Nutritional and Phytochemical Composition of Vaccinium padifolium Sm Wild Berries and Radical Scavenging Activity

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Abstract: Blueberries have a well-deserved reputation as a potential functional food, supported by studies which have identified and quantified various nutrients and bioactive phytochemicals with known benefits for human diet and health. Wild blueberries have attracted particular attention due to the levels and concentrations of those phytonutrients. This study aims to evaluate for the first time the chemical composition of Madeira Island’s endemic Vaccinium padifolium Sm wild berry. Results show that this fruit contains high values of total soluble phenolic content (around 4 g GAE kg\(^{-1}\) FW), as well as significant values of total monomeric anthocyanin content (around 3 g eq. cyanidin kg\(^{-1}\) FW) and DPPH scavenging activity (around 86.72%). Additionally, results reveal that this fruit has water content of about 88% as well as low sugar content (17.98 and 29.73 g kg\(^{-1}\) for glucose and fructose, respectively). Results also confirm that this wild blueberry is a good source of dietary fiber, fat and minerals. The high level of terpenoid compounds stands out in the aroma profile analysis.

Keywords: antioxidant capacity, nutritional composition, Vaccinium padifolium Sm, volatile profile, wild blueberry

Practical Application: This study is in line with the efforts of the scientific community to identify new sources of phytonutrients that are beneficial to human health, characterizing the wild Madeira blueberry in terms of phytonutrients that suggest there may be health benefits associated with its consumption. The findings of this research are very important for both the commercial and agricultural sectors that produce this fruit, and for consumers who seek phytonutrient-rich foods.

Introduction

The Vaccinium padifolium Sm, commonly known as Madeira whortleberry (MWB), or Uveira da Serra in Portuguese, is an endemic species of Madeira Island (Portugal). It is a deciduous shrub that produces a wild berry fruit (Figure 1) traditionally consumed in liqueur, jam, and used in infusions. Madeira Island has diverse microclimates as well as a moderate to high exposure to UV radiation, which can have a significant impact on agriculture, an important sustainable economic factor in the region. It is known that plants respond actively to stress by producing protective compounds, an example being key enzymes to secondary metabolites, mainly UV-absorbing phenolics, such as flavonoids (Dao and others 2011; Song and others 2015), which may in turn increase the content of some bioactive compounds in fruits. There is currently an increased interest in the consumption of fruits that contain naturally derived bioactive compounds, because of their positive impact on disease prevention and other health-related benefits. Fruits, such as berries, are rich in phenolic compounds, which can protect against cancers, cardiovascular diseases, diabetes, hypertension, asthma, and even infection, if consumed in abundance (Dasgupta and Klein 2014). Blueberries are recognized for their nutritional and beneficial health effects. They present a low glycemic index (<56) in general and also figure in the group of fruits with high amounts of anthocyanins and other polyphenols, which have been reported to have good antioxidant properties (Routray and Orsat 2011; Dasgupta and Klein 2014). For these reasons, it is important to study the MWB and compare it with other wild and cultivated blueberries around the world.

The available literature on physicochemical studies on MWB is mainly concerned with anthocyanin quantification and identification (Cabrita and Andersen 1999; Cabrita and others 2000). However, there are several studies on wild and cultivated blueberries around the world, considering proximate composition (Pallas and others 2013; Reque and others 2014; Souza and others 2014), phenolic composition and antioxidant activity (Pallas and others 2013; Bett-Garber and others 2015), volatile profile (Beaulieu and Stein-Chisholm 2014; Du and Rouseff 2014; Beaulieu and others 2016), organic acids (Mikulic-Petkovsek and others 2012, 2015; Bett-Garber and others 2015), anthocyanins (Routray and Orsat 2011; Bett-Garber and others 2015; Veberic and others 2015), nutraceutical value and properties promoting health (Norberto and others 2013; Manganaris and others 2014). Studies have also been done on the composition and quality of blueberries during the harvest season and at different locations (Mikulic-Petkovsek and others 2015; Zorenc and others 2016) and even when processed into different products (Syamaladevi and others 2012; Zorenc and others 2016).
The goal of this work was to study the *Vaccinium padifolium* Sm berry from Madeira Island (Portugal) in more detail, by determining the proximate composition (moisture, fat, protein, total mineral (ash), and total dietary fiber), sugars and organic acids, the content of total soluble solids (%Brix), total soluble phenolics and total monomeric anthocyanin, as well as its antioxidant capacity and aromatic profile.

Materials and Methods

Chemical reagents

The eluents for HPLC were acetonitrile HPLC grade, obtained from Fisher Scientific (Loughborough, UK) while methanol HPLC grade was from Panreac Química S.A. (Barcelona, Spain). Sulphuric acid (95–97%) was provided by Sigma-Aldrich (St. Louis Mo., U.S.A.). These were previously filtered with Pall membrane filters (0.20 μm). Ultra-pure water (18 MΩ) was obtained using a Milli Q-System (Millipore, Milford, Mass., U.S.A.).

For the total phenolic content assay, Folin-Ciocalteu’s reagent and sodium carbonate anhydrous were obtained from Panreac (Barcelona, Spain) while gallic acid was supplied by Sigma-Aldrich (St. Louis Mo., U.S.A.). For the DPPH assay, 2,2-Diphenyl-1-pikryl-hydrazyl was obtained using a Distillation and Titration Unit (model Velp Scientifica, Italy) air oven at 105 °C for 24 h for moisture content determination and the second portion was frozen at −85 °C to avoid deterioration until proximate analysis was conducted (content in fat, protein, total mineral (ash), and total dietary fiber). The third portion of fresh fruit was prepared for the remaining assays, that is, the sugars and organic acids, total soluble solids (%Brix), total monomeric anthocyanins (TAC), total soluble phenolics (TSP), and volatile profile. The extractions were made in triplicate and analyses in duplicate for all assays.

Proximate composition

The nutritional composition of the MWB was analyzed with regard to its content: moisture, fat, protein, total mineral (ash) and total dietary fiber. The content of total soluble solids (%Brix) was also determined.

Moisture content was determined through dehydration using a Heratherm OMS180 (Thermo Scientific, Germany) air oven at 105 °C for 24 h, method AOAC 925.10 (AOAC 2005).

Fat content was determined by an adaptation of the gravimetric method (Bligh and Dyer 1959), adding methanol/chloroform/water at 2:2:1 (V/V/V) to the sample, crafting the extract lysis using an ultrasound bath (Ultrasons–H, PSelecta) for 40 min and centrifuging (Centrifuge 5430R, Eppendorf, Germany) at 5000 rpm for 10 min. Then, the lower layer containing the chloroform with the restrained fat was separated from the methanol and water upper layer. The chloroform was then evaporated by a rotary evaporator (Hei-VAP Advantage, Heidolph, Germany) and the lipid residue was weighed.

Protein content was determined with quantification of total nitrogen by the Kjeldahl method AOAC 945.18-B (AOAC 2005), using a Distillation and Titration Unit (model Velp Scientifica UDK 152, Europe). Factor N × 6.25 was applied to convert total nitrogen to protein content.

Sample collection and storage

To ensure the representability of results, samples were collected from three different locations within the Ecological Park in the mountains north of the capital city of Funchal, Madeira Island (Portugal) and labeled as lots 1 (32°42′29.26″N 16°53′14.03″W), lot 2 (32°42′41.94″N 16°53′49.75″W) and lot 3 (32°42′21.36″N 16°53′0.09″W), situated 1353, 1409, and 1286 m above sea level, respectively. Lot 1 is 992 m from lot 2 and 545 m from lot 3, plus the ends (lot 2 and 3) are 1443 m apart from each other. About 300 g of *Vaccinium padifolium* Sm samples were randomly harvested, from each one of the above-mentioned locations, in the beginning of October 2015, when the fruit is firmly mature and usually consumed by the general population. All samples were collected manually in individual stainless-steel containers to avoid the fruit being damaged by squashing, and then they were transported in cooler boxes. On the same day, each sample was divided into three portions: one portion was immediately dehydrated at 105 °C for 24 h for moisture content determination and the second portion was frozen at −85 °C to avoid deterioration until proximate analysis was conducted (content in fat, protein, total mineral (ash), and total dietary fiber). The third portion of fresh fruit was prepared for the remaining assays, that is, the sugars and organic acids, total soluble solids (%Brix), total monomeric anthocyanins (TAC), total soluble phenolics (TSP), and volatile profile. The extractions were made in triplicate and analyses in duplicate for all assays.

Figure 1-Madeiran whortleberry’s shrubs (*Vaccinium padifolium* Sm) in blossom (left) and the wild berries at ripening stages (right).
Protein stands for the absorbance value measured at \( \lambda \text{ nm} \) in buffers at pH 1.0 – (A

\( \times \)

28000 \( \varepsilon \)) 19

Assessment of

\( \times \)

\( \lambda \text{ nm} \)).

Fat concentration was performed on quartz cuvettes with an optical thickness of 10 mm, using ultrapure water as blank. All measurements were carried out in triplicate. Concentration of TSP was calculated using a standard curve of aqueous solutions of gallic acid (50 to 750 mg/L) and expressed as grams gallic acid equivalents (GAE) per kilogram of fresh weight (FW).

Total monomeric anthocyanins (TAC). Assessment of TAC was carried out by the pH differential method according to AOAC as described by Lee and others (2005). Briefly, 1 g of fresh fruit was pressed into a fine paste in a mortar, homogenized with 30 mL of 95% ethanol/1.0 M HCl solution (85:15, v:v). The extract was transferred to a 50 mL volumetric flask, completing the volume with the ethanol–HCl solution and stored for 12 h at 4 °C. It was later centrifuged in an Eppendorf centrifuge, model 5702 (Hamburg, Germany), at 4000 rpm for 15 min at room temperature and then filtered. Absorbance was measured at 520 and 700 nm in buffers at pH 1.0 (potassium chloride, 0.025M) and 4.5 (sodium acetate, 0.4 M). Pigment concentration is expressed as grams cyanidin 3-glucoside (cy-3-glc) and malvidin 3-glucoside (mv-3-glc) equivalents per kilogram of fresh weight and calculated as follows:

\[
\text{TAC (mg/g)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}
\]

where A = (A320 nm – A700 nm) pH 1.0 – (A320 nm – A700 nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin 3-glucoside or 493.4 g/mol for malvidin 3-glucoside; DF = dilution factor; 1 = cuvette pathlength in cm; \( \varepsilon = 26900 \text{ L/mol.cm, molar extinction coefficient for cyanidin 3-glucoside and} \) \( \varepsilon = 28000 \text{ L/mol.cm, molar extinction coefficient for malvidin 3-glucoside.} \) \( 10^3 \) = factor to convert g to mg.

Antioxidant capacity by the DPPH assay. Sample extraction was made as described for TSP. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was conducted according to the Brand-Williams and others (1995) technique with some modifications. The stock solution (0.06 mM) was prepared by mixing 2.5 mg of DPPH radical with 100 mL of methanol. Solution absorbance was adjusted at 0.7 ± 0.03 in 515 nm using the dual beam spectrophotometer Shimadzu UV-Vis 2600 (Kyoto, Japan). 3.9 mL of DPPH radical was mixed with 100 µL of the sample extract or Trolox as a standard (methanol was used as blank). The decrease in absorbance at 515 nm was measured at 30-s intervals for 30 min, which was the time established for stabilization. Results were calculated using the following standard curve of methanol solutions of Trolox as a standard (1 to 20 mM) and expressed as mmol equivalent Trolox per kilogram of FW and percentage of scavenging activity was calculated as follow:

\[
I(\%) = \left( \frac{A_{515\text{min}}}{} - \frac{A_{515\text{min}}}{} \right) \times 100\%
\]

where \( A_{515\text{min}} \) and \( A_{515\text{min}} \) stands for the absorbance value measured at the beginning of the reaction and after 30 min of reaction of Trolox standards.
### Table 1—Chemical composition of the wild berry fruit from the endemic shrub *V. padifolium* Sm and comparison with literature.

| Blueberries | Soluble solids (°Brix) | Moisture (g kg⁻¹) | Ash (g kg⁻¹) | Protein (g kg⁻¹) | Total lipid (g kg⁻¹) | Fiber (g kg⁻¹) | Sugars (g kg⁻¹) | Organic acids (g kg⁻¹) |
|-------------|------------------------|-------------------|-------------|-----------------|---------------------|-------------|----------------|---------------------|
| **Lot 1**   | 8.17 ± 0.32ᵃ 890.12 ± 44.36ᵇ | 17.04 ± 1.71ᵇ 6.50 ± 0.06ᵇ | 23.76 ± 1.21ᵇ 15.60 ± 0.87ᵇ | 18.15 ± 0.19ᵇ 29.15 ± 0.14ᵇ | 3.56 ± 0.13ᵇ 2.58 ± 0.05ᵇ | 3.83 ± 0.16ᵇ 2.81 ± 0.09ᵇ | 0.34 ± 0.02ᵇ |
| **Lot 2**   | 8.23 ± 0.12ᵃ 884.61 ± 0.73ᵃ | 19.01 ± 1.67ᵃ 5.19 ± 0.04ᵃ | 51.91 ± 2.82ᵃ 21.61 ± 3.68ᵇ | 18.68 ± 0.64ᵃ 32.86 ± 0.78ᵃ | 3.83 ± 0.16ᵇ 2.81 ± 0.09ᵇ | 0.46 ± 0.01ᵇ |
| **Lot 3**   | 8.30 ± 0.10ᵃ 880.40 ± 3.76ᵃ | 14.11 ± 1.81ᵇ 6.89 ± 0.10ᶜ | 39.08 ± 3.03ᶜ 9.17 ± 1.58ᶜ | 17.13 ± 0.04ᵇ 27.20 ± 3.01ᵇ | 2.39 ± 0.10ᶜ 3.69 ± 0.08ᶜ 0.72 ± 0.12ᵇ |
| **Madeira Whortleberry’s fruit** | | | | | 3.60 ± 0.13ᵇ 2.58 ± 0.05ᵇ | |
| **Literature** | | | | | | | | |
| (Souza and others 2014) | 14.67 ± 0.58 | 877.00 | 0.80 | 4.80 | 1.9 | 19.00 | – | – | – |
| (Ehlenfeldt and others 1994) | – | – | – | – | – | – | 9.37 | 2.37 | 0.48 |
| (Mikulic-Petkovsek and others 2012) | – | – | – | – | – | – | 10.30 | – | 0.59 |
| (Wang and others 2008) | – | – | – | – | – | – | 45.53; 29.72 | 97.06; 79.26 | 3.47; 3.14 | 0.043; 0.03 |
| (Reque and others 2014) | 10.67 | 857.80 | 18.00 | 4.10 | 7.30 | – | – | – | – |
| (Ehlenfeldt and others 1994) | – | – | – | – | – | – | 9.37; 1.74 | 2.37; 8.78 | 0.48; 5.02 |
| (Mikulic-Petkovsek and others 2012) | – | – | – | – | – | – | 20.50 | 22.40 | 5.70 | – | 2.71 |
| (USDA - United States Department of Agriculture, Agricultural Research Service 2016) | – | 842.1 | 2.40 | 7.40 | 3.30 | 24.0 | 48.80 | 49.7 | – | – |

Values representing Madeira Whortleberry’s fruit are average of three individual samples (n = 3), expressed as mean ± standard deviation. Different letters (a, b, c) within columns denote statistically significant differences (P < 0.05) by Holm-Sidak test.
### Table 2—Energy value (per 100 g of edible portion in FW basis) and daily value intake contribution of the three populations of MWB (%).

| Nutritional facts | lot 1 | lot 2 | lot 3 |
|-------------------|------|------|------|
| Energy (kJ / kcal) | 158.20/37.92 | 243.95/58.93 | 217.10/52.23 |
| % Daily | 3.30 | 1.04 | 1.38 |
| Value* | Total Protein | 6.24 | 8.64 | 3.68 |
| Value* | Total Dietary Fiber | 6.24 | 8.64 | 3.68 |
| Value* | Total Fat | 3.40 | 7.41 | 5.59 |
| Value* | Total Minerals | ** | ** | ** |

*Percent Daily Values are based on an 8400 kJ/2000 kcal diet.
**Percent Daily Values not specified.

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### Aroma profile

The samples were prepared in triplicate by adding of 50 mL of ultra-pure water to 10 g of fresh fruit pressed into a fine paste in a mortar. The juice was homogenized and sonicated for 10 min, at room temperature. For SPME, 1 μL of 3-octanol (Internal Standard, 500 mg/L) was added to 10 mL of each sample, in a 20 mL vial containing 3 g of NaCl. The vial was then immediately capped and vortexed prior to automated SPME analysis. Extraction was performed by exposing the 50 μm/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxan SPME fiber (DVB/ CAR/ PDMS, bipolar, adsorbent) into the vial for 30 min at 60 °C, keeping the sample under continuous stirring.

In order to carry out the analysis of volatiles, the TRACE GC Ultra equipped with the TriPlus auto sampler in SPME mode and the mass spectrometer detector ISQ single quadrupole (electronic impact ionization mode) from Thermo Scientific (Hudson, N.H., U.S.A.) was used. Compounds were desorbed at 240 °C by inserting the fiber into the GC injector for 5 min. The column used was a Factor Four capillary column, VF-5 ms 60 m × 0.25 mm ID DE = 0.25 (Varian, USA) and the carrier gas was He at 1 mL/min. The transfer line and ion source temperatures were both kept at 240 °C. The oven temperature program started at 40 °C for 2 min then increased up to 240 °C at 3 °C/min and finally kept at 240 °C for 15 min. The mass range 30 to 300 m/z was recorded and the identification of compounds was done by comparison of the mass spectra obtained with those present in Wiley 6.0 and NIST08 library databases. Furthermore, Kovats indexes were also obtained and compared with the online database, PheroBase. To get an idea of each volatile concentration, the amount of each volatile compound was expressed in terms of Internal Standard, namely relative concentration.

### Statistical analysis

Significant differences were evaluated by the analysis of variance (one-way ANOVA with Holm-Sidak method) using the statistical software SigmaPlot 12.0.

### Results and Discussion

#### Proximate composition

In general terms, the MWB showed high water content (88.50% of its constitution), medium content of dietary fiber, fat and ash and low carbohydrate and protein content (Table 1), which is in accordance with the generally proximate composition of fruits (Rosa and others 2010). Energy content and daily value intake contribution are shown in Table 2.

The MWB presented low carbohydrate content (calculation based on Food and Agriculture Organisation of the United Nations (2003)), around 79.70 g kg⁻¹ FW, which represents 3.07% of the daily value. The energy content ranged from 37.92 kcal (158.20 kJ) to 58.93 kcal (243.95 kJ) per 100 g of fresh weight portion (FW). Daily carbohydrate average intake in adults is 260 g/person (Council Regulation (EU) 1169/2011 2011). According to the Inst. of Medicine (US) Panel on Macronutrients, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2005), our results suggest that this berry may be a suitable fruit for diets that require a low glycemic index, such as those used to control diabetes. In addition, the °Brix average value around 8.23% also indicates low soluble solid content available as sugars in the fruit juice, when compared with the literature (10.67% and 14.70%) in Table 1. The MWB has a water content of 88.50%, which is in accordance with other blueberry species found in the literature (Table 1). According to Navarra (2004) the high water content of these fruits can be a good regulator and stabilizer of body temperature and a good base solution for electrolytes (sodium and potassium).

Protein content for MWB (around 6.20 g kg⁻¹ FW) is within the values shown in the literature (Table 1). The average daily protein intake in adults is 50 g/person (Council Regulation (EU) 1169/2011 2011). About 100 g of fresh fruit from lots 2 and 3 can supply 1.04% up to 1.38% of daily protein intake, respectively. Fiber content of MWB ranged from 9.17 to 21.61 g kg⁻¹ FW (Table 1). According to the 25 g/person recommended daily intake of dietary fiber (Navarra 2004), MWB can contribute from 3.68% to 6.64% of daily fiber needs. These values are within the values stated in literature (Table 1). Fat content in MWB ranged from 23.76 up to 51.91 g kg⁻¹ FW (Table 1). Considering that the average daily fat intake in adults is 70 g/person (Council Regulation (EU) 1169/2011 2011), MWB can contribute about 5.47% to daily fat energy needs (Table 2). This value is significantly higher than those observed in literature on cultivated berries, which have values ranging from 1.90 to 7.30 g kg⁻¹ FW (Table 1). For mineral content, MWB revealed values between 14.11 and 19.01 g kg⁻¹ FW, which are in the highest range according to the literature (Table 1). The daily percentage values of mineral intake are not specified.

#### Organic acids and sugars

Concerning sugars, glucose and fructose were quantified as they are the sugars most frequently found in berries. The mean values in MWB are approximately 17.99 and 29.73 g kg⁻¹, respectively, a lower content than usually observed for cultivated blueberries (Table 1). Regarding the organic acids studied, citric, succinic and malic were the main organic acids present in MWB, with around 3.26, 3.03, and 0.51 g kg⁻¹, respectively, which are comparable with the values found in the literature (Table 1), citric acid being the most abundant in blueberries. Sugars and organic acids are the main soluble constituents of berries and have a great impact on the organoleptic properties of the fruit, as well as contributing to its nutritive value as a source of micronutrients and phytochemicals (Mikulic-Petkovsek and others 2012).

#### TSP and TAC

The values obtained for TSP (Table 3), around 4.00 g kg⁻¹ FW, when compared to literature reveals a medium-high content in the MWB, while those obtained for TAC, around 3.00 g eq. cyanidin kg⁻¹ FW, suggest a high content in these bioactive compounds. Results for TAC (Table 3) were expressed through cy-3-glc and mv-3-glc since these are the most widely reviewed in the literature and also because mv-3-glc was found to be the most abundant monomeric anthocyanin (relative amount of 25%) in MWB according to Cabrita and Andersen (1999). In general,
it is shown that wild blueberries present a higher content of these bioactive compounds than cultivated blueberries.

When compared to other berry fruits the total phenolic content of MWB is in accordance with values found in literature, whereas regarding TAC presents considerably higher values (Table 4).

Antioxidant activity

The values obtained for the DPPH assay (around 116.56 mmol eq Trolox kg$^{-1}$ FW or about 86.72% scavenging activity) reveal a very high antioxidant activity for MWB when compared to other results found in the literature (Table 3). These results are in agreement with high values of total monomeric anthocyanin content, since anthocyanins are the major contributors to the antioxidant capacity of blueberries, responsible for about 84% of TAC (Skrovankova and others 2015).

Aroma profile

A total of 72 volatiles were identified in the MWB samples. In order to simplify data analysis, these compounds were grouped into the following chemical families: alcohols (15), esters (7), aldehydes (8), fatty acids (4), and terpenoids (38). The predominance of terpenoids in the samples studied is in accordance with most studies (8), fatty acids (4), and terpenoids (38). The predominance of terpenoids in the samples studied is in accordance with most studies (Du and Rouseff 2014). The main difference found lies in the ultraviolet radiation exposure, typical on Madeira Island, which can cause the conversion of some C$_6$C$_9$ aldehydes into alcohols, as previously mentioned by Eichholz and others (2011).

A comparison of the three different MWB populations show that the main differences are found only in terms of concentration, since the predominant chemical compounds, as well as those that appear in small concentrations, are generally the same in the three lots. Together with terpenoids, the higher alcohols

### Table 3—Total soluble phenols, total monomeric anthocyanins and antioxidant activity through DPPH radical scavenging of the wild Madeira berry and comparison with literature.

| Blueberries | TSP (g GAE kg$^{-1}$ FW) | TAC (g eq cyanidin kg$^{-1}$ FW) | TAC (g eq malvidin kg$^{-1}$ FW) | DPPH (mmol eq Trolox kg$^{-1}$ FW) | DPPH (% scavenging activity) |
|-------------|--------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------------------|
| Lot 1       | 4.26 ± 0.17$^a$          | 2.76 ± 0.08$^a$                | 2.92 ± 0.09$^a$                | 122.08 ± 6.51$^a$               | 88.82 ± 2.33$^a$            |
| Lot 2       | 4.21 ± 0.18$^a$          | 2.95 ± 0.13$^{ab}$             | 3.11 ± 0.14$^{ab}$             | 117.76 ± 2.56$^{ab}$            | 87.32 ± 1.01$^{ab}$         |
| Lot 3       | 3.45 ± 0.12$^b$          | 3.34 ± 0.32$^b$                | 3.53 ± 0.34$^b$                | 109.84 ± 2.44$^b$               | 84.01 ± 1.10$^b$            |

| Literature | Cultivated highbush blueberries (Vaccinium corymbosum) |
|------------|-------------------------------------------------------|
| (Giovannelli and Buratti 2009) | 2.51 to 3.10 | 0.92 to 1.29 | – | – | – |
| (Koca and Karadeniz 2009) | 0.77 to 8.20 | – | 0.18 to 0.29 | – | – |
| (Búzna and others 2011) | 4.25 to 6.52 | 1.01 to 1.63 | – | – | 30.00 to 47.00 |
| (Souza and others 2014) | 3.05 | 0.30 | – | – | – |
| (Rodrigues and others 2011) | 2.74 | 4.62 | – | 12.44 | – |
| (Dragović-Uzelac and others 2010) | 2.64 to 5.28 | 1.00 to 2.50 | – | 56.30 to 76.00 | – |
| Rodrigues and others (2011) | 4.37 | 2.20 | – | 16.40 | – |

| Literature | Wild blueberries (Vaccinium myrtillus) |
|------------|---------------------------------------|
| (Giovannelli and Buratti 2009) | 5.77; 6.14 | 3.30; 3.44 | – | – | – |
| (Koca and Karadeniz 2009) | 3.08 to 5.42 | – | 0.59 to 294 | – | – |
| (Búzna and others 2011) | 8.19; 6.73 | 3.06; 2.52 | – | – | 59.79; 49.93 |
| (Liu and others 2011) | 6.03 | 1.77 | – | – | – |

Values representing Madeira Whortleberry’s fruit are average of three individual samples ($n = 3$), expressed as mean ± standard deviation. Different letters ($a$ and $b$) within columns denote statistically significant differences ($P < 0.05$) by Holm-Sidak test.

### Table 4—Comparison between total soluble phenols and total monomeric anthocyanins values of the wild Madeira berry and values found for other berry fruits in literature.

| Blueberries | TSP (g GAE kg$^{-1}$ FW) | TAC (g eq cyanidin kg$^{-1}$ FW) |
|-------------|--------------------------|---------------------------------|
| Lot 1       | 4.26 ± 0.17$^a$          | 2.76 ± 0.08$^a$                |
| Lot 2       | 4.21 ± 0.18$^a$          | 2.95 ± 0.13$^{ab}$             |
| Lot 3       | 3.45 ± 0.12$^b$          | 3.34 ± 0.32$^b$                |

| Literature | Madeira Whortleberry’s fruit |
|------------|------------------------------|
| (Fredes and others 2014) | 6 | 1 |
| (Souza and others 2014) | 8.5 | 0.59 |
| (Ballistreri and others 2013) | 0.84 to 1.62 | 0.06 to 0.94 |
| (Souza and others 2014) | 3.14 | 0.27 |
| (Hayaloglu and Demir 2015) | 0.58 to 1.15 | – |
| (Cao and others 2015) | – | 0.059-0.98 |
| (Silva and others 2007) | – | 0.20 to 0.60 |
| (Pinto and others 2008) | 2.05-3.18 | – |
| (Crecente–Campos and others 2012) | 2.74 | – |
| (Souza and others 2014) | 6.22 | 0.16 |
| (Fredes and others 2014) | 7.00 | 0.30 |

Values representing Madeira Whortleberry’s fruit are average of three individual samples ($n = 3$), expressed as mean ± standard deviation. Different letters ($a$ and $b$) within columns denote statistically significant differences ($P < 0.05$) by Holm-Sidak test.
Figure 2–An estimate of the concentration of each volatile family identified in the three lots of MWB.

are the majority compounds, namely cis-3-hexen-1-ol, 2-hexen-1-ol, 2-ethylhexanol, (E)-2-hexenol, linalool, L-α-terpineol, α-citronellol, and geraniol. Nonanoic acid and (E)-2-hexanal was also seen to be abundant in MWB cultivars. In the supplemental material (Suppl. 1), the description of the aroma descriptors of all volatile compounds identified is also presented, the fresh, green, fruity, fatty, and floral being the most predominant. Individual concentration estimates suggest that there are some compounds that can be presented above their odor threshold, such as three aldehydes, namely the cis-3-hexenal, hexanal, nonanal, (Du and others 2011; Beaulieu and Stein-Chisholm 2014) and a norisoprenoid, the β-damascenone (Leffingwell and Associates 2016). Accordingly, these compounds can have a great impact on the MWB aroma, which is in accordance with other studies (Du and Roussef 2014) that highlight the importance of these compounds in the typical blueberry aroma. Additionally, considering that all the quantified alcohols are below their odor threshold, these compounds may only contribute to the fruit aroma by interacting with other volatiles and with the fruit matrix, as concluded by other authors (Du and others 2011). A similar contribution is expected for ester compounds.

Considering terpenoids, although β-damascenone is the only compound above its odor threshold, the diversity of compounds and differences in terms of individual amounts may contribute to the characteristic odor of each lot of MWB, as mentioned above.

In terms of potential health-related benefits, terpenoids are known to have great bioactive and medicinal proprieties (Eichholz and others 2011), a fact that reinforces the advantages of consuming fruits rich in these compounds. In the MWB samples, D-limonene is one of the compounds appearing in higher concentrations, ranging from 0.669 to 1.988 μg/100 g FW in lots 1 and 3, respectively. Other major terpenoids found in the studied fruits was geraniol, presenting concentrations ranging from 3.510 to 9.651 μg/100 g FW, in lots 2 and 3. To complete the previous discussion, analysis data on the volatiles was also submitted to a multivariate data analysis to clarify whether there are differences among the three lots that are not evident according to Figure 2. Figure 3 presents the scores plot of the first two principal components of the PCA model computed, showing in this case that there are some differences in the volatile profile of the three lots, which weight more than the sample variability. The differences found in terms of volatile profile of this variety, produced at different locations of a given region, are in accordance with other studies (Beaulieu and Stein-Chisholm 2014). Thus, slight differences in terms of aroma profile are expected for MWB grown at different locations subject to different microclimates, which is very characteristic of Madeira Island. In this context, ultraviolet exposition, the particular soil composition and water supply may explain these differences, since the three lots of MWB studied belongs to the same variety, the same ripeness stage and same post-harvest treatment until analysis.

Conclusions

This is the first study on the nutritional and physicochemical profile of the wild Madeira blueberry, for which there has been increased recorded interest and consumption in recent years. Our study demonstrates the presence of 88.50% water in the composition of the fruit as well as a high level of some bioactive compounds: total soluble phenolics (about 4 g kg−1 FW) and total monomeric anthocyanins (around 3 g eq. cyanidin kg−1 FW). The scavenging activity assessed with DPPH radicals was around 86.72%. Additionally, MWB showed a low sugar content (17.98 and 29.73 g kg−1 for glucose and fructose, respectively), a good source of dietary fiber, fat, and minerals (ash). These values showed that this fruit can provide nutritive properties along with good hydration. Regarding the aroma compounds, terpenoids and higher alcohols were the most plentiful in this wild berry.

Notwithstanding the preliminary nature of this study, the results demonstrate the presence of phytonutrients and bioactive compounds in the composition of the wild Madeira blueberry which are usually associated with the health benefits of their consumption.

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Authors’ Contributions

Maria J. Carvalho designed the study and along with Ana C. Vieira applied the sample treatments and the chromatographic and spectroscopic analyses. Carla S. Gouveia studied the
nutritional composition of the fruit. The results were used under the supervision of Dr. Ana C. Pereira, who also applied the chemometric study. All the above-mentioned authors drafted the manuscript. This work was supervised by Prof Dr. Miguel Á. Carvalho and Prof Dr. José C. Marques.

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