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# Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data

Brígida-María Ruiz-Rodríguez <sup>a</sup>, Patricia Morales <sup>b</sup>, Virginia Fernández-Ruiz <sup>a</sup>, María-Cortes Sánchez-Mata <sup>a,\*</sup>, Montaña Cámara <sup>a</sup>, Carmen Díez-Marqués <sup>a</sup>, Manuel Pardo-de-Santayana <sup>c</sup>, María Molina <sup>d</sup>, Javier Tardío <sup>d</sup>

<sup>a</sup> Dpto, Nutrición y Bromatología II, Facultad de Farmacia, Universidad Complutense de Madrid (UCM), Pza Ramón y Cajal, s/n, E-28040 Madrid, Spain

<sup>b</sup> Dpto, de Ciencias de la Salud, Universidad Internacional Valenciana (VIU), **C**/José Pradas Gallén, s/n 2<sup>a</sup> planta, 12000 Castellón de la Plana, Spain

<sup>c</sup> Dpto, Biología (Botánica), Facultad de Ciencias, Universidad Autónoma de Madrid (UAM), C/Darwin, 2 E-28049 Madrid, Spain

<sup>d</sup> Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca "El Encín", Apdo. 127. E-28800 Alcalá de Henares, Spain

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# ABSTRACT

*Arbutus* wild berries have been traditionally collected and appreciated in the Mediterranean region, although it is actually considered an underutilized fruit-tree species. Increasing the scarce knowledge about its nutritional composition and natural production may interest a broad range of scholars, such as ethnobotanists, chemists, nutritionists and anthropologists. The present study aims to provide original data on the nutritional value and the biomass production of wild strawberry-tree fruits, studying the variation of these characteristics in fruits harvested in different years, from two different Spanish areas.

Macro and micronutrient composition of mature *Arbutus unedo* fruits have been analyzed, with particular attention to the content of some bioactive compounds (fiber, vitamin C as ascorbic and dehydroascorbic acids, total phenolics, carotenoids, including lycopene) and the organic acids profile. The contribution to recommended dietary allowances (RDAs) of this exotic fruit has also been calculated. Fruit crop volume per tree has been estimated as well in the wild strawberry-tree populations surveyed to provide a general framework for discussing the agronomic potential of the species.

A wide variability in the nutrient composition of strawberry-tree fruits was found which shows that the analysis of many different samples from different origins and seasons are required to provide average reliable data about the chemical composition of wild fruits. From the results obtained, strawberry-tree fruits can be considered a very good source of health promoting compounds as vitamin C and dietary fiber (202.6 mg/100 g and 42.6% minimum contribution to RDAs, respectively). They are also rich in total available carbohydrates, sugars, potassium and secondary metabolites, such as phenolic compounds, being poor in lipids and Na. These results, together with its high production may help to reinforce its consumption, as an alternative to the fruits available in the market or a source of bioactive compounds for dietary supplements or functional foods.

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# 1. Introduction

The consumption of locally grown, wild or semi-wild edible plants has been important for most human cultures (e.g., Sharif Ali et al., 2008), and especially in the Mediterranean region, making an important contribution to the health of local communities (Heinrich, Nebel, Leonti, Rivera, & Obón, 2006; Tumino, Frasca, Giurdanell,

 \* Corresponding author. Tel.: + 34 91 3941799; fax: + 34 91 3941798. *E-mail addresses*: brigidamariaruiz@estumail.ucm.es (B.-M. Ruiz-Rodríguez), patricia.morales@campusviu.es (P. Morales), vfernand@farm.ucm.es (V. Fernández-Ruiz), cortesm@farm.ucm.es (M.-C. Sánchez-Mata), mcamara@farm.ucm.es (M. Cámara), cardimar@farm.ucm.es (C. Díez-Marqués),

manuel.pardo@uam.es (M. Pardo-de-Santayana), m.molina.simon@madrid.org (M. Molina), javier.tardio@madrid.org (J. Tardío). Lauria, & Krogh, 2002). They often contain higher amount of nutrients and bioactive compounds than many cultivated species, especially those which have been under cultivation for many generations (Trichopoulou et al., 2000). However, while many *in vivo* studies have evaluated the beneficial effect of the "Mediterranean diet", the nutritional impact of such local foods has been largely overlooked.

The strawberry-tree (*Arbutus unedo* L., Ericaceae) is a circum-Mediterranean species that also lives in Ireland and Macaronesian islands (Villar, 1993). It has been used as food and medicine since Greek times (Font Quer, 1961, Teofrasto, 1988). These applications and its ornamental use have recently attracted scientific interest (e.g., Celikel, Demirsoy, & Demirsoy, 2008).

The leaves and fruits of this species have been medicinally used in the Mediterranean for its antiseptic, diuretic and laxative effects and also to treat cardiovascular pathologies such as arterial hypertension,

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atherosclerosis and thrombosis (González-Tejero, 1990; Ziyyat et al., 2002). Its fruits have also shown antimicrobial activity (Kivçak, Mert, & Denizci, 2001). Bioactive compounds such as polyphenols, aromatic acids, iridoids, monoterpenoids, phenylpropanoids, sterols, triterpenoids and flavonoids present in its leaves and bark, may explain its pharmacological activity (Pallauf, Rivas-Gonzalo, Castillo, Cano, & Pascual-Teresa, 2008; Ziyyat et al., 2002). The biological activity of *A. unedo* leaves has been widely studied, while that of its fruits has been less surveyed.

Its spherical (about 2 cm in diameter) fleshy edible fruits have been traditionally eaten in Spain and other Mediterranean countries (e.g. Dogan, Baslar, Ay, & Mert, 2004; Pardo-de-Santayana et al., 2007; Redzic, 2006). The ripe fresh fruits get a tasty flavor if taken in an advanced stage of maturity. The combination of their sugar and organic acid content is responsible for their food sensory characteristics. Due to their high fermentable sugar content they have been traditionally used to obtain alcoholic beverages. Their pectin content makes them appropriate for the production of gels, as jellies, jams and compotes. These three main ways of consumption have been widely reported in different Spanish regions (Tardío, Pardo-de-Santayana, & Morales, 2006).

However, the edible use of the strawberry-tree fruits is currently not widespread. This may be due not only to its restricted growing area and the lack of commercial plantations, but also because of the fruits only reach a really pleasant flavor if consumed overripe. The reasons for the deficiency in commercial plantations could have been influenced by its heterogeneity, the lack of selection and varietal identification in order to obtain higher production rates or better fruit quality (Celikel et al., 2008). Breeding programs to obtain A. unedo cultivars with high fruit quality have rarely been attempted, with the exception of some trials made in China (Cai-Huang, 1997). Likewise, yield parameters have been scarcely assessed (e.g., Herrera, 1998; Ogaya & Peñuelas, 2007) despite the great interest involved both for agronomic characterization and sustainable harvesting. Some other studies have been made on the ecological, morphological and pomological characteristics of A. unedo genotypes in Spain, Italy and Turkey (Celikel et al., 2008; Chiarucci, Pacini, & Loppi, 1993; Herrera, 1987; Mulas, Cani, Brigaglia, & Deidda, 1998; Mulas et al., 2004).

On the other hand, food industries are demanding new food ingredients for developing commercial foods. Exotic or unusual foods, such as the strawberry-tree fruits, may have great potential as a source of unusual colors and flavors, as well as bioactive compounds. They could also be considered as interesting high-value nutraceuticals, being a source of bioactive compounds for dietary supplements or functional foods. In fact, the interest in the health benefits of incorporating strawberry-tree fruits or their fruit extracts into yogurts, pie and pastry fillings, cereal or meat products has been recently described (Alarcão-E-Silva, Leitão, Azinheira, & Leitão, 2001; Ganhao et al., 2010).

Nevertheless, there are only a few works on the chemical composition of strawberry-tree fruits from Turkey, Algeria, Croatia, Portugal and Italy (Alarcão-E-Silva et al., 2001; Ayaz et al., 2000; Barros et al., 2010; Celikel et al., 2008; Özcan & Haciseferoğullari, 2007; Pawlowska et al., 2006). Only García-Alonso, de Pascual-Teresa, Santos-Buelga, and Rivas-Gonzalo (2004) and Pallauf et al. (2008) studied strawberry-tree fruits of Spanish origin. Some of these works have a limited number of samples or compounds determined, and many of them are focused on antioxidants more than on nutritional aspects. The lack of studies including a complete nutritional characterization of *A. unedo* fruits has been also indicated by Barros et al. (2010).

The influence of geographical and seasonal variation in nutritional parameters of fruits has already been studied in different fruits (Fang et al., 2008; San José, 2010). However, no data have been found about the variability of the macro and micronutrients composition of strawberry-tree fruits. This may be especially important in this

species, since it has a long reproductive period. The fruits are harvested in late autumn but flower buds formation and flowering takes place respectively in spring and autumn of the preceding year. In addition, plant species are drought-sensitive (Peñuelas, Sardans, Ogaya, & Estiarte, 2008), thus climatic differences may affect production rates and nutritional composition.

Therefore, to contribute to increase the knowledge about the nutritional and production potential of strawberry-tree fruits, as an alternative to other fruits available in the market or as a new ingredient for the food industry, this study aims: a) to characterize their nutritional value, b) to estimate the fruit biomass production, and c) to study the variations of these characteristics in fruits harvested from different areas and seasons.

# 2. Materials and methods

#### 2.1. Plant material

Strawberry-tree (*A. unedo*) berries were gathered in their optimal ripening status, which takes place in November–December, in three different seasons (2007, 2008 and 2009) and from two localities with different environmental conditions, San Martin de Valdeiglesias (Madrid, center of Spain) and Salorino (Cáceres, west of Spain). Each sample had at least 500 g of fruits that were gathered from different trees randomly selected in both natural forests. They were packed in plastic bags and carried to the laboratories in a cold system within the day. All the selected berries presented a healthy external appearance.

#### 2.2. Analytical methods

Fresh fruits were homogenized in a laboratory blender. Aliquots were taken to analyze dry matter, pH, titratable acidity, organic acids and vitamin C. Other determinations were performed in freezedried samples. Triplicate subsamples were taken for each analytical procedure. Dry matter (DM) was determined by desiccation to constant weight at  $100 \pm 2$  °C following AOAC procedures (Horwitz & Latimer, 2005); pH was measured by potentiometer (MicropH-2000, Crison Instrument) over an homogenized sample 1/10 (w/v) in distilled water (Horwitz & Latimer, 2005); titratable acidity (TA) was determined by titration with 0.1 N NaOH until pH of 8.1 was reached.

#### 2.2.1. Total available carbohydrate (TAC) determination

The analysis of TAC was carried out by a colorimetric method using anthrone reagent, as described by Osborne and Voogt (1986) using 0.5 g of freeze-dried sample. Samples were pre-treated with 15 mL of 52% (v/v) HClO<sub>4</sub> and 10 mL of distilled water and kept for 18 h in the dark. After this period, samples were filtered and the volume of the filtrate was adjusted to 250 mL. Finally, the solution was further diluted to 8% (v/v), and 5 mL of 0.1% (w/v) anthrone solution in 70% (v/v) H<sub>2</sub>SO<sub>4</sub> was added to 1 mL of extract. Samples were kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green color, and absorbance was measured at 630 nm on a UV/Vis Spectrometer EZ210 (Perkin Elmer, Waltham, MA, USA) equipped with Lambda software PESSW ver. 1.2. The absorbance of the sample solution was compared to a 10–100 mg/mL concentration range standard glucose calibration curve.

#### 2.2.2. Soluble sugars

Soluble sugars were determined by High Performance Liquid Chromatography (HPLC) according to Sánchez-Mata, Peñuela-Teruel, Cámara-Hurtado, Díez-Marqués and Torija-Isasa (1998). Triplicate subsamples of 0.5 g of freeze-dried berries were extracted with 80% (v/v) ethanol in a water 55–60 °C for 45 min with constant stirring. The ethanol was evaporated by using a rotary vacuum evaporator (Büchi R-114) set to 40 °C and the concentrate made up to 25 mL with distilled water. Then, the samples were passed through a previously washed (5 mL of methanol followed by 5 mL of water) Sep-Pak C18 cartridge (Waters, Milford, MA, USA). Two milliliters of filtrate was mixed with 8 mL of acetonitrile and the mixture was filtered through a 0.45 (µm Millipore PVDF membrane (Millipore, Bedford, MA, USA)) before injection (100 µL aliquots) into the HPLC, equipped with PU II isocratic pumping system (Micron Analitica, SA, Spain), a Rheodyne valve, and a differential refractometer R401 detector (Jasco, Madrid, Spain). The chromatographic column used was a Luna 5 µ NH<sub>2</sub> 100 R (250 mm × 4.60 mm) (Phenomenex, Torrance, CA, USA). The mobile phase was acetonitrile:water (80:20), at a flow rate of 0.9 mL/min.

All chromatograms (Fig. 1) were processed using Cromanec XP software (Micronec, Spain). The resultant peak areas in the chromatograms were plotted against calibration curves obtained from multiple standards solutions (external standard method), in a concentration range of 0.1 to 1 mg/mL for each compound.

### 2.2.3. Soluble and insoluble dietary fiber assay

AOAC enzymatic–gravimetric methods (993.19 and 991.42) were used for soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) analysis (Horwitz & Latimer, 2005). In brief, freeze-dried samples were treated with alpha-amylase, protease and amyloglucosidase. The soluble and insoluble fractions were separated by vacuum filtration. Waste from the digests was dried at 100 °C, and ash and protein contents were determined in the residue. Total fiber is the sum of soluble and insoluble fiber.

#### 2.2.4. Total proteins

Total proteins were determined as nitrogen content by the Kjeldahl method. An amount of 0.7 g of freeze-dried sample was digested in sulfuric acid,  $NH_3$  was distilled over N/10  $H_2SO_4$  and the excess of sulfuric acid was titrated against N/10 NaOH. Total nitrogen content was converted to protein content by using the conversion factor 6.25 (Horwitz & Latimer, 2005).

### 2.2.5. Lipids

A Soxtec Sistem HT 1043 Extraction Unit Tecator was used. The crude fat was determined by extracting 0.5 g of freeze-dried sample with petroleum ether. Containers were removed and dried at 105 °C, cooled and weighted.

# 2.2.6. Ash content and mineral composition

The method 930.05 of AOAC procedures was used (Horwitz & Latimer, 2005). A sample of 500 mg was incinerated with high pressure in a microwave oven (Muffle Furnace mls1200) for 24 h at 550 °C, and ashes were gravimetrically quantified. The residue of incineration was extracted with HCl (50% v/v) and HNO<sub>3</sub> (50% v/v)

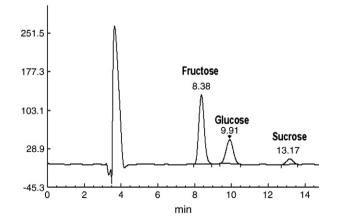
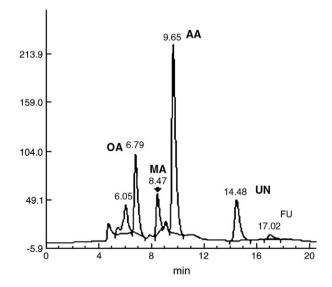


Fig. 1. HPLC profile of soluble sugars in strawberry-tree fruits harvested in 2007, location 1. Chromatographic conditions: Luna  $NH_2$  100 R (250 mm × 4.60 mm, 5 µm) column; mobile phase: acetonitrile/water (80/20); detection: refraction index; flow rate = 0.9 mL/min.

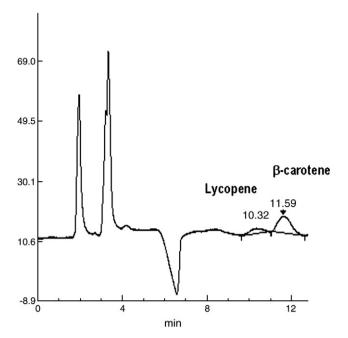
and made up to an appropriate volume with distilled water, where Fe, Cu, Mn and Zn were directly measured at the suitable wavelength for each element, using standard solutions for calibration purposes. An additional 1/10 (v/v) dilution was performed in LaCl<sub>2</sub> (1.8%, w/v) for Ca and Mg determination, and CsCl<sub>2</sub> (0.2%, w/v) for Na and K analysis. All measurements were performed in atomic absorption spectroscopy (AAS) in the Analyst 200 Perkin Elmer equipment.

# 2.2.7. Vitamin C and organic acids content

Content of individual organic acids (oxalic, malic, and ascorbic) were quantified by High Performance Liquid Chromatography (HPLC), based on the methods proposed by Vazquez-Odériz, Vázquez Blanco, López Hernández, Simal Lozano, and Romero-Rodríguez (1994) and Arella, Deborde, Lahély, Bourgignon, and Hasselmann (1996). An amount of 5 g of homogenized fresh fruits were extracted in 25 mL 4.5% (w/v) *m*-phosphoric acid, with magnetic shaking (P-Selecta, Asincro) during 15 min in darkness. Extracts were filtered through Albet no. 1242 paper; a portion was filtered through a 0.45 µm PVDF membrane, for injection into HPLC (AA and organic acids determination), while other aliquot of 3 mL of filtrate was added of 2.5 mL of 4% (w/v) L-Cistein, adjusted to pH 7 with 20% (w/v) K<sub>2</sub>HPO<sub>4</sub>, and let stand by for 5 min to allow the reduction of dehydroascorbic acid to ascorbic acid. After that they were adjusted again to pH 3 with 20% (w/v) metaphosphoric acid and completed with distilled water to a final volume of 10 mL, prior to filtration through a 0.45 m PVDF membrane and injection in the chromatographic system. The instrumental equipment was a liquid chromatographer (Micron Analítica, Madrid, Spain) equipped with an isocratic pump (model PU II), an AS-1555 automatic injector (Jasco, Japan), a Sphereclone ODS(2) (250×4.60 mm, 5 µm) Phenomenex column, a UV-visible detector (Thermo Separation Specta Series UV100); and using Cromanec XP software (Micronec, Spain). The mobile phase was 1.8 mM  $H_2SO_4$  (pH = 2.6). For AA analysis a flow rate of 0.9 mL/min and UV detection at 245 nm was used, while conditions for organic acids were 0.4 mL/min and at 215 nm. The identification of each compound was made by comparison of the retention times of each chromatographic peak (see Fig. 2) with those of standards products prepared from oxalic, malic, ascorbic and fumaric acids diluted in 4.5% (w/v) *m*-phosphoric acid. Quantification was performed by construction of calibration lines for each compound, after verification of recovery rates of the analytical method.



**Fig. 2.** HPLC profile of organic acids, including ascorbic acid in *Arbutus unedo* fruits harvested in 2009, location 1. Chromatographic conditions: Sphereclone ODS(2), (250×4.60 mm, 5 µm) column; mobile phase 1.8 mM H2SO4 (pH=2.6);  $\lambda_{detection}$  = 215 nm; flow rate = 0.4 mL/min: OA: Oxalic Acid; MA: Malic Acid; AA: Ascorbic Acid; FU: Fumaric acid; UN: Uhknown.



**Fig. 3.** HPLC profile of carotenoids in *Arbutus unedo* fruits harvested in 2008, location 1. Chromatographic conditions: µBondapack C18 (300 mm×2 mm, 10 µm) column mobile phase methanol/ACN 90:10 (v/v) + TEA 9 µM; column temperature 30 °C;  $\lambda_{detection} = 475$  nm; flow rate 0.9 mL/min.

# 2.2.8. Carotenoids content

These compounds were analyzed following the method of Olives-Barba, Cámara-Hurtado, Sánchez-Mata, Fernández-Ruiz, and López-Saenz-de-Tejada (2006). An amount of 0.3 g of freeze-dried samples were mixed with 50 mL of extraction solvent consisting on hexane/ acetone/ethanol (50:25:25 v/v/v), magnetic stirred during 30 min and 5 mL of water were added. After waiting 30 min for separation, the upper layer was separated and an aliquot of 10 mL of the extract was evaporated to dryness in a Büchi Labortechnik AG (Flawil, Switzerland) rotatory evaporator; the residue was dissolved to a final volume of 5 mL of a mixture of THF/ACN/methanol (15:30:55 v/v/v). The final solution was filtered through 0.45 µm membrane filters and 100 µL were injected for HPLC analysis in a Micron Analitica, S.A. chromatographer (Madrid, Spain), equipped with a PU II isocratic pumping system; a Jasco (Tokyo, Japan) AS-1555 autosampler; a ERC-Gecko-2000 (Riemerling, Germany) column heater; a Thermo Separation (San José, CA, USA) Spectra Series UV100 UV-Vis detector and Cromanec XP software (Micronec, Spain). The analytical column was a Bondapack C18 (300 mm×2 mm), 10 µm of pore size, from Waters (Milford, MA, USA), working at 30 °C. The mobile phase flow rate was 0.9 mL/min and the absorbance was monitored at 475 nm. The identification of each compound was made by comparison of the retention times of each peak (see Fig. 3).

### 2.2.9. Total phenolics

A 0.5 g representative sample of freeze-dried fruits was extracted with 20 mL of acidic methanol/water (50:50, v/v; pH 2). The tube was centrifuged at 4000 rpm for 15 min and the supernatant was recovered. An amount of 20 mL of acetone/water (70:30, v/v) was added to the residue, and the tubes were shaken and centrifuged again. Methanol and acetone extracts were combined and used to determine total phenols (Perez-Jimenez, Arranz, & Saura-Calixto, 2006). The determination was performed according to the Folin-Ciocalteu procedure (Singleton & Rossi, 1965): briefly 0.5 mL were introduced into test tubes, 0.5 mL of Folin-Ciocalteu's reagent and 10 mL of sodium carbonate (7.5%, w/v) were added and flasks were made up to 50 mL with distilled water. The mixture was incubated in the dark for 60 min and absorbance was measured at 750 nm. Total phenolic content was expressed as gallic acid equivalents (GAE).

#### 2.3. Fruit production estimations

Yield estimations were carried out in the same locations where the strawberry-tree fruits were gathered. Tree measurements, including total height and stem diameter, and local abundance of the species were calculated. To determine average fruit mass, 100 ripe fruits from each location were weighed. Likewise, average number of fruits per branch and average number of branches per tree were calculated in 25 specimens from each location to estimate fruit production per tree and fruit production per hectare. According to the nutritional composition and yield estimations of strawberry-tree fruits, the energetic contribution was also expressed as kcal per fruit, kcal per tree, and kcal per hectare.

#### 2.4. Statistical analysis

All the analyses were carried out in triplicate. Analysis of variance (ANOVA), followed by Duncan's test, was conducted using Statgraphics Plus 5.1 software, to analyze data at the 95% confidence level.

### 3. Results and discussion

The results of the nutrient composition and energy values (expressed on fresh weight) of the wild strawberry-tree fruits analyzed in 2007, 2008 and 2009 in the two locations are given in Tables 1–5. Total average values, taking into account the samples by triplicate from the three years of study in both locations (n=18), are summarized in Table 6. As shown in Tables 1 and 6, the main components of the fruits were moisture and carbohydrates. The average content of water in strawberry-tree fruits was of 56% (w/w), a similar value to those described by Özcan and Haciseferoğullari (2007) and Barros et al. (2010). However, as shown in Table 1, significant differences were revealed in the fruit moisture from different locations and years, probably due to different environmental conditions, such as water availability, sunlight and wind exposition, which contribute to fruit desiccation. Samples collected in 2009 in

Table 1

Macronutrient composition (g/100 g on fresh weight; mean  $\pm$  SD, n = 3) and energy values (kcal/100 g on fresh weight) of *Arbutus unedo* L fruits. Different letters means significant differences in each row (P<0.05).

	2007		2008		2009	
	Location 1	Location 2	Location 1	Location 2	Location 1	Location 2
Available carbohydrates	$26.64 \pm 0.76 \text{ d}$	31.55 ± 1.13 e	$21.07 \pm 0.32$ c	$26.96 \pm 0.65 \text{ d}$	$17.89 \pm 1.04 \text{ b}$	$14.11 \pm 0.58$ a
Proteins	$0.93 \pm 0.04$ abc	$1.11 \pm 0.04$ ab	$0.72 \pm 0.10$ a	$1.19 \pm 0.18 \text{ b}$	$0.86 \pm 0.05 \text{ ab}$	$0.58 \pm 0.05$ a
Lipids	$0.58 \pm 0.04$ a	$0.58 \pm 0.00 \text{ b}$	$0.50 \pm 0.06$ b	$0.78 \pm 0.06$ c	$0.76 \pm 0.05 \text{ c}$	$0.30 \pm 0.02$ a
Soluble fiber	$2.93 \pm 0.35$ bc	$2.17 \pm 0.06$ a	$2.42 \pm 0.07$ ab	$3.71 \pm 0.36$ e	$3.37 \pm 0.14$ de	$3.07 \pm 0.17$ cd
Insoluble fiber	$7.86 \pm 0.28$ a	$13.26 \pm 0.66$ c	$14.55 \pm 0.36$ d	$18.55 \pm 0.22 \text{ f}$	16.21 ± 0.25 e	$9.11 \pm 0.49$ b
Moisture	53.22 ± 2.99 ab	$47.45 \pm 3.86$ a	$59.32 \pm 1.53$ b	$46.82 \pm 1.72$ a	$60.17 \pm 0.39$ b	71.89±0.70 c
Energy	115.57 c	135.93 d	91.67 b	119.60 c	81.87 b	61.48 a

	2007		2008		2009	
	Location 1	Location 2	Location 1	Location 2	Location 1	Location 2
Fructose	$12.34 \pm 1.17 \text{ b}$	$11.34 \pm 0.10 \text{ b}$	$12.10\pm0.84~\text{b}$	$12.69 \pm 1.96 \text{ b}$	$5.83 \pm 0.20$ a	$3.65 \pm 0.43$ a
Glucose	$6.50 \pm 0.06$ c	$6.13 \pm 0.64$ c	$5.30 \pm 0.51$ c	$4.94 \pm 1.11$ bc	$2.78 \pm 0.18$ ab	$2.34 \pm 0.13$ a
Sucrose	$0.34 \pm 0.08$ a	$0.48 \pm 0.02 \text{ b}$	n.d.	n.d.	<loq_< td=""><td><loqtraces< td=""></loqtraces<></td></loq_<>	<loqtraces< td=""></loqtraces<>
Total	18.84 b	17.47 b	17.41 b	17.64 b	8.61 a	5.98 a

n.d. = non detected (LOD sucrose = 0.03 g/100 g). LOQ sucrose = 0.11 g/100 g.

location 2 had much higher water content, that differed significantly from previous years (P<0.05). Overall, moisture content was lower than most of the conventional fruits (about 75–95%), being only similar to some other wild berries as for example, cranberries (*Vaccinum oxycoccus* L.), according to Souci, Fachmann, and Kraut (2008).

Macronutrients content, including total carbohydrates, was coincident with values reported by Barros et al. (2010), although no data on the distribution of these carbohydrates in digestible or nondigestible have been reported by the referred authors. Carbohydrate fraction was composed by TAC (23.55 g/100 g as an average value) and dietary fiber (16.21 g/100 g as an average value). Both parameters showed high variability between years and locations (P<0.05). The comparison with other fruits showed that the ratio TAC/fiber is highly variable among the different species. The proportion estimated in strawberry-tree fruits was near 60/40, in agreement with Herrera (1987), while in other fruits the fiber values are often much lower than TAC, reaching a ratio of 90/10 (Souci et al., 2008). This nutritionally interesting profile, with a high fiber proportion in the carbohydrate fraction, is only similar to that of *Ribes nigrum* L.

Annual TAC values found in the fruits gathered in 2007 were higher than those of 2008 and specially 2009, probably due to the influence of physiological factors involved in the ripening process. Carbohydrate content was inversely correlated to moisture levels (P<0.05), which suggest that TCA, as the main component of strawberry-tree fruits, were highly influenced by water content in the samples (dilution effect), while other components are more independent of moisture content in the fruits.

Soluble sugars were the major components of TCA, with a percentage ranging between 42 and 80%, in agreement with Ayaz et al. (2000). As shown in Table 2, the sugar composition revealed a high contribution of fructose followed by glucose, and sucrose in minor proportion. The latter was even not detected in 2008 and only traces, i.e., less than the limit of quantification (LOQ) of the analytical method, were found in 2009. It is also remarkable the stability in the content of total and major individual sugars (fructose and

glucose) in 2007 and 2008. Those parameters were significantly lower in 2009 but also stable between the two localities in this year (P<0.05). Although different factors may be involved in soluble sugars content in the fruits, the higher moisture content in 2009 samples could be related to this fact, in the same way as it happens to TCA.

Comparing with other species, the strawberry-tree fruits showed an unusual profile of soluble sugars. The lower amounts of sucrose found in these fruits may be due to the enzymatic hydrolysis of this sugar into glucose and fructose during ripening process. For that reason, fruits with a high invertase activity, such as prickly pear fruit, kaki or pomegranates, reach a glucose/fructose ratio near 1 (Ayaz et al., 2000). A ratio higher than 1 has been found in some wild fruits, such as wild red-bilberries (Vaccinium vitis-idaea L.) or blackthorn fruits (Crataegus monogyna Jacq.), which usually have more than 3-fold more glucose than fructose (Barros et al., 2010; Souci et al., 2008). On the contrary, all the strawberry-tree samples analyzed in this study showed an inverse behavior, with a ratio glucose/fructose between 0.4 and 0.6 (similar to the results reported by Barros et al., 2010), which means that fructose represented about double than glucose, with independence of the total soluble sugar content. This means that other factors different from the hydrolysis of sucrose may be involved in soluble sugars contents in strawberry-tree fruits. Moreover, this profile can be related to the intense and pleasant sweet taste of strawberry-tree fruits when they are completely ripe, since fructose is the sweetest of all the naturally occurring carbohydrates (Hanover & White, 1993).

As can be seen in Table 6, strawberry-tree fruits can be considered a very interesting source of dietary fiber, as a 100 g serving can provide 42.6% of the daily amount required for men and 64.8% of the daily amount required for women (Trumbo, Schlicker, Yates, & Poos, 2002). As far as we know, no data has previously been reported on the distribution of the fiber fraction in these fruits. Insoluble fiber constituted 72–85% of total fiber. The content of soluble fiber, including pectins, was also remarkable, since the values described in this study were higher than those found in most fruits, even those of pectin-rich fruits such as apples, citrics, apricots, peaches or plums

#### Table 3

Ashes content (g/100 g on fresh weight) and mineral and trace element (mg/100 g on fresh weight) content in *Arbutus unedo* L. fruits (mean  $\pm$  SD, n = 3). Different letters means significant differences in each row (*P*<0.05).

	2007		2008	2008		2009	
	Location 1	Location 2	Location 1	Location 2	Location 1	Location 2	
Ashes	$0.726 \pm 0.046$ a	$1.048 \pm 0.082$ b	$0.689 \pm 0.066$ a	$1.058 \pm 0.049 \text{ b}$	$0.976 \pm 0.037 \text{ b}$	$0.685 \pm 0.015$ a	
Na	$6.26 \pm 2.30$ ab	$9.94 \pm 1.14$ b	$7.57 \pm 0.31$ ab	$8.46 \pm 1.11$ ab	$4.33 \pm 0.26$ a	$8.55 \pm 1.58$ ab	
К	$79.72 \pm 11.73$ a	$109.38 \pm 9.97 \text{ b}$	$174.71 \pm 11.78$ bc	$323.14 \pm 23.46$ d	215.78 ± 12.13 c	$161.19 \pm 1.74 \text{ b}$	
Ca	$49.61 \pm 3.80$ a	$51.03 \pm 3.35$ a	67.78±5.32 b	$86.30 \pm 4.17 \text{ c}$	$104.12 \pm 3.47 \text{ d}$	$40.54 \pm 1.05$ a	
Mg	$9.56 \pm 1.03$ a	$12.23 \pm 0.50$ a	$19.11 \pm 0.81$ b	45.85 ± 5.53 c	$17.97 \pm 0.56$ b	$13.16 \pm 0.30$ ab	
Cu	$0.087 \pm 0.010$ ab	$0.103 \pm 0.010$ b	$0.198 \pm 0.001 \text{ c}$	$0.208 \pm 0.004$ c	$0.083 \pm 0.005 \text{ ab}$	$0.073 \pm 0.003$ a	
Fe	$0.805 \pm 0.113$ b	$0.860 \pm 0.094$ b	$0.931 \pm 0.051$ b	$1.856 \pm 0.080$ c	$0.505 \pm 0.026$ a	$0.354 \pm 0.001$ a	
Mn	$0.043 \pm 0.008$ a	$0.065 \pm 0.002$ ab	$0.085 \pm 0.006 \text{ b}$	$0.178 \pm 0.014$ c	$0.075 \pm 0.001$ b	$0.038 \pm 0.005$ a	
Zn	$0.473 \pm 0.055$ c	$0.555 \pm 0.038$ c	$0.488 \pm 0.042$ c	$0.762 \pm 0.029 \text{ d}$	$0.362 \pm 0.020$ b	$0.188 \pm 0.007$ a	

Acidity parameters: pH, titratable acidity (mL N/10 NaOH per 100 g fresh matter) and organic acids content (mg/100 g on fresh weight) in *Arbutus unedo* L, fruits (mean  $\pm$  SD, n = 3). Different letters means significant differences in each row (P<0.05).

	2007		2008	2008		2009	
	Location 1	Location 2	Location 1	Location 2	Location 1	Location 2	
рН	$3.47 \pm 0.12$ bc	$3.28\pm0.04~\mathrm{ab}$	$3.38\pm0.07~\mathrm{abc}$	$3.43 \pm 0.12$ bc	$3.49 \pm 0.01 \text{ c}$	$3.21 \pm 0.04$ a	
Titratable acidity	$123.99 \pm 8.04$ c	$140.45 \pm 9.37$ c	69.63 ± 7.90 ab	$89.29 \pm 4.68 \text{ b}$	56.39 ± 5.39 a	$77.09 \pm 1.10$ ab	
Oxalic acid	66.33±6.71 a	$139.01 \pm 26.13$ b	$117.71 \pm 5.06$ b	$146.75 \pm 16.11$ b	$48.44 \pm 2.75$ a	$60.93 \pm 4.45$ a	
Malic acid	$253.26 \pm 21.95$ ab	$231.38 \pm 7.32$ ab	$227.11 \pm 41.41$ ab	$203.34 \pm 24.12$ a	$314.94 \pm 45.84$ b	$299.69 \pm 29.37$ a	
Citric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	
Fumaric acid	n.d.	n.d.	<loq_< td=""><td>n.d.</td><td><math>0.919 \pm 0.172 \text{ b}</math></td><td><math>0.539 \pm 0.070</math> a</td></loq_<>	n.d.	$0.919 \pm 0.172 \text{ b}$	$0.539 \pm 0.070$ a	

n.d. = non detected (LOD citric acid = 0.06 mg/100 g, LOD fumaric acid = 0.045 mg/100 g). LOQ fumaric acid = 0.150 mg/100 g.

among others (Souci et al., 2008). Insoluble fiber showed a wider variability range (7.86–18.55 g/100 g) than soluble fiber fraction (2.17–3.71 g/100 g) in the samples coming from different years and locations (Table 1). The distribution of fiber fractions agrees with the contents found in other wild and cultivated fruits such as strawberries, *Crataegus monogyna* or *Vaccinium oxycoccus* berries (Souci et al., 2008).

Other minor components of strawberry-tree fruits were proteins (0.58-1.19 g/100 g) and lipids (0.30-0.78 g/100 g) which showed annual fluctuations, but without a significant influence of the moisture content of the fruits (Table 1).

On the basis of the proximate analysis, it can be calculated that a fresh portion of 100 g of strawberry-tree fruits assures, on average, 101 kcal. Energy value seems to be influenced mainly by TAC, as it was the main macronutrient of the fruits (positive correlation, p<0.05). In this way, samples gathered in 2009 provided less energy (72 kcal/100 g as an average value) compared to those gathered in 2008 and 2007 (106 and 126 kcal/100 g respectively) (Table 1).

The spider diagram in Fig. 4 shows the proximate composition profile (g/100 g of fresh weight) of *A. unedo* fruits gathered in 3 different years. The statistical differences (one way ANOVA) in energy contribution, carbohydrates and moisture content are clearly represented when the year of study was considered as an independent factor.

Mineral composition of strawberry-tree fruits ranged between 0.7 and 1% (w/w) on fresh weight. Values were lower than those presented by Özcan and Haciseferoğullari (2007) for the same species (2.82%). Micronutrients characterization was carried out by analyzing mineral (Na, K, Ca, and Mg) and trace elements (Cu, Fe, Zn, and Mn). As can be observed in Table 3, it is quite variable between years and locations. Among macroelements, K was the main mineral element, showing the highest values (79.72–323.14 mg/100 g), followed by Ca (40.54–104.12 mg/100 g). The content of Mg was not high, ranging between 9.56 and 45.85 mg/100 g, while Na presented the lowest values (4.33–9.94 mg/100 g).

Regarding the microelements, as can be seen in Table 3, the highest values were reached by Fe (0.354–1.856 mg/100 g) and Zn (0.188–0.762 mg/100 g). Manganese and Cu appeared as the minor microelements, with values between of 0.038–0.178 mg/100 g and 0.073–0.208 mg/100 g, respectively. Microelements, also called "trace elements", include a wide number of compounds with physiological activity and some of them accomplish decisive functions to maintain human health (Palmer, Venkateswaran, Fleshner, Klotz, & Cox, 2008). The biological activities of Cu, Fe, Zn and Mn, are strongly associated with the presence of unpaired electrons that allow their participation in redox reactions. It is assumed that these trace metals play a key role in the protection mechanisms by scavenging free radicals.

The mineral profile of strawberry-tree fruits was similar to other wild berries described by previous authors (Souci et al., 2008). As shown in Table 6, the highest contributors to mineral intake were Cu (13.9% of RDA) and Fe (11.1% of RDA for men). In addition Ca content should be highlighted, as it was found to be higher than most of the habitual wild fruits, except for *Rosa canina* L. fruits (257 mg/100 g, according to Souci et al., 2008). On the contrary, Mn content was lower than other berries, such as gooseberries or grapes.

Total ashes content was highly stable in the samples; however, the behavior of the different mineral elements studied was quite different. In this way, it is interesting to remark the importance of the range of variation, which can be estimated by the heterogeneicity index (maximum value/minimum value), as defined by Allane and Benamara (2010). According to this index, Ca and Na behave as the most stable elements, with no statistically significant differences when comparing strawberry-tree berries gathered in different years (heterogeneicity index of 2.0–2.4). On the contrary, the year of harvest significantly influenced the fruit content of K, Mg, Cu, Fe, Mn and Zn, being higher in 2008, than in 2007 and 2009, and with the widest range of variation (heterogeneicity index of 3.0–5.6). Micronutrients may vary largely depending on environmental conditions, as precipitation, humidity and soil composition, which may influence

Table 5

Antioxidant compounds (mg/100 g on fresh weight; mean $\pm$ SD, n = 3) in Arbutus unedo	L. fruits. Different letters means significant differences in each row ( $P$ <0.05).
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	2007		2008		2009	
	Location 1	Location 2	Location 1	Location 2	Location 1	Location 2
Vitamin C	$133.2 \pm 6.4$ a	217.2 ± 4.0 b	$122.0 \pm 1.1$ a	262.7 ± 1.5 c	$155.2 \pm 9.7$ a	$203.8 \pm 12.9 \text{ b}$
Ascorbic acid	$111.8 \pm 10.6$ a	$123.4 \pm 18.9$ ab	$93.1 \pm 0.7$ a	164.4 ± 3.3 c	$155.2 \pm 9.7 \text{ bc}$	$203.8 \pm 12.9 \text{ d}$
Dehydroascorbic acid	$26.7 \pm 2.6$ a	$83.1 \pm 10.3 \text{ b}$	$28.9 \pm 0.3$ a	$90.8 \pm 5.6 \text{ b}$	n.d-*	n.d-*
β-carotene	$0.398 \pm 0.031$ a	$0.652 \pm 0.018$ b	$0.257 \pm 0.066$ a	$0.684 \pm 0.124$ b	$0.243 \pm 0.027$ a	$0.808 \pm 0.072$ b
Lycopene	n.d.	<loq< td=""><td><math>0.153 \pm 0.054</math> a</td><td><loq.< td=""><td><math>0.154 \pm 0.015</math> a</td><td><math>0.209 \pm 0.073</math> b</td></loq.<></td></loq<>	$0.153 \pm 0.054$ a	<loq.< td=""><td><math>0.154 \pm 0.015</math> a</td><td><math>0.209 \pm 0.073</math> b</td></loq.<>	$0.154 \pm 0.015$ a	$0.209 \pm 0.073$ b
Total phenolics (mg GAE/100 g)	1973.0±151.5 c	1973.6±122.6 c	1736.4±80.4 c	1954.6±198.4 c	$1351.2 \pm 123.3 \ b$	$951.7 \pm 49.0$ a

GAE = Gallic acid equivalents.

n.d.- = non detected (LOD lycopene = 0.040 mg/100 g). LOQ lycopene = 0.133 mg/100 g. \* The same value was found for AA and total vitamin C.

Nutritional composition of *Arbutus unedo* fruits (on fresh weight) and contribution to the Recommended Dietary Allowances (RDA) referred by Trumbo et al. (2002).

Constituents	Average value $(n=18)$	Range (Min-Max)	RDA (minimum)	% RDA
Moisture (g/100 g)	56.48	46.82-71.89	_	_
Energy (kcal/100 g)	101.00	61.48-	_	-
		135.93		
Protein (g/100 g)	0.899	0.581-1.187	$56 \text{ g/d}^{a}$	1.6 <sup>a</sup>
Lipids (g/100 g)	0.609	0.299-0.779	46 g/d <sup>b</sup>	1.9 <sup>b</sup>
Available carbohydrates	23.55	14.11-31.55	130 g/d	18.1
(g/100 g)	20100	1 11 1 0 100	1008/4	1011
Fructose (g/100 g)	10.36	3.64-14.54	-	-
Glucose (g/100 g)	5.51	2.34-6.49	-	-
Sucrose (g/100 g)	0.412	Traces-0.483	-	-
Total fiber (g/100 g)	16.21	10.04-22.27	38 g/d <sup>a</sup>	42.6 <sup>a</sup>
(-1, -1) = (-1, -1)	2.05	2 1 7 2 7 1	25 g/d <sup>b</sup>	64.8 <sup>b</sup>
Soluble fiber $(g/100 g)$ Insoluble fiber $(g/100 g)$	2.95 13.26	2.17-3.71 7.86-18.55	_	-
Ashes (g/100 g)	0.864	0.685-1.058	_	_
Na $(mg/100 g)$	7.52	4.33-9.94	_	_
K (mg/100 g)	177.3	79.72-	_	-
		323.14		
Mg (mg/100 g)	19.62	9.56-45.85	420 mg/d <sup>a</sup>	4.6 <sup>a</sup>
			310 mg/d <sup>b</sup>	6.3 <sup>b</sup>
Ca (mg/100 g)	66.54	40.54-	1.000 mg/d	6.6
F ( (100 )	0.005	104.12	0 (13	44.43
Fe (mg/100 g)	0.885	0.354-1.856	$8 \text{ mg/d}^{a}$	11.1 <sup>a</sup> 4.5 <sup>b</sup>
Cu (mg/100 g)	0.125	0.073-0.208	18 mg/d <sup>b</sup> 900 μg/d	4.5 13.9
Mn (mg/100 g)	0.081	0.038-0.178	$2.3 \text{ mg/d}^{a}$	3.5 <sup>a</sup>
····· (····g/ 100 g)	01001	01000 01170	$1.8 \text{ mg/d}^{b}$	4.5 <sup>b</sup>
Zn (mg/100 g)	0.471	0.188-0.762	11 mg/d <sup>a</sup>	4.2 <sup>a</sup>
			8 mg/d <sup>b</sup>	5.8 <sup>b</sup>
Oxalic acid (mg/100 g)	96.51	48.44-	-	-
		146.75		
Malic acid (mg/100 g)	254.9	203.3-299.6	-	-
Fumaric acid (mg/100 g)	0.729	0.489-1.114	-	-
Ascorbic acid (mg/100 g) Dehydroascorbic acid	141.9 45.9	93.1–203.8 Traces–90.8	-	-
(mg/100 g)	45.9	Traces-90.8	-	-
Total vitamin C (mg/100 g)	182.4	122.0-262.7	90 mg/d <sup>a</sup>	202.6% <sup>a</sup>
			75 mg/d	243.2%
Total phenolics	1656	951-1973	_	_
(mg GAE/100 g)				
$\beta$ -carotene(mg/100 g)	0.520	0.219-0.890	0.9 mg RAE/d <sup>a</sup>	9.6 <sup>a</sup>
			0.7 mgRAE/d <sup>b</sup>	12.3 <sup>b</sup>
Lycopene(mg/100 g)	0.173	0-0.262	-	-

Traces: <LOQ; RAE = retinol activity equivalents. <sup>a</sup> RDA for males. <sup>b</sup> RDA for females.

these levels, as they could induce the response of the plant to physiological stress situations, in which the minerals could act as cofactors regulating the metabolic pathways of the plant (Peñuelas et al., 2008).

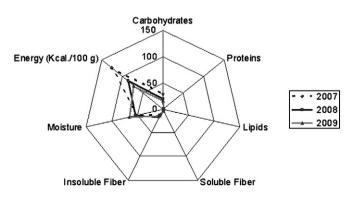


Fig. 4. Proximate composition profile (g/100 g of fresh weight) of Arbutus unedo L fruits gathered in 3 different years.

Strawberry-tree fruits did not show too high acidity (Table 4), as it has been found in other wild fruits as those of *Rosa canina*. The sugars/ acids ratio provides its unique flavor that makes this fruit suitable for a great number of food applications. pH values were low and stable (3.2–3.5), in agreement with Cavaco, Longuinho, Quintas, and De Carvalho (2007). Nevertheless, titratable acidity (92 mL N/10 NaOH per 100 g of fresh sample, on average) was very variable among years and locations, since there is no direct relationship between both parameters due to the logarithmic scale of pH values. The fruits gathered in 2007 had titratable acidity values higher than those collected in 2008 and 2009. According to the water content of the samples, a significant negative correlation (P<0.05) was found between moisture and titratable acidity.

Organic acids also showed significant variations between seasons (Table 4), possibly related to the metabolism of the plant. Non-volatile acids are natural components of many fruits and vegetables. Particularly, organic acids play an important role in maintaining the fruit quality and the nutritive value of different fruits. There are only a few studies on the chemical composition of non-volatile organic acids fraction of strawberry-tree fruits, and the compounds reported are quite variable. Ayaz et al. (2000) reported fumaric and malic acids as the major ones in Turkish A. unedo var. ellipsoidea Aznov. fruits, and Alarcão-E-Silva et al. (2001) found quinic and malic acids as the main compounds in Portuguese strawberry-tree fruits. In agreement with these studies, malic acid was present in the fruits analyzed in this study in high levels (0.2–0.3%, Table 4), higher than those found by Ayaz et al. (2000), and lower than those reported by Alarcão-E-Silva et al. (2001). Malic acid is predominant in different conventional fruits as well as other wild berries, such as Rubus fruticosus L. and Vaccinium myrtillus L., with levels above 0.8 g/100 g (Souci et al., 2008). Other remarkable point regarding organic acid composition of strawberrytree fruits was the absence of quantifiable amounts of citric acid, in agreement with the previous authors. Citric acid is a major acid in many conventional fruits but is also absent from other wild berries as Vaccinium myrtillus or Rubus fruticosus fruits (Rodríguez, Odériz, Lozano, & Hernández, 1992).

Fumaric acid has been reported as a major acid in *A. unedo var. ellipsoidea* fruits by Ayaz et al. (2000), but other authors as Alarcão-E-Silva et al. (2001) agree with the present study in the low or inexistent levels of fumaric acid in strawberry-tree fruits. It was only detected in very small amount in the samples harvested in 2009 (Table 4).

Differently from previous studies, oxalic acid was quantified in variable amounts (0.05–0.15 g/100 g), being higher than in other fruits, with some exceptions as *Averrhoa carambola* L. (0.04–0.68 g/ 100 g). However, it is important to highlight the low toxicity of oxalic acid, with a minimal lethal dose for an adult of 5 g. Its effect in the reduction of the availability of dietary Ca and the formation of kidney calculus is well known. Several authors (Guil, Torija, Giménez, Rodríguez-García, & Jiménez, 1996) recommended an oxalic acid/Ca relation not higher than 2.5 in foods to avoid negative effects. In the *A. unedo* berries analyzed in this study, oxalic acid/Ca ratio was 1.45, so despite the high oxalic acid concentration in these fruits, it is not considered as to decrease Ca availability. There was no significant correlation between titratable acidity and the content of major acids (malic and oxalic), which may suggest that some other compounds may be involved in the acidity of the fruits.

Table 5 describes the antioxidant compounds determined, including vitamin C, phenolics and carotenoids. Results refer exclusively to the raw fruits, since the cooking process partially decrease the content of available antioxidants. Therefore, although strawberry-tree fruits are sometimes consumed as processed, they may be a better source of antioxidants if consumed fresh (Pallauf et al., 2008).

The nutritional importance of vitamin C as an essential watersoluble vitamin is well established: ascorbic acid (AA) is a cofactor in numerous physiological reactions, and also has a high redox potential, alone and coupled to other antioxidants. Previous studies in *A. unedo*  fruits showed very variable amounts of vitamin C, and only a few studies reported values of ascorbic acid in fresh fruits (Alarcão-E-Silva et al., 2001; Hisar, 1947) as it has been performed in the present study. The analysis showed vitamin C levels considerably high (122–262 mg/ 100 g; Table 5), similar to other wild fruits as rose fruits, which have been used as a source of vitamin C in teas and other products (Kharazmi, 2008). It can be stated that vitamin C is one of the most interesting contributors to nutrient intake in *A. unedo* fruits, since just one serving of 50 g of raw fruits provides the totality of RDA for adults (Table 6).

The nutritional interest of ascorbic acid (AA) comes not only from its activity as vitamin C, in conjunction with its oxidized form, dehydroascorbic acid (DHAA), but also because it is a potent antioxidant either in the food or the human body by the destruction of oxygen free radicals. There is only one study reporting the contribution of AA to total vitamin C in freeze-dried strawberry-tree fruits (Pallauf et al., 2008). The values obtained are lower than those described in this study (93.1-203.8 mg/100 g; in fresh fruits). Regarding DHAA, nothing was found in the literature for A. unedo fruits. The analysis revealed a high and significant variation between the DHAA content in 2007 and 2008 in both locations, being in 2009 less than the limit of detection (LOD) of the analytical method (Table 5). Vitamin C content was more influenced by the location than by the year of harvest. Data obtained during the tree years confirm the presence of ascorbic acid as a major vitamin, being always higher than 56% of total vitamin content (Fig. 5).

The content of carotenoids ( $\beta$ -carotene and lycopene) has been also quantified. The  $\beta$ -carotene, vitamin A precursor, was the most abundant (0.243–0.808 mg/100 g), while very low values of lycopene were described (Table 5). The values obtained in the present study for  $\beta$ -carotene were higher than those reported by Pallauf et al. (2008), resulted in 0.025 mg/100 g. According to Barros et al. (2010), lycopene was not detected in strawberry-fruits and  $\beta$ -carotene was present in low amounts. Likewise, Alarcão-E-Silva et al. (2001) only found  $\beta$ -carotene, with an increase in its content during the ripening process. Carotenoids may be responsible of yellow colors in the flesh of the fruits, but the external red color is mainly due to the presence of other phenolic pigments, identified as 3-glucosylcyanidin (Proliac & Raynaud, 1981).

Phenolic acids and their derivatives are widely distributed in plants, and many of them are essential metabolites (Krygier, Sosulski, & Hogge, 1982). They occur naturally in combination with other compounds, usually in the form of esters. The predominant phenolic acid previously identified and quantified in mature A. unedo fruits was gallic acid, with levels of 10.7 mg/g of dry weight (Ayaz et al., 2000). Anthocyanins composition is characterized by the presence of cyanidin and delphinidin glycosides, responsible of the color of the fruits (Pallauf et al., 2008). The quantity and the composition of phenolic compounds present in foods are influenced by the genotype, extraction procedure, and environmental conditions. Since phenolic compounds are known to play an important role as antioxidants in human nutrition, subtle differences in phenolic composition may be of considerable importance from a nutritional standpoint. Phenolic content in the strawberry-tree fruits analyzed ranged between 951.7 and 1973.6 mg GAE/100 g (Table 5), which is a very high level, in the range of rose fruits (Barros et al., 2010), and higher than many fruits considered as rich in phenols, such as blueberries, with 670 mg GAE/100 g (Marinova, Ribarova, & Atanassova, 2005). Statistical differences between 2007 and 2008 were not detected, while it decreased significantly in 2009 (P<0.05), confirming the influence of environmental conditions on the total phenolic content.

Overall, the antioxidant compounds which showed more seasonal stability in strawberry-tree fruits were total vitamin C and  $\beta$ -carotene. The influence of the year of harvesting was statistically significant (*P*<0.05) in ascorbic acid, dehydroascorbic acid, phenolics and lycopene composition, which are more labile compounds, sensitive

#### mg/100 fresh weight

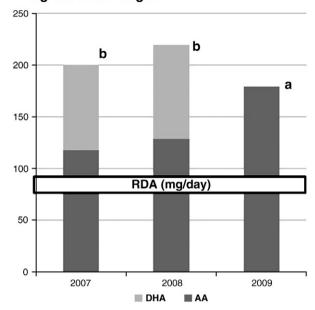


Fig. 5. Average vitamin C values of wild strawberry-tree fruits gathered in different years.

to light exposition, temperature and other environmental factors. Likewise, some correlations (P<0.05) appeared among the antioxidant compounds: ascorbic acid was positively correlated to  $\beta$ -carotene (0.6669) and negatively correlated to phenolics (-0.7122), which was also negatively correlated to lycopene (-0.8376). The explanation of these relationships should be further studied in the light of the complex synergistic/antagonic actions of the different compounds involved in antioxidant metabolism of plants (Masibo & He, 2009).

The statistical study showed that the year of harvest, and thus the environmental factors associated with climate conditions, has a stronger influence on most of the chemical composition of strawberry-tree fruits than geographical location.

Regarding fruit production at the two locations of *A. unedo* surveyed, significant differences were also described (Table 7). In location 1, strawberry-tree specimens were bigger, according to tree diameter values. Therefore, fruit production per tree was also higher in this location (6.42 kg/tree). Nevertheless, fruit production per hectare was lower (46.25 kg/ha in location 1, against 538.61 kg/ha in location 2) since the species density was clearly higher in location 2. Fruit mass was similar, ranging between 3.10 and 3.62 g of fresh weight. Consistently, each fruit provides approximately the same figure of kcal per 100 g of fresh portion (near 100 kcal/100 g), equivalent to the consumption of approximately 30 fruits. Energy values of strawberry-tree relating to fruit production per tree and fruit production per hectare were estimated for the first time in the present study.

#### 4. Conclusions

A wide variability in nutrient composition of strawberry-tree fruits was found in different parameters of the chemical composition, depending on the year or location of harvest. Results have shown that an individual sample may not be fully representative. The analysis of many different samples from different origins and seasons, are required to provide average reliable data about the chemical composition of wild fruits. On the other hand, proteins, lipids, soluble and insoluble fiber, ashes, Na, Ca, pH and total vitamin C, may be more constant in the composition of the fruits. Population characteristics and fruit production did not seem to have a clear influence on fruit composition.

Population characteristics and fruit production of Arbutus unedo L. at the two locations surveyed. According to nutritional composition, energy contribution per fruit, per tree and per hectare is given.

	Location 1	Location 2
Population characteristics		
Tree diameter (cm)	$24.18 \pm 2.63$ a	$7.52 \pm 0.45$ b
Tree density (number of trees/ha)	$7.20 \pm 3.44$ a	$206.40 \pm 32.49 \text{ b}$
Yield parameters		
Fruit mass (g)	$3.10 \pm 0.09$ a	$3.62 \pm 0.09 \text{ b}$
Fruit production per tree (kg/tree)	$6.42 \pm 1.19$ a	$2.61 \pm 0.42$ b
Fruit production per hectare (kg/ha)	$46.25 \pm 18.62$ a	$538.61 \pm 59.73$ b
Energy contribution		
kcal/fruit	3.13	3.66
kcal/tree	6485	2637
kcal/ha	46,722	544,104

Values are expressed as mean  $\pm$  standard error from 3-yr sampling, in fruit mass, and 2-yr in fruit production per tree and per hectare.

In each row, different letters mean significant differences (P<0.05).

In the light of the results presented, and even despite of the natural variability in the nutritional composition of the fruits, strawberry-tree can be considered a very good source of vitamin C and dietary fiber (202.6 and 42.6% minimum contribution to RDAs, respectively) as well as of polyphenols. It is also rich in TAC, sugars, potassium, being poor in lipids and Na and with a good oxalic/Ca ratio. This fact, together with the availability of the species in the Mediterranean region and its high fruit production, means that these wild berries could become a source of health promoting compounds with promising industrial uses.

The contribution of exotic wild food plants to the total dietary intake has not yet been completely estimated. In this sense, the information on the composition of strawberry-tree fruits might be of use to consumers and food technologists. The results of the present study suggest that the use of traditionally consumed wild fruits may be invigorated by their incorporation in the contemporary diets. Further research of the chemical composition in these unusual and valuable fruits could be a desirable feature for selecting A. unedo genotypes with improved fruit nutritional quality.

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