeira Island	July 2014
		Porto Moniz, Madeira Island	August 2014
R. grandifolius	Wild blackberry	Santo da Serra, Madeira Island	September 2014
		Pico do Arieiro, Madeira Island	September 2014
S. lanceolata	Madeiran elderberry	Pico das Pedras, Madeira Island	October 2014
V. cylindraceum	Azorean blueberry	Flores Island, Azores Archipelago	August 2014
V. padifolium	Madeiran blueberry	Pico do Arieiro, Madeira Island	September 2013
		Pico do Arieiro, Madeira Island	September 2014

[a] Species non-native to Portugal.

2.4. Calibration procedure

For the quantitative analysis of the samples, calibration curves were built using eight different concentrations of each element (Llorent-Martínez et al., 2013). Standard solutions were prepared in 10% (v/v) sub-boiling HNO_3 by diluting a multi-element standard solution containing all the elements. The calibration curves were built between the quantification limit (QL) of each analyte and 2000 $\mu g\,L^{-1}$ in all cases except Hg. Considering that memory effects may occur for Hg, the highest concentration for this element was 5 $\mu g\,L^{-1}$.

2.5. Statistical analysis

All samples were assayed in triplicate (three independent analyses of the same pooled sample), and results are given as mean ± standard deviation. Statistical analysis was carried out using SPSS software for Windows, IBM SPSS Statistics 20 (SPSS, Inc., USA). Analysis of variance (ANOVA) was applied to indicate significant differences between samples. ANOVA is used to detect variables which do not vary significantly as a function of the factors being studied and is useful for comparing (testing) three or more means (groups or variables) for statistical significance (Sarembaud et al., 2007).

The significance of differences between means was evaluated using a pairwise multiple comparison procedure (Tukeys post hoc test) at a 5% level. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Validation of the method

The procedure to digest the dry berries has been adapted from a previous publication (Llorent-Martínez et al., 2013). The validation has been carried out by the analysis of a certified reference material (SRM 3281, cranberries in powder form), and by recovery experiments. Three aliquots of the certified reference material were independently analyzed, and the comparison between the experimental and certified levels did not show significant differences (student's t statistical test; $p = 0.05$). The results obtained are shown in Table 3.

Recovery experiments were performed for all the elements between 100 $ng\,g^{-1}$ and 10 $mg\,g^{-1}$ spiked concentrations. These experiments were carried out at low levels for trace elements and high levels for the most abundant elements (Al, Ca, Fe, K, Mg, Mn, Na, P and Zn). The observed recoveries (data not shown) ranged between 85 and 110%, with relative standard deviations (RSDs) lower than 10% in all cases. These results confirmed that no significant metal losses occurred during the digestion procedure.

Table 3
Analysis of the certified reference material SRM-3281 (cranberries in powder form).

Element	Certified value ($mg\,kg^{-1}$)	Observed value ($mg\,kg^{-1}$)	t_{calc}[a]
Ca	528 ± 7	540 ± 20	0.98
Cu	3.52 ± 0.09	3.41 ± 0.05	0.83
Fe	27.7 ± 0.7	29 ± 3	0.21
Mg	446 ± 4	460 ± 10	0.48
Mn	21.9 ± 0.2	21.4 ± 0.3	0.41
P	835 ± 17	870 ± 50	0.16
K	8020 ± 130	8300 ±200	0.24
Na	259 ± 3	270 ± 10	0.19
Zn	6.9 ± 0.2	6.8 ± 0.4	0.04

Results are given as mean ± standard deviation ($n = 3$).
[a] $p = 0.05$, $t_{Student}$ (theoretical value) = 2.78.

3.2. Analytical parameters

Measurements were carried out using the full quantitative mode analysis in the quadrupole ICP-MS. The complete list of the monitored isotopes is shown in Table 1 (more than one isotope was selected when possible to confirm the results).

The correlation coefficients for all the calibration curves were at least 0.998, showing good linear relationships throughout the ranges of concentrations studied. The method detection limits

Table 4
Method detection limits (MDLs).

Element	MDL ($\mu g\,g^{-1}$)	Element	MDL ($\mu g\,g^{-1}$)
Ag	0.035	Mn	0.04
Al	0.34	Mo	0.02
As	0.15	Na	0.4
Ba	0.035	Ni	0.15
Ca	25	P	1
Cd	0.03	Pb	0.012
Co	0.01	Sb	0.015
Cr	0.2	Se	0.3
Cu	0.15	Sn	0.015
Fe	2	Ti	0.035
Hg	0.03	Tl	0.015
K	10	V	0.075
Mg	0.4	Zn	0.1

(MDLs) for all the selected elements are presented in Table 4. These values are not instrumental detection limits, but MDLs, which take into account the entire procedure and refer to concentrations in real samples, not in the final solutions.

The repeatability of the analytical method was evaluated by the analysis of the CRM during the same day, performing five independent analyses, whereas the intermediate precision was evaluated in three different days, with a total of six replicates. RSDs were lower than 10% for all the elements.

3.3. Inorganic content of the analyzed berries

The results obtained for all the elements detected in the analyzed berries are given in Tables 5 & 6. The ranges of concentrations (min-max values) are shown in Table 5, whereas the average concentrations are shown in Table 6. Results showed that a wide variation was observed in the quantitative composition of minerals from different berry species ($p < 0.05$). The results obtained for each berry will be discussed in the following subsections.

The main goal of this study was to determine the mineral contents of the different berries, and compare them with other commercial berries in terms of their nutritional value. Hence, the RDA values for minerals provided by the Commission of the European Communities (Commision of the European Communities, 2008) were used to calculate the amount of minerals ingested through the consumption of each of the analyzed berries. For commercial berries, the manufacturers usually provide a recommended daily amount to be ingested. For the target berries in this work, calculations were made considering a daily consumption of 30 g of fresh berries, which is a normal amount for similar foodstuffs. The results obtained are shown in Table 7.

In all cases, the levels of different toxic elements (Ag, As, Cd, Hg, Pb, Sb, Sn, and Tl) were below the MDL, or very low concentrations were observed. According to The Commission of the European Communities (Commision of the European Communities, 2006), the maximum levels for Cd and Pb are 0.05 and 0.2 mg kg^{-1}, respectively, which are higher than the values detected in this study. Hence, none of these berries would represent a risk to human health from this point of view.

Table 5
Variation (min-max value) of minerals and trace elements concentration levels in the analyzed berry species; expressed in μg g^{-1} (fresh weight).

	E. umbellata[a]	*M. faya*[b]	*R. grandifolius*[a]	*V. padifolium*[a]
Ag[1]	N.D.	N.D.	N.D.	N.D.
Al	13–86	11–200	15–260	20.0–30.9
As[2]	N.D.	N.D. Detected	N.D.	N.D.
Ba	1.0–1.2	0.14–1.6	3.4–6.5	3.0–3.2
Ca	925–1150	750–1140	1300–2100	770–940
Cd[3]	N.D.	N.D.	N.D.	N.D.
Co	0.03–0.04	0.03–0.31	0.04–0.06	0.03–0.14
Cr	Detected	Detected – 2.7	Detected – 1.1	Detected
Cu	5.2–7.4	1.0–3.6	3.1–5.9	2.6–5.3
Fe	28–40	23–302	34–273	13.2–20.5
Hg[3]	N.D.	N.D.	N.D.	N.D.
K	7600–8200	3400–5800	4300–5700	3000–3900
Mg	410–440	420–770	1350–1650	515–550
Mn	8.1–12.1	3.7–16	23.8–43.2	39–49
Mo	0.5–0.8	0.06–0.34	0.10–0.13	N.D.
Na	60–73	1300–2600	56–88	52–66
Ni	1.2–1.6	0.6–3.1	0.8–1.6	1.0–1.1
P	730–1000	275–440	610–950	510–610
Pb[4]	N.D.	N.D.–0.05	N.D.	N.D.
Sb[5]	N.D.	N.D. – 0.6	N.D.	N.D.
Se[6]	N.D.	N.D.	N.D.	N.D.
Sn[5]	N.D.	N.D.	N.D.	N.D.
Ti	1.4–5.2	1.8–50.5	2.2–48.3	0.33–0.79
Tl[5]	N.D.	N.D.	N.D.	N.D.
V[7]	Detected	Detected – 0.79	Detected – 0.72	N.D.
Zn	4.5–17.4	3.4–6.2	5.7–11.3	4.6–6.6

N.D.= Not detected.
MDLs: [1] 0.035 μg g^{-1}; [2] 0.15 μg g^{-1}; [3] 0.03 μg g^{-1}; [4] 0.012 μg g^{-1}; [5] 0.015 μg g^{-1}; [6] 0.3 μg g^{-1}; [7] 0.075 μg g^{-1} [a]n = 2 [b]n = 8; 3 replicates each.

3.3.1. Elaeagnus umbellata

E. umbellata (autumn olives) berries were collected in Funchal (Madeira Island) in October 2013 and 2014. *E. umbellata*, also known as Japanese silverberry or autumn olive, is a deciduous shrub or small tree, typically up to 3.5 m tall. Indigenous to Eastern Asia, it is also present in other continents. It grows small, round berries, which ripen to red, dotted with silver or brown color (Hussain, 2011; Khattak, 2012). Powders or extracts from the berries can be used in beverages, sauces, jams, or other products, and the berries are incorporated in the diet in some areas of Asia (Pei et al., 2015).

In general, the levels of the different nutritional elements in the berries collected in 2013 and 2014 were unaffected by collection year. Exceptions were observed for K, Mn, Mo, P and Zn ($p < 0.05$); the latter decreased approximately 3-fold from 2013 to 2014. The average levels of the most important nutritional elements varied between 7900 μg g^{-1} of K and 0.03 μg g^{-1} of Co. Concentrations decreased in the following order: K > Ca > P > Mg > Na > Fe > Mn > Cu > Ni > Mo > Co. Vanadium was detected, although it could not be quantified. The contribution to the RDA (Table 7) was lower than 10% for Ca, Fe, Mg, P, and Zn. However, contributions between 10 and 20% are expected for Cu, K, and Mn, and higher than 40% for Mo, making these berries a potential candidate as food supplement considering its mineral content.

Previous studies on this species (Hussain, 2011; Khattak, 2012; Tag et al., 2014) have also reported K, P, Mg and Ca as the main minerals, but at lower concentrations than those here observed. Different cultivars and edafoclimatic conditions might be the reason for the observed variations.

3.3.2. Myrica faya

M. faya (candleberry-myrtle) berries were collected in seven different locations in Madeira Island (Table 2) in July-August 2014, and one sample in July 2013. *Myrica faya* Aiton belongs to the genus Myrica, in the family *Myricaceae*. Native to Macaronesia region (Azores, Cape Verde and Madeira archipelagos, Canary Islands), it is an evergreen shrub or small tree, usually about 8 m tall. Its fruits are small edible berries, red to purple in color when ripe. These berries can be directly consumed, although they present a slightly bitter taste, so they are mainly used to produce jams and liquors (Spínola et al., 2014).

Taking into account that berries were collected in different locations (with different climatic conditions) and in two different years, the levels of the elements vary among the analyzed samples (Table 5). Significant differences ($p < 0.05$) were found among samples collected in different locations (except for Ba and Cu levels). The concentration of Fe, K, Mo, Ni and Pb in Machico samples seemed to be affected by the year of collection ($p < 0.05$). For an easier discussion, only the average values (Table 6) will be used. It can be observed that the levels of nutritional elements decreased in the following order: K (4400 μg g^{-1}) >Na > Ca > Mg > P > Fe > Mn > Zn > Cu > Ni > V > Co > Mo (0.11 μg g^{-1}). In this case, the contribution to the RDA would be lower than 10% for all the elements, except Mn (13%) and Fe (24%), which would contribute significantly to the diet. However, the good levels of antioxidants (Spínola et al., 2014) combined with the presence of a high number of inorganic nutrients (including V and Mo, absent in other berries) would make *M. faya* berries an interesting candidate as a new food supplement.

Table 6
Average concentration levels of minerals and trace elements in the analyzed berry species; expressed in $\mu g\,g^{-1}$ (fresh weight).

	E. umbellata	*M. faya*	*R. grandifolius*	*S. lanceolata*	*V. cylindraceum*	*V. padifolium*
Ag[1]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Al	30 ± 30^{a}	80 ± 50^{a}	100 ± 100^{a}	4.7 ± 0.5^{a}	37 ± 3^{a}	25 ± 5^{a}
As[2]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ba	1.1 ± 0.1^{a}	0.5 ± 0.3^{a}	5 ± 1^{c}	3.3 ± 0.3^{b}	5.6 ± 0.5^{c}	3.1 ± 0.1^{b}
Ca	1000 ± 100^{a}	900 ± 100^{a}	1700 ± 400^{c}	1200 ± 100^{ab}	1400 ± 100^{c}	860 ± 80^{ab}
Cd[3]	N.D.	N.D.	N.D.	N.D.	N.D.	Detected
Co	0.03 ± 0.01^{a}	0.2 ± 0.1^{b}	0.05 ± 0.01^{a}	0.016 ± 0.005^{a}	0.008 ± 0.001^{a}	0.08 ± 0.03^{a}
Cr	Detected	0.6 ± 0.5^{a}	0.6 ± 0.4^{a}	0.12 ± 0.01^{a}	0.08 ± 0.01^{a}	Detected
Cu	6.0 ± 0.9^{c}	1.6 ± 0.5^{a}	5 ± 1^{bc}	3.5 ± 0.3^{ab}	3.7 ± 0.3^{b}	4 ± 1^{bc}
Fe	32 ± 5^{a}	110 ± 50^{b}	120 ± 90^{b}	28 ± 5^{a}	19 ± 1^{a}	$17 \pm 3a$
Hg[3]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
K	7900 ± 300^{c}	4400 ± 500^{ab}	5000 ± 700^{b}	15100 ± 400^{d}	4700 ± 300^{ab}	3500 ± 400^{a}
Mg	420 ± 10^{a}	550 ± 100^{a}	1500 ± 200^{b}	1590 ± 50^{b}	420 ± 20^{a}	530 ± 10^{a}
Mn	11 ± 1^{a}	9 ± 3^{a}	30 ± 10^{b}	12 ± 1^{a}	131 ± 5^{c}	44 ± 5^{b}
Mo	0.7 ± 0.1^{b}	0.11 ± 0.05^{a}	0.12 ± 0.01^{a}	Detected	N.D.	N.D.
Na	70 ± 6^{a}	2000 ± 400^{c}	70 ± 10^{a}	65 ± 3^{a}	560 ± 30^{b}	60 ± 5^{a}
Ni	1.4 ± 0.2^{b}	1.6 ± 0.6^{b}	1.1 ± 0.3^{ab}	0.42 ± 0.05^{a}	0.36 ± 0.03^{a}	1.1 ± 0.1^{ab}
P	800 ± 100^{b}	330 ± 50^{a}	800 ± 200^{b}	2010 ± 70^{c}	760 ± 30^{b}	560 ± 40^{ab}
Pb[4]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sb[5]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Se[6]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sn[5]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ti	1.7 ± 0.3^{a}	20 ± 10^{a}	20 ± 20^{a}	0.69 ± 0.05^{a}	0.44 ± 0.03^{a}	0.5 ± 0.2^{a}
Tl[5]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
V[7]	Detected	0.3 ± 0.2^{a}	0.4 ± 0.3^{b}	Detected	N.D.	Detected
Zn	10 ± 6^{ab}	4.4 ± 0.5^{ab}	9 ± 3^{b}	5.9 ± 0.4^{b}	5.3 ± 0.3^{b}	5.4 ± 0.9^{a}

N.D.= Not detected.
MDLs: [1] 0.035 $\mu g\,g^{-1}$; [2] 0.15 $\mu g\,g^{-1}$; [3] 0.03 $\mu g\,g^{-1}$; [4] 0.012 $\mu g\,g^{-1}$; [5] 0.015 $\mu g\,g^{-1}$; [6] 0.3 $\mu g\,g^{-1}$; [7] 0.075 $\mu g\,g^{-1}$.
Results are given as mean ± standard deviation.
Values not sharing the same letter are significantly different at $p < 0.05$ probability level; letter *a* corresponds to the lowest value, whereas letter *e* to the highest value; *ab* means that the values are within the range of *a* and *b*.
Number of samples indicated in Table 2; 3 replicates for each sample.

Table 7
Percentage (%) of contribution to RDA for a daily consumption of 30 g of fresh berries, using average levels from Table 6.

	RDA (mg)	% RDA					
		E. umbellata	*M. faya*	*R. grandifolius*	*S. lanceolata*	*V. cylindraceum*	*V. padifolium*
Ca	800	3.8	3.4	6.5	4.4	5.4	3.2
Cu	1	18	4.8	15	10.5	11.1	12
Fe	14	6.9	23.6	25.7	6	4.1	3.6
K	2000	11.8	6.6	7.5	22.6	7.1	5.2
Mg	375	3.4	4.4	12	12.7	3.4	4.2
Mn	2	16.5	13.5	45	18	193	66
Mo	0.05	42	6.6	7.2	–	–	–
P	700	3.4	1.4	3.4	8.6	3.3	2.4
Zn	10	3	1.3	2.7	1.8	1.6	1.6

Potassium, calcium and magnesium have also been reported as dominant elements in *Myrica rubra* (bayberry) (Chen et al., 2004; Cheng et al., 2008; Fang et al., 2006). *M. faya* presents higher levels of K than *M. rubra*, but the contents of other element are lower or similar than in *M. rubra*.

3.3.3. Rubus grandifolius

R. grandifolius (wild blackberries) berries were collected in September 2014 in two different locations in Madeira Island (Table 2).

Rubus grandifolius Lowe is a species endemic to Madeira archipelago. Its berries, known as *amoras*, physically similar to other blackberries, are consumed fresh or processed as jam, juice or liquor, and are used in folk medicine to treat diverse diseases (Gouveia-Figueira and Castilho, 2015).

For most of the elements (except Ba and Mo), the levels observed in berries collected in Santo da Serra (600 m above mean sea level) were higher than those found in Pico do Arieiro (1000 m above mean sea level) berries, probably due to the different soil characteristics. Significant differences were found for Ba, Ca, K, Mg, Mn, P and Zn contents in samples collected in different locations ($p < 0.05$). The levels of nutritional elements decreased in the following order: K (5000 $\mu g\,g^{-1}$) >Ca > Mg > P > Fe > Na > Mn > Zn > Cu > Ni > V > Mo > Co (0.05 $\mu g\,g^{-1}$). The contribution to the RDA of minerals of wild blackberries is significant for Cu (15%), Fe (26%), Mg (12%) and Mn (45%). In addition, the level of V and the additional contribution to the RDA of other minerals, although lower than 10%, makes it important to carry out additional research in terms of organic compounds (results to be published elsewhere).

The levels of minerals found in the present study were higher than those reported for other *Rubus* species (blackberry and raspberry) (de Souza et al., 2014; Ekholm et al., 2007; Nile and Park, 2014).

3.3.4. Sambucus lanceolata

Berries from *S. lanceolata* (Madeiran elderberry) were collected in Santana (Madeira Island) in October 2014. *Sambucus lanceolata* Banks ex Lowe is a species endemic to Madeira Island, whose

common name in English is "Madeira elder", and "sabugueiro" in Portuguese. It is a small tree or shrub, up to 7 m tall, with small yellowish round fruits that turn black when ripe. They have been used in folk medicine as diuretic (Press and Short, 1994; Rivera and Obón, 1995).

In this case, samples could not be collected in different locations or years, so only the average levels will be discussed. The levels of the nutritional elements decreased in the following order: K (15,100 $\mu g\,g^{-1}$) >P > Mg > Ca > Na > Fe > Mn > Zn > Cu > Ni > Co (0.016 $\mu g\,g^{-1}$). The levels of Mo and V were lower than the MDL. In terms of contribution to the mineral RDA, percentages higher than 10% are expected for Cu, K, Mg and Mn, and close to 10% for P. Lower mineral concentrations were documented in other *Sambucus* species (Nile and Park, 2014). Hence, in a similar way to *R. grandifolius*, there is ongoing research on the organic composition to determine the potential health benefits of these berries.

3.3.5. Vaccinium cylindraceum

Vaccinium cylindraceum Sm. (Azorean blueberry) berries were collected in August 2014 in Terceira Island (Azores archipelago), where it is endemic as a shrub belonging to the *Ericaceae* family. Up to 5 m tall, it grows blue-black berries of cylindrical form that are used to make candy and jams (Schäfer, 2005).

Samples from Azorean blueberry could not be collected in different locations or years, so only the average levels will be discussed, in a similar way to *S. lanceolata*. The quantified levels of the nutritional elements decreased in the following order: K (4700 $\mu g\,g^{-1}$) >Ca > P > Na > Mg > Mn > Fe > Zn > Cu > Ni > Co (0.008 $\mu g\,g^{-1}$) >. The levels of Mo and V were below the MDL. The contribution to the RDA of most elements were below 10%, except for Cu (11%) and Mn (>190%). The high levels of Mn are in contrast with the levels of this element found in the rest of the berries here analyzed and with the levels reported for other berries. Therefore, additional research is required to confirm the high Mn content here observed. In this sense, our research group will collect samples from different harvests (locations and years) and draw conclusions from the results obtained.

3.3.6. Vaccinium padifolium

Vaccinium padifolium (Madeiran blueberry) berries were collected in September 2013 and 2014 in Pico do Arieiro (Madeira Island). It is a semi-evergreen shrub or small tree up to 3 m tall, endemic to Madeira Island, which grows berries of blue-black color when ripe (Press and Short, 1994). Berries are edible and used to make jams and food preserves; they are used in local ethnopharmacology for cough, colds, bronchitis and dysentery, and exported for commercial production of ophthalmic specialities (Cabrita and Andersen, 1999; Rivera and Obón, 1995).

The levels of some minerals in berries collected in 2013 and 2014 were similar, but not for Al, Ca, K, Mg, Mn and P contents ($p < 0.05$). The concentrations of the nutritional elements decreased in the following order: K (3500 $\mu g\,g^{-1}$) >Ca > P > Mg > Na > Mn > Fe > Zn > Cu > Ni > Co (0.08 $\mu g\,g^{-1}$). As in *V. cylindraceum*, K, Ca and P were the major components. The levels of Mo were below the MDL, whereas V was detected, but not quantified. The contribution to the RDA of minerals was lower than 10% for all

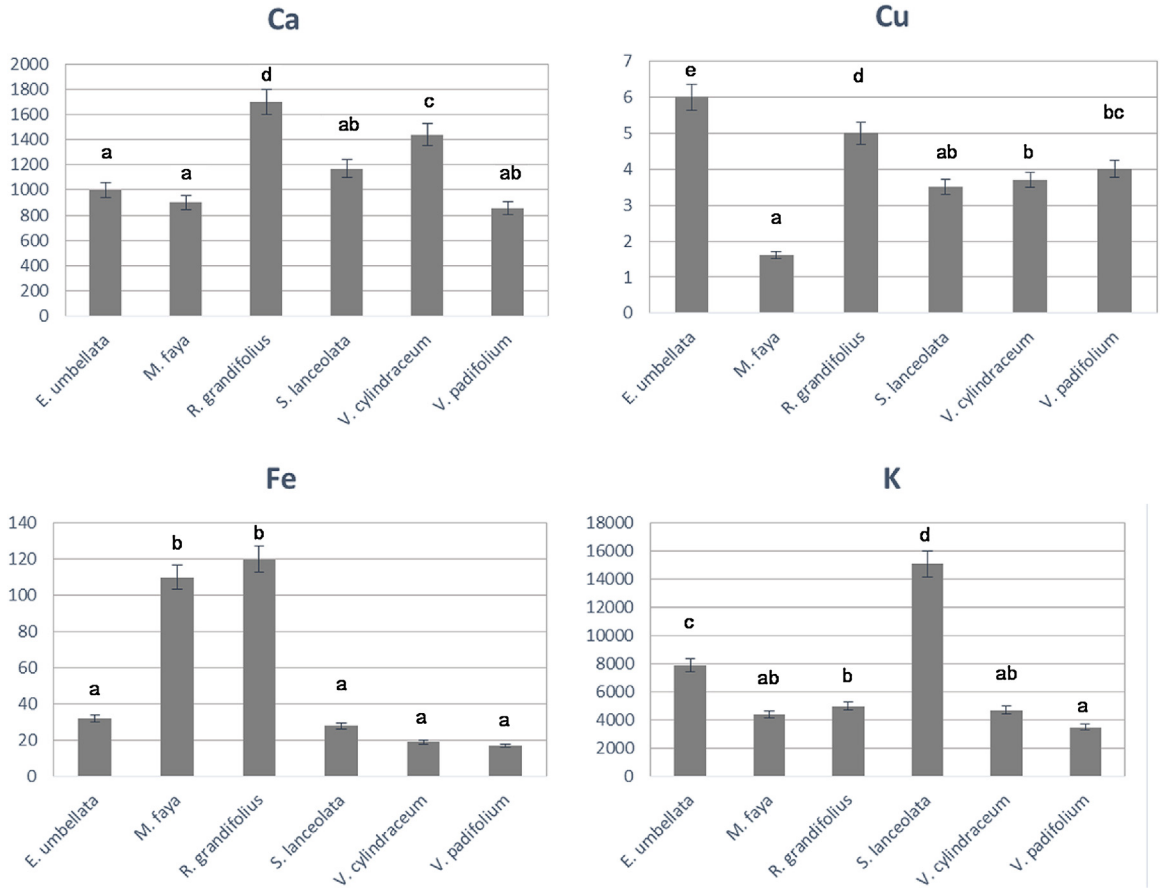

Fig. 1. Comparison of the average contents of Ca, Cu, Fe, and K in the analyzed berry species, expressed in $\mu g\,g^{-1}$ (fresh weight). Number of analyzed samples indicated in Table 2; 3 replicates for each sample. Columns not sharing the same letter are significantly different at $p < 0.05$ probability level; letter *a* corresponds to the lowest value, whereas letter *e* to the highest value; *ab* means that the values are within the range of *a* and *b*.

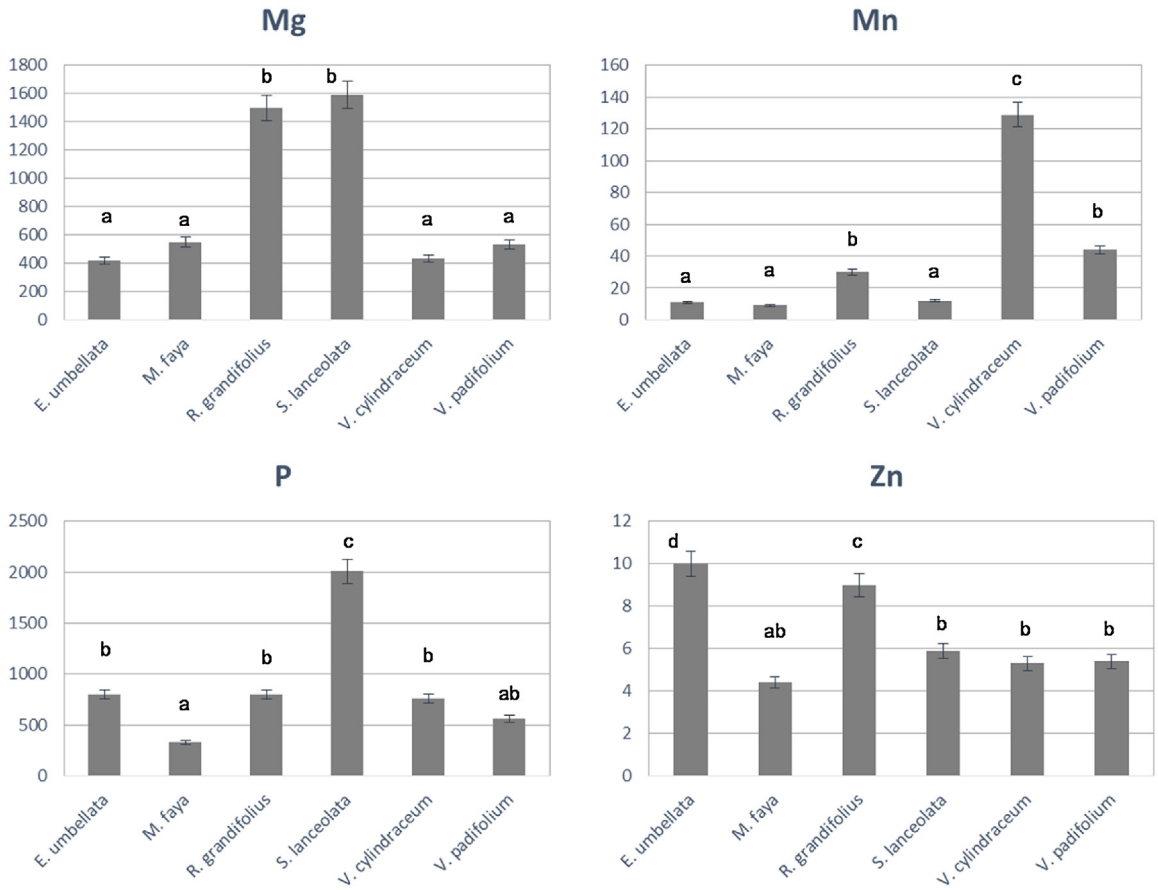

Fig. 2. Comparison of the average contents of Mg, Mn, P, and Zn in the analyzed berry species, expressed in $\mu g\,g^{-1}$ (fresh weight). Number of analyzed samples indicated in Table 2; 3 replicates for each sample. Columns not sharing the same letter are significantly different at $p < 0.05$ probability level; letter *a* corresponds to the lowest value, whereas letter *e* to the highest value; *ab* means that the values are within the range of *a* and *b*.

elements, except Cu (12%) and Mn (66%), which makes these berries less interesting in terms of their mineral content than the previous ones.

In general, most of the analyzed berries presented levels of minerals high enough to provide a significant contribution to the RDA of minerals, although it depends on the berries and the most abundant elements in each species. A comparison between the different berries can be observed in Figs. 1 & 2 for Ca, Cu, Fe, K, Mg, Mn, P, and Zn.

A daily consumption of 30 g of berries was assumed for each of the analyzed berries. Hence, the levels of nutrients determined in each species are proportional to their contribution to the RDA of minerals. The highest levels of Ca were observed in wild blackberries and Azorean blueberries ($p < 0.05$), while wild blackberries and candleberry-myrtles were rich in Fe (Fig. 1 & 2). Autumn olives presented the highest levels of Cu and Zn. Madeiran elderberries exhibited the highest content in K, and P. Wild blackberries and Madeiran elderberries were the best sources of Mg. Azorean blueberries showed the highest levels of Mn, much higher than in the other berries (Fig. 1 & 2). In general, all the analyzed berries presented balanced levels of minerals, so their incorporation in a varied diet should be encouraged.

3.4. Comparison with other berries

A comparison between the nutritional levels here observed and those reported for other similar berries has been performed, although it should be mentioned that data are presented here as $\mu g\,g^{-1}$ of fresh weight while other authors reported values as $\mu g\,g^{-1}$ of dry weight, so the comparison is not straightforward. Other authors' data from blueberries, cranberries, blackcurrants, strawberries, bilberries, lingonberries, maqui, and goji berries have been selected (Brauch et al., 2016; de Souza et al., 2014; Ekholm et al., 2007; Elisabetta et al., 2013; Hua et al., 2014; Llorent-Martínez et al., 2013; Nile and Park, 2014). The levels of all the minerals were within range of those reported for maqui (Brauch et al., 2016), goji berries (Llorent-Martínez et al., 2013), bilberries (Elisabetta et al., 2013), blueberries (Elisabetta et al., 2013; Hua et al., 2014) and strawberries (Hua et al., 2014), although higher than some reports in blueberries (de Souza et al., 2014; Nile and Park, 2014), strawberries and cherries (de Souza et al., 2014), cranberries and blackcurrants (Ekholm et al., 2007; Nile and Park, 2014).

4. Conclusions

The inorganic composition of six non-commercial wild berries has been determined by ICP-MS after microwave digestion. This is the first work reporting in detail the inorganic content of *M. faya*, *R. grandifolius*, *S. lanceolata*, *V. cylindraceum* and *V. padifolium* berries. The mineral composition was diverse among the different berries and, in general, the analyzed berries presented low concentrations of Co, Ni, Mo and Cu, but proved to be good sources of K, Ca, P, Na and Mg. Moreover, only very low contents of toxic elements were detected, ensuring the absence of risk for human health. The results obtained have been discussed using RDA data for minerals, observing that *R. grandifolius* and *S. lanceolata* were the samples that would provide the highest contribution to the diet in terms of

inorganic nutrients. The targeted berries are used by local population in a domestic perspective, probably due to the lack of knowledge about their potential health benefits, safety and their validation as commercial assets. Food markets provide several types of similar fruits, generally cultivated abroad, at very high prices. Hence, the consumption and marketing of the analyzed berries deserves promotion, representing an opportunity for growers and collectors to reach the appropriate niche market to increase their revenues.

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