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# **Phenols, Volatile Compounds, Organic Acids and Antioxidant activity of Strawberry tree (*Arbutus unedo* L*.)* fruits belonging to five genotypes growing in Morocco**

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# **Abstract:**

This study aims to identify the individual phenolics and volatile compounds, as well as the organic acids of strawberry tree (*Arbutus unedo* L.) genotypes fruits. The antioxidant activities were also assessed using three methods (DPPH, ABTS and ꞵeta carotene bleaching assays) significant differences (p˂0.05) were observed among all the genotypes. Total phenols varied from 25.37 to 39.06 mg GAE/g dried weight (DW), total flavonoids ranged between 3.30 and 7.07 mg RE/g DW, and anthocyanins varied from 0.15 to 0.64 mg cya-3-glu/100 g DW. Moreover, the antioxidant activities were in the range of 3.33-21.08, 2.25-19.58, and 1.08-13 mg ascorbic acid equivalent /g DW for DPPH, ABTS and βeta carotene bleaching assays, respectively. 17 phenolics compounds were identified by HPLC in *A. unedo* fruits. Gallocatechol and catechin were the most abundants compounds. Among the volatile compounds identified, hexadecanoic acid was the most abundant in all the genotypes fruits. The principal component analysis revealed that the ﬁrst two components formed 66.47% of the total inertia.

**Keywords:** *Arbutus unedo* L., antioxidant activity, volatile compounds, organic acid, polyphenolic profiles, Morocco.

**1. Introduction**

The strawberry Strawberry tree (*Arbutus unedo* L.), is evergreen shrub belonging to *Ericaceae*  family endemic to Mediterranean region and North Africa (Sulusoglu and Cavusoglu., 2011). *A. unedo* is a Medicinal plants naturally grown as population or solitary tree in countries such as Morocco, Tunisia, Algeria, Turkey, Syria, Greece, Croatia, France, Portugal and Spain (Serce et al. 2010). It considered as an important source of molecules with high antioxidant potential, due mainly to polyphenols concentrated in its fruit, which play a major role in safeguarding health, because of their biological functions, such as antimutagenicity, anticarcinogenicity and antiaging (Rodríguez et al., 2013). The *A. unedo* fruit is suitable for the production of alcoholic beverages, jams, jellies and marmalades (Pallauf et al., 2008) but also for medicinal purposes (Ruiz-Rodriguez et al., 2011). In Morocco, it is known as “Sasnou” and it is widely used in traditional medicine as antiseptics, diuretics and laxatives, more recently, in the therapy of hypertension and diabetes (Bnouham et al., 2007). Both fruits and leaves have been used for medicinal purposes for centuries (Ruiz-Rodriguez et al. 2011). Moreover, *A. unedo* fruits are a very good dietary source of antioxidants, including phenolic compounds (e.g. anthocyanins and other ﬂavonoids, gallic acid derivatives and tannins), vitamins C and E and carotenoids (Ayaz et al., 2000; Alarcão-E-Silva et al., 2001; Males et al., 2006; Pawlowska et al., 2006; Pallauf et al., 2008; Fortalezas et al., 2010; Tavares et al., 2010). These bioactive plant compounds have been used since ancient times as both primary and supplemental treatment for various ailments as well as to support normal physiological functions (Shehzad et al., 2018). These phenolic compounds can amplify the body’s defense system to eliminate cancer cells and block angiogenesis, which is the formation of new blood vessels, essential for tumor development (Shehzad et al., 2018). Consumption of food rich in flavonoids decreased risk factors for heart disease (Mennen et al., 2004). Flavanols and procyanidins in particular may confer vascular benefits by increasing the available pool of nitric oxide, reducing platelet aggregation (Gentileet al, 2012). An increased interest in using naturally occurring phytochemicals from plants for the prevention and treatment of different chronic human diseases was reported in many studies. Among phytochemicals, both phenolic compounds from a large number of plant foods, spices, and beverages have been shown to inhibit or attenuate cancer and cardiovascular diseases (Pandey and Rizvi, 2009) as *A. unedo* fruit is a source potential of phytochemicals. Previous phytochemical studies on the plant showed the presence of three anthocyanins: delphinidin 3-O-galactoside, cyanidin 3-O-galactoglucoside, and cyanidin 3-O-galactoside (Maccarrone et al., 1990). The total content of phenols has been estimated by Alarcao-E-Silva et al., (2001) as 14.6 mg/g dried fruit. There is so far little data in the literature on antioxidants found in *A. unedo* L., although the fruits were reported very high on antioxidants when compared with 27 other fruits. (Garcia-Alonso et al., 2004).

In Morocco, most of those fruits remained underexploited due to the lack of awareness of their potential, market demand and value addition and very few studies have been devoted toward *A. unedo* fruits. To the author’s knowledge, this is the first known report of the volatile profile and phenolic compounds of Moroccan *A. unedo* fruits. Thus, this study aimed at assessment of *A. unedo* fruits spontaneously growing in Moroccan agroecosystems in terms of their main biochemical characteristics, volatile compounds and antioxidant potency in a comparative scheme of five prospected Moroccan genotypes. The specific objectives of this study are : i) to assess the quality of *A. unedo* fruits (pH, titratable acidity, total soluble solide and organic acids), ii) to evaluate the antioxidant activities of *A. unedo* fruits using three methods (DPPH, ABTS and ꞵeta carotene bleaching assays), iii) to quantify the individual phenolics and volatile compounds of *A. unedo* fruits and iv) to determine the correlations between all abovementioned parameters.

**2. Materials and methods**

***2.1. Plant material***

Fruits of strawberry tree (*A. unedo* L.) of five genotypes (Chefchaoun, Moulay Driss Zerhoun, Laanoucer, Ksiba and Tahnaout) were harvested during the period between October and November of 2019 from several regions of Morocco where they grow naturally (Table 1). At each site, random samples of fruits were harvested at their fully ripened stage, and transferred to the laboratory for physicochemical and phytochemical analysis. Fruits were frozen at -80 °C, freeze-dried, and ground, then kept in appropriate conditions for subsequent use.

***2.2. Chemicals and reagents***

2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, rutin, βeta carotene, Folin Ciocalteu reagent, ascorbic acid, sodium carbonate (Na₂CO₃) and standards of organic acids were obtained from Sigma Aldrich, St. Petersburg, Russia, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was from HIMEDIA, potassium iodate was from Scharlau. Standard compounds (phenolic acid standards: ellagic, gallic, and chlorogenic acids; ﬂavonoids standards: rutin, quercetin-3-O-glusoside and quercetin-3-O-galactoside) were obtained from Extrasynthese (Genay, France), the water was distilled and filtered through a Milli-Qapparatus filter.

***2.3. Physico-chemical analyzes***

Total soluble solids (TSS) were assessed according to (AOAC, 2002) by triplicate with a digital refractometer (Atago N1; Atago Co. Ltd., Tokyo, Japan) at 20 °C and expressed as %. Total titratable acidity (TA) was also determined acoording to (AOAC, 2002) by triplicate using an automatic titration device (877 Titrino plus, Metrohm ion analyses CH9101, Herisau, Switzerland) with 0.1 N NaOH up to pH 8.1, using 1 mL diluted juice in 25 mL distilled H2O, and the results were expressed as g malic acid per 100 g fw (Celikel, et al. 2008). The pH was measured using a pH meter according to the method described by (AOAC, 2002). Weigh 10 g of the fruit cut into small pieces, add 100 ml of distilled water and mix for 5 min until juice was obtained. The measurement was made by immersing the pH meter electrode in the solution.

***2.4. Organic Acids and ascorbic acid profiles***

The samples (0.5 g) were extracted with 5 mL of Milli-Q water by incubation for 30 min under ultra-sonication at 25 and kHz 20 °C as described by Hernández et al. (2016). Next, the slurry was centrifuged at 15,000 g for 20 min (Sigma 3–18 K; Sigma, Osterode am Harz, Germany), and the supernatant was filtered through a 0.45 μm Millipore filter and used for analysis. All extractions were carried out in triplicate. The chromatographic analysis was carried out according to Hernández et al. (2016). Thus, 10 μL of extract were injected into a Hewlett-Packard HPLC Series 1100 (Wilmington DE, USA) with an autosampler and an UV detector, set at 210 nm and coupled with a refractive index detector (HP 1100, G1362A). A column (Supelcogel TM C- 610 H column 30 cm x 7.8 mm) and apre-column (Supelguard 5cm x 4.6 mm; Supelco, Bellefonte, PA) were used for the analyses of both organic acids and ascorbic acid. The elution buffer consisted of 0.1% phosphoric (V/V) at a flow rate of 0.5 mL min−¹, and organic acid absorbance was measured at 210 nm using a diode-array detector (DAD). Calibration curves were used for the quantification of organic acids and ascorbic acid showing good linearity (r2 ≥ 0.999). The results were expressed as g 100 g−1 of dry weight (DW).

***2.5.******Phytochemical composition***

*2.5.1. Extraction Procedure*

One gram of powder from each sample was mixed with 25 mL of ethanol (1:25, w/v) at 25°C for 15 min using an IKA T-18 digital Ultra-Turrax homogenizer. The homogenate was then centrifuged for 10 min at 6,000 rpm and the supernatant was removed from the residue. The latter was homogenized with ethanol and the supernatant removed as above. The supernatants were then combined and filtered.

*2.5.2. Total Phenols*

Total phenols content (TPC) of *A. unedo* was determined by the reduction of phosphotungstic-phosphomolybdic acid (Folin–Ciocalteu’s reagent) to blue pigments, in alkaline solution according to Folin as described by Ben Salem et al. (2018). Briefly, 100 µL of diluted sample (1/100) with ethanol was added to 400 µL of 1/10 diluted Folin Ciocalteu reagent. After 5 min, 500 µL of 10% (w/v) sodium carbonate solution was added. After 1 h of incubation at room temperature, absorbance at 765 nm (spectrophotometer Spectraphysic Jasco V-630, Japan) was measured in triplicate. Total polyphenols content was expressed as mg gallic acid equivalents per g dry weight of *A. unedo* fruit (mg GAE/g DW).

*2.5.3. Total Flavonoids*

Total flavonoids content (TFC) was measured using the colorimetric method with aluminum chloride (Lamaison and Carnat, 1990). One mL of the sample was diluted separately then mixed with 1 mL of a 2% aluminum chloride solution. The mixture was incubated at room temperature for 15 min. Rutin was used to develop the calibration curve. The absorbance was measured at 430 nm (spectrophotometer Spectraphysic Jasco V-630, Japan). The results were expressed as mg rutin equivalents per dry weight of *A. unedo* fruit (mg RE/g DW).

*2.5.4. Total Anthocyanins*

Total anthocyanin content (TAC) of samples was determined using the pH diﬀerential method with some modiﬁcations according to Jakobek et al. (2007); Giusti and Wrolstad (2001). One mL of aliquot of each *A. unedo* extract sample was added separately to 980 μl of KCl buﬀer (pH 1.0) and NaOAc buﬀer (pH 4.5). The absorbance was measured at 510 nm and 700 nm (spectrophotometer Spectraphysic Jasco V-630, Japan) for both sets of pH 1.0 and pH 4.5 solutions, using 50% ethanol as a blank after 15 min of incubation at room temperature. The TAC was calculated using equation (1), and the results were expressed as mg of cyanidin-3-glucoside equivalents in 100 g of dry weight.

TAC = (*A*\**MW*\**DF* \**1000* / *Ɛ*\**L*)  (1)

where, A : Absorbance = [(A 510 nm-A 700 nm)] pH 1.0 - [(A 510 nm-A 700 nm)] pH 4.5 ; MW : molecular weight (449.2 g mol-¹) ; DF : dilution factor ; Ɛ : molar absorptivity coefficient of cyanidin-3-glucoside (26900 L mol-¹ cm-¹).

***2.6. Determination of Antioxidant Activities***

The antioxidant activity (AA) was evaluated using three different assays: (i) DPPH assay, (ii) ABTS assay and (iii) the βeta carotene bleaching test. The antioxidant activity was determined in triplicate and the results were presented as a mean ± standard deviation.

*2.6.1. DPPH Free Radical Scavenging Capacity*

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the samples was determined according to Ben Salem et al. (2018). Thus, DPPH solution was prepared by dissolving 0.1 g of DPPH in 1 L methanol (0.1 g L-¹). Then, one mL of this solution was added to 125 µL of each extract. The mixture was stirred thoroughly and incubated in the dark at room temperature for 10 min. The absorbance of both sample and control was measured at 517 nm using a Lambda EZ 150 (spectrophotometer Spectraphysic Jasco V-630, Japan), and the DPPH radical scavenging activity was calculated using the following equation (2):

DPPH scavenged (%) = {(Ac - As) / Ac} \* 100 (2)

where, Ac and AS refer to the control and sample absorbances, respectively.

IC50 value (mg equivalent ascorbic acid /g dry weight) defines the inhibitory concentration at which tested radicals were scavenged by 50%. It was calculated by plotting inhibition percentage of each test against the sample extract dilutions.

*2.6.2. ABTS Assay*

The ABTS• [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid] radical scavenging assay were determined according to Dorman and Hiltunen (2004). Thus, 990 µL of eachextract was incubated in 10 µL ABTS (7 mM)-ETOH and 2.45 mM potassium persulfate solution after sonicated at 20°C for 15 min during 16 h in the dark. The mixtures were incubated for 18 h in the darkness at room temperature. The ethanol was used to dilute the stock solution of ABTS until absorbance of 0.70 ± 0.05 was reached at a wavelength of 734 nm. The antioxidant activity results were expressed as mg equivalent ascorbic acid per g dry weight (DW).

*2.6.3. βeta Carotene Bleaching Assay*

The ꞵeta carotene blanching assay was determined according to Barros et al. (2010). ꞵeta carotene (0.5 mg) in 1 mL of chloroform was taken in a amber bottle and mixed with 200 mg of linolenic acid and 600 mg of Tween 80 (polyoxyethelene sorbitan monopalmitate). The chloroform was removed under nitrogen, and the resulting solution was immediately diluted with 30 mL of triple distilled water and the emulsion was mixed well for 1 min. The emulsion was further diluted with 120 mL of oxygenated water and used for assay. To each sample extract (0.5 mL), 2.5 mL of the prepared emulsion mixture was added and then vigorously mixed. A control consisting 0.5 mL of ethanol and 2.5 mL of emulsion was also analyzed. The absorbance of reaction mixture was read immediately (t = 0) at 470 nm against blank, consisting of emulsion mixture, except β-carotene, and at the 60 min interval for 2 h (t = 120). The tubes were incubated in a water bath at a temperature of 50 ◦C between measurements. Color measurement was monitored until the β-carotene color disappeared. The linoleic acid peroxidation inhibition uses the following Equation (3):

AA = 100 [1- (Ao - At) / (Aoo- Aot)] (3)

where, Ao and Aoo refer to the absorbance measured at the beginning of samples and control incubation, respectively. At and Aot are the final absorbance of samples and control, respectively.

***2.7. Extraction and Determination of Polyphenolic Compounds***

*2.7.1. Extraction Method*

Samples (1 g) were mixed with 10 mL of methanol: water (80:20, v/v) and then, the mixtures were sonicated during 30 min, and macerated one hour in refrigeration (4 ºC). After the time, the samples were centrifuged for 10 min, 8000 g at 4 ºC. The supernatants were collected and the pellets were mixed with 10 mL of acetone: water (70:30, v/v) and the same steps were repeated (sonication, maceration and centrifugation). Then, the supernatants were combined and evaporated to dryness using a rotary evaporator R-205 (Büchi, Flawil, Switzerland) under reduced pressure, at 40 ºC. 5 mL of methanol were added to the residue, and the mixture was well shaken in a stirrer for 2 min. Due to the high sugar content present in the samples, which could interfere with the HPLC column, the samples were loaded onto a C18 Sep-Pak cartridge, previously conditioned with 5 mL of methanol, 5 mL of pure water, and then with 5 mL of 0.01 mol L-¹ HCl. The cartridge was washed with 5 mL of pure water and then eluted with acidified methanol (0.1 g L-¹ HCl). The collected fractions were stored at -20 ºC until further use.

*2.7.2. Determination of Polyphenolic Compounds*

Polyphenolic profiles of all samples were determined by High Performance Liquid Chromatography (HPLC) according to Genskowsky et al. (2016**)**. A volume of 20 µL of the samples were injected into a Hewlett-Packard HPLC series 1200 instrument (Woldbronn, Germany) equipped with a diode array detector (DAD) and a C18 column (Mediterranea sea 18, 25 × 0.4 cm, 5 micrometers particle size) from Teknokroma, (Barcelona, Spain). Polyphenolic compounds were analyzed in standard and sample solutions using a gradient elution at 1 mL min-¹. The mobile phases were composed by formic acid in water (1:99, v/v) as solvent A and acetonitrile as solvent B. The chromatograms were recorded at 280, 320, 360 and 520 nm. Polyphenolic compounds identification was carried out by comparing UV absorption spectra and retention times of each compound with those of pure standards injected under the same conditions. The compounds were quantified through calibration curves of standard compounds injected under the same conditions. Phenolic acid standards were dissolved in methanol at diﬀerent concentrations between 10 and 200 μg mL-¹ ; ﬂavonoids standards were dissolved in methanol at diﬀerent concentrations between 1 and 250 μg mL-¹ . Quantiﬁcation of anthocyanins was carried out based on linear curves of authentic standards. A cyanidin 3-glucoside calibration (concentration between 1 and 250 μg mL-¹ ) was used for cyanidin derivatives.

***2.8.******Volatile compound analysis***

*2.8.1. Extraction and GC–MS analysis*

Static headspace extraction of volatile compounds was performed by using solid phase microextraction (SPME) with a 65 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber. The analysis of *A. unedo* components was carried out by gas chromatography-mass spectrometry (GC-MS) using a gas chromatography Agilent 7890 A with masse selective detector 5975 Network MSD and coupled to an automatic sampling system MPS (Gerstel), a polyethylenglycol capillary column VF-WAXms (30 m x 0.25 mm i.d. x 0.25 μm film thickness) and a split/splitless injector, and the Library pal 600 k. About 1 g of the investigated sample was placed into a 20 mL vial closed with a screw and heated to 60°C for 20 min and the fiber was then exposed to strawberry headspace. After 20 min, the SPME fiber was automatically with drawn from the vial and introduced into the GC injector. Working conditions were: splitless mode with injector temperature at 250°C, the oven temperature program was 50°C for 4 min , rising at 5°C/min to 230°C (held for 10 min ); then rising at 10°C/min to 250°C; and finally, 3 min at 250°C, a constant flow of 1 ml/min (helium) was set up. Mass spectra were recorded in EI mode at 70 eV, scanning the 35-395 m/z range. The interface and source temperatures were 230 and 250°C, respectively.

***2.9. Statistical analysis***

Prior to the statistical analyses, data were tested for normality and homogeneity of variance using SPSS software v22 (IBM, SPSS Statistics, Armonk, New York, United States). The means were evaluated according to descriptive statistics represented as Mean ± SE. Data analysis was performed using IBM SPSS v22. Analysis of variance (One-way ANOVA) was performed to test significant differences among the samples. The differences among means were estimated with Duncan new multiple range test (DMRT). Correlation coefficients and their levels of significance were calculated using Pearson correlation. Principal component analysis was carried out using correlation matrix. In addition, a scatter plot was created according to the first two principal components (PC1 and PC2).

**3. Results and discussion**

***3.1. Physicochemical parameters***

The results for titratable acidity, pH and total soluble solids for all genotypes were summarized in Table 2. Significant variations were observed among genotypes (p<0.001).The titratable acidity ranged from 0.65 to 1.01 g malic acid/100 g FW with an average of 0.83 g malic acid/100 g FW. The highest value was recorded in "TAH" (1.01 g malic acid/100 g FW) while the lowest value was observed in "MDZ" (0.65 g malic acid/100 g FW). The titratable acidity of *A.unedo* fruits reported in this study was higher than those found by other authors; Ozan and Haciseferoğullari (2007) and Vidrih et al. (2013). They reported values of 0.51% and 0.4% respectively. However, The results were lower than the ones recorded by Doukani and Hadjer (2015) (2.14%) in Algerian *A. unedo* genotypes. Also, Celikel et al. (2008) recorded titratable acidity value ranged between 0.80 and 1.59%. The significant difference in acidity could most probably be due to the climate factor and the process of fruit ripening (Messaid. 2008).

The pH values varied from 2.44 "KSB" to 3.92 "LAN" with an average of 3.36. These values were approximately similar with those revealed by Ruiz Rodriguez et al. (2011) and González et al. (2011). They recorded 3.47 and 3.50 respectively. However, the results of this study were lower than those found by Serçe et al. (2010) and Ozan and Haciseferoğullari (2007) who reported 5.57 and 4.6 respectively. The low pH value can have a big advantage manufacturing. The differences depend on many factors including the climate, region and ripeness of the fruit (Huberson. 2008; Messaid. 2008)

The total soluble solids of *A. unedo* fruits varied from 14.83% "LAN" to 18.53% "KSB" with an average of 16.87%. Similar results were reported by Doukani and Tabak., (2015). They recorded values ranged from 16.66 to 17.66%. The values of this study were higher than those found by Muller et al. (2010) and Serçe et al. (2010) who reported (8.1%) and (11.9%) respectively. The fruit of *A. unedo* L. has higher soluble solids content than that of *Arbutus andrachnae* (14%), blackberries (9.5%) and raspberries (6.2%) (Seker and Toplu., 2010). These differences can be related to the climate, soil type and the process of fruit ripening (Serçe et al., 2010).

***3.2. Organic Acids***

The results obtained for organic acids content were reported in Table 3. Significantdifferences (P < 0.001) were observed among the genotypes. Four organic acids were identified by HPLC for all *A. unedo* genotypes. Citric acid, malic acid, ascorbic acid and succinic acid were identified in the investigated samples. Citric acid was determined as the major organic acid, followed by malic acid. The citric acid content ranged between 1.74 g/100 g "KSB" and 5.32 g/100 g "LAN" with an average of 3.17 g/100 g. The results of citric acid content in this study were higher than those reported by Serçe et al. (2010) and Doukani and Hadjer (2015) who recorded 0.03 g/100 g and 8.56 mg/100 g respectively. However, Ruiz-Rodriguez et al. (2011) reported a total absence of citric acid. The malic acid content ranged from 1.53 g/100 g "KSB" to 2.87 "TAH" g/100 g with an average of 2.19 g/100 g. The results of malic acid content in this study were higher than those reported by Serçe et al. (2010) and Doukani and Hadjer (2015) who recorded 0.34 g/100 g and 282.3 mg/100 g values respectively. However, the results obtained in this study were lower than those recorded by Alarcão-E-Silva et al. (2001). They reported 5.99 g/100 g in *A. unedo* fruits from Portugal. The ascorbic acid content varied from 0.28 g/100 g "KSB" to 1.00 g/100 g "TAH" with an average of 0.72 g/100 g. The results obtained were higher than those recorded by other authors; Pallauf et al. (2008) recorded 6.03 mg/100 g in Spanish *A.unedo* fruits. Ascorbic acid values recorded in this study were also higher than those reported by Alarcaoe-Silva et al. (2001); Pimpão et al. (2013) and Morales et al. (2013). They recorded 346 mg/100 g, 89 mg/100 g and 182 mg/100 g respectively. The succinic acid content ranged from 0.49 g/100 g "CHF" to 4.66 g/100 g "LAN" with an average of 1.52 g/100 g. In an other study, Doukani and Hadjer (2015) recorded traces of succinic acid in Algerian *A. unedo* fruits. Comparing our results with those of other authors, some organic acids were absents in our fruits, notably: oxalic, fumaric, lactic, suberic and quinic acids. Fumaric (0.15 g/100 g), lactic (0.05 g/100 g), suberic (0.023 g/100 g) and quinic (7.35 g/100 g) acids were detected and quantified by Ayaz et al. (2000) in Turkish *A. unedo* fruits. In other studies, Ruiz-Rodriguez et al. (2011) and Morales et al. (2013) recorded values of oxalic acid 0.05-0.15 g/100 g and 0.09 g/100 g respectively. The presence and composition of organic acids can be affected by various factors such as: growing conditions, maturity, season, geographical origin and soil type.

***3.3. Phytochemical Composition***

*3.3.1. Total Phenols*

The total phenols content (TPC) of *A. unedo* fruits were reported in Table 4. Significant differences (p=0.044) were observed among the genotypes. The total phenols content ranged between 25.37 mg/g DW "KSB" and 39.06 mg GAE/g DW "LAN", with an average of 30.98 mg/g DW.. Previous studies indicated a wide variation on total phenolic content among *A. unedo* genotypes, grown in diverse agro climatic conditions including Spain, Croatia, Algeria and Turkey, The TPC of *A. unedo* fruits reported in this study was higher than those reported by other authors ; Doukani and Tabak (2015) and Isbilir et al. (2012. They recorded a range of 7.02 to 14.74 mg GAE/g and 14.29 mg GAE/g in Algerian and Turkish *A. unedo* genotypes respectively. In another study, Seker and Toplu (2010) reported a TPC ranging from 17.7 to 25.8 mg GAE/g). According to these results, and despite natural variations, total phenols content in fruits of *A. unedo* grown in Morocco fruits was always over 39.06 mg GAE/g DW, indicating that it could be considered as an excellent source of polyphenols content which is of great importance in the light of the fact that modern diets are often lacking of bioactifs compounds.

*3.3.2. Total Flavonoids*

The results of the total flavonoids content were summarized in Table 4. A significant variation was observed (p = 0.002) among genotypes. The total flavonoids content ranged between 3.30 "KSB" and 7.07 mg GAE/g DW "TAH", with an average of 5.20 mg GAE/g DW. These concentrations were higher than those recorded by Pallauf et al. (2008) (0.32 mg/100 g), Bouzid et al. (2014) (2.18-6.54 mg EC/g) and by Jurica et al. (2017) (0.23-0.28 mg EQ/g). These differences could be attributed to the used methods and experimental conditions.

*3.3.3. Total Anthocyanins*

The total anthocyanins content was reported in Table 4. A statistically significant variation (p = 0.024) was observed among the genotypes studied. The anthocyanins values varied from 0.15 mg equivalent cya-3-glu/100 g DW "KSB" to 0.64 mg equivalent cya-3-glu/100 g DW "MDZ" with an average of 0.34 mg equivalent cya-3-glu/100 g DW. These values were lower than the ones recorded by Pallauf et al. (2008) (3.77 mg equivalent cya-3-glu/100 g DW).

***3.3. Antioxidant Activities***

The results obtained for antioxidant activity based on the radical scavenging capacity DPPH, ABTS and βeta carotene were reported in Table 5. Significant differences (p˂0.001) were observed among the genotypes. The average antioxidant activities values were 8.93, 7.82 and 5.58 mg ascorbic acid equivalents/g dry weight as determined by DPPH, ABTS, and βeta carotene assays, respectively. All genotypes presented scavenging effects against DPPH radical ranging from 3.33 to 21.08 mg ascorbic acid equivalent/g DW. The fruits collected from ‘‘LAN’’presented the lowest IC 50 value, revealing the highest radical scavenging activity among the samples and therefore, the highest antioxidant activity. These results were higher than those recorded by other authors. They reported that the value of scavenging activity (DPPH) of *A.unedo* fruitgrown in Tunisia was3.2 mg BHT equivalent/g DW (Ben Salem et al. 2018). Fonseca et al. (2015) reported also, a value of IC 50 ranging from 1.87 to 3.93 mg trolox equivalent/g DW in Portuguese *A. unedo* fruit. However, the results obtained in this study were lower than the values reported by Barros et al. (2010). They analysed the antioxidant activity of three wild fruits and they recorded values of scavenging activity (DPPH) 22.35, 29.85 and 21.4 mg trolox equivalent/g DW for *A. unedo* , *Prunus spinosa and Rosa canina* sl. respectively. The antioxidant activity determined by βeta carotene assay ranged between 1.08 and 13 mg ascorbic acid equivalent/g DW. The fruits of genotype ‘‘LAN’’ had significantly the lowest ABTS value, 1.08 mg ascorbic acid equivalent/g DW and therefore, the highest antioxidant activity. The results obtained in this study were lower than those reported by other authors; Isbilir et al. (2012) analysed the bleaching activity of βeta carotene. They recorded IC 50 values varied from 9.25 to 15.85 mg/g DW in Turkish fruits. In an other study, Barros et al. (2010) analysed the antioxidant activity throught βeta carotene bleaching method of three wild fruits (*A. unedo* , Prunus spinosa and Rosa canina sl.) and they recorded values 38.7, 49.3 and 19.8 mg trolox equivalent/g DW, respectively. Free radical scavenging activity of samples was determined by ABTS radical cation decolorization assay (Table 5). The value of ABTS assay ranged between 2.25 and 19.58 mg ascorbic acid equivalent/g DW. The fruits of genotype ‘‘LAN’’ revealed also the lowest ABTS value, 2.25 mg ascorbic acid equivalent/g DW and therefore, the highest antioxidant activity. The antioxidant capacity of *A. unedo* fruits determined in this study was higher than the amount presented by Ben Salem et al. (2018) who recorded (5.1 mg trolox/ g DW) in Tunisian *A. unedo* fruits. The *A. unedo* fruits had strong antioxidant activity for the βeta carotene assay. The different antioxidant levels observed in this study may reflect a relative difference in the ability of antioxidant compounds in extracts to reduce the free radical DPPH, ABTS, and oxidative bleaching of βeta carotene in vitro systems.

Antioxidant activity was widely studied on *A. unedo* fruits by using different antioxidant determining methods such as ABTS, TEAC, FRAP, DPPH etc. The studies indicated that type of extraction of phenols present in fruits of *A. unedo* influenced the antioxidant activity (Pallauf, et al. 2008; Barros, et al. 2010; Fortalezas, et al. 2010; Seker and Toplu, 2010; Mendes, et al. 2011; Ruiz-Rodríguez, et al. 2011; Isbilir, et al. 2012; Morales, et al. 2013). In addition, several studies reported that *A. unedo* fruit was found to be a powerful antioxidant plant more than other fruit such as pomegranate (Gil et al., 2000), red and green grape and apple juices, (Santini et al, 2014), pomace (Maragò et al., 2015), grape (Schempp et al., 2015 and Liu et al., 2018) which can be explained by the higher composition of strawberry, pomegranate, grape and apple in polyphenols.

***3.4. Profile of Polyphenolic Compounds***

The results of polyphenolic compounds were summarized in Table 6 and 7. Significant variations were observed at p <0.001 among all genotypes. 17 phenolic compounds were identified in *A. unedo* fruits. Gallocatechol was the dominant compound in the genotypes. The concentration of gallocatechol differed between genotypes. The highest level reported in "TAH" (65.31 mg/100 g DW) and the lowest in "CHF" (16.15 mg/100 g DW). Catechin was the dominant compound in the genotypes "CHF" and "MDZ". "CHF" had the highest concentration (49.36 mg/100 g DW) of catechin, and "LAN" had the lowest concentration (22.09 mg/100 g DW). Among the phenolic acid group, chlorogenic acid was significantly higher in the genotypes. The highest level was observed in "TAH" (27.42 mg/100 g DW), and the lowest in "CHF" (5.55 mg/100 g DW). Other minor compounds such as quercetin-3-xyloside, quercetin-3-galactoside, quercetin-3-glucoside, rutin, cyanidine-3-glucoside, cyanidine-3-5-diglucoside and cyanidine-3-arabinoside were also identified. Concerning the last two compound which are cyanidine 3,5 diglucoside and cyanidine 3 arabinoside, they were identified only in three genotypes (CHF, MDZ and TAH). The lowest amounts were recorded in "CHF" (0.61 mg/100 g DW) and (0.36 mg/100 g DW), respectively whereas the highest ones were recorded in "TAH" (3.30 mg/100 g DW) and (1.64 mg/100 g DW), respectively. Our results agreed with those of Ganhão et al. (2010) who reported catechin, gallic acid, ellagic acid, chlorogenic acid, rutin and cyanidin-3-glucoside in Spanish *A. unedo* fruits. In other study, Ayaz et al. (2000) identiﬁed six phenolics acids in Turkish *A. unedo* fruits, namely gallic acid (10.7 ± 0.04 mg/g DW), protocatechuic acid (0.6 ± 0.03 mg/g DW) and gentisic acid (1.9 ± 0.11 mg/g DW). However, Mendes et al. (2011) had identified other phenolic compounds in Portugaise *A.unedo* fruits. These compounds are gallic acid glucoside, galloylquinic acid, quinic acid derivative, proanthocyanidin dimer, galloylshikimic acid, digalloylquinic acid, digalloylshikimic acid, catechin monomer, proanthocyanidin trimer, strictinin ellagitannin, ellagitannin derivative, galloyl derivative, trigalloylshikimic acid, myricetin rhamnoside, quercetin glucoside, gallotannin and ellagic acid rhamnoside. In Italy, the phenolic compounds of *A. unedo* fruits included anthocyanins (delphinidin-3-O-galactoside, cyanidin-3-O-glucose and cyanidin-3-O-arabinoside); 4-arbutin, β-D-glucogalline; 3-O-galloylquinic acid; gallic acid, 4-O-β-D-glucopyranoside; 5-O-galloylquinic acid; 5-O-galloylshikimic acid; and 3-O-galloylshikimic acid (Pawlowska, et al. 2006).

***3.5. Volatile Compounds Characterization***

In this study, 25 volatile compounds were identified in *A.unedo* fruits using HS-SPME method combined to GC-MS analysis. Results of volatile compounds were reported in Table 8. . The volatile compounds present in all the genotypes were hexadecanoid acid, tetradecanoid acid, hexadecanoic acid, methyl ester, dodecanoic acid and phenol. Hexadecanoid acid was the most abundant in *A. unedo* fruits, ranging from 27.68 % in the "CHF" genotype to 52.18 % in the "MDZ" genotype. Moreover, 9-octadecenoic acid (Z)- was the second most abundant compound, ranging from 1.18 % for the "CHF" genotype to 37.60 % for the "LAN" genotype followed by tetradecanoid acid which varied from 6.90 % in the "LAN" genotype to 18.04 % in the "TAH" genotype. Other minor compounds such as octadecanoic acid, methyl ester, hexadecanoic acid, ethyl ester, 3-dodecene, (E) and benzene (2 Methyl-2-propeny) were also identified and the content was not exceed 1 %. Benzene (2 Methyl-2-propeny) was only presented in the "KSB" genotype and in very low amount (0.46 %). Additionally, hexadecanoic acid, ethyl ester and octadecanoic acid methyl ester were only identified in "TAH" genotype. According to the results of Oliveira et al. (2011), Alcohols are the main component of the volatile fraction of Turkish *A. unedo* fruits and the main volatile compound identified was (Z)-3-hexen-1-ol. This volatile compound was also identified in strawberries and their products (Barron and Etiévant. 1990; Hamilton-kemp et al. 1996; Hakala et al. 2001).

***3.6. Correlation among variables***

In order to identify the relations between biochemical traits, all variables were subjected to bivariate correlation using the Pearson coefficient. Signiﬁcant correlations at the level of 0.05 or 0.01 are summarized in the Table 9 and 10. In the current study, the correlation value was found between DPPH and total anthocyanins (r = 0.931; p<0.05) as well as between malic acid and titrable acidity (r = 0.763; p<0.01). Citric acid was also correlated to titrable acidity (r = 0.522; p<0.05), pH (r = 0.751; p<0.01) and soluble solids (r = -0.949; p<0.01). The results reported also, correlations between DPPH and anthocyanins (r = 0.645; p<0.01). Moreover, this study revealed correlations between ABTS and ascorbic acid (r = 0.526; p<0.05), anthocyanins (r = 0.748; p<0.01), and DPPH (r = 0.883; p<0.01). In addition, it conveyed correlations between βeta carotene and ascorbic acid (r = 0.514; p<0.05), anthocyanins (r = 0.554; p<0.05), DPPH (r = 0.950; p<0.01) and ABTS (r = 0.864; p<0.01). Also, cyanidin-3-glucoside was correlated to anthocyanins (r = 0.680; p<0.01). In this study, no correlation was observed between the antioxidant activity and total phenols. These results must be interpreted with caution as the Folin-Ciocalteu method used over estimates the concentration of phenolic containing compounds such as ascorbic acids and vitamins could interfere during total phenols evaluation and that do not give significant correlation. Furthermore, the synergism between the antioxidants in the mixture makes the antioxidant capacity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants. However, different works have reported good linear correlations between antioxidant activity test and total phenols (Su and Chien, 2007; Liu, et al. 2008; Anastasiadi, et al. 2010; Serçe, et al. 2010). The correlation coefﬁcients may provide information on the parameters that are potentially important in assessing *A. unedo* genotypes (Norman, et al. 2011). Signiﬁcant and strong correlated traits can be used to predict other ones, and could be considered of importance for genotypes characterization and discrimination (Podgornik, et al. 2010).

***3.7. Principal Component Analysis***

The aim of this analysis was to identify the main factors to reduce the number of effective parameters to use in classification of the *A. unedo* genotypes based on their biochemical, antioxidant capacity and volatile compounds. In our study, only a principal component loading of more than |0.5| was considered as being significant for each factor. Total variance was explained by four components. The first two components was explained 66.47% of the total variability observed (Table 11 ).

The first component accounted for 39.85 % of the total variance, which is strongly influenced by the gallic acid derivative (0.89), chlorogenic acid (0.89), ellagic acid derivative I (0.96), ellagic acid derivative II (0.86), ellagic acid (0.95), rutin (-0.93), cyanidin-3,5-diglucoside (0.87), tetradecanoic acid (0.88), limonene (0.97) and pentadecanoic acid (0.90). The second component accounted for 26.63 % of the total variance and is mainly influenced by gallic acid (0.91), gallocatechol (0.91) and 9-octadecenoic acid (Z) (0.88). Generally, these results were in accordance with those reported in previous *A. unedo* biochemical studies (Gündoğdu, et al. 2018; Colak, 2019). They have reported that the biochemical attributes are important in order to evaluate the variation in traits of *A. unedo* genotypes. Scatter plot was prepared according to the first two principal components: PC1 and PC2 (respectively 39.85 and 26.63 % of total variance) that discriminate between the genotypes according to their volatile compounds and biochemical characteristics (Figure 1 ). Starting from negative to positive values of PC1, the distribution of genotypes indicated an increased in the succinic acid and the most of phenolic compounds. Whereas, starting from negative to positive values of PC2, total soluble solids, malic acid and the most of volatile compounds decreased in their values. However, the distribution of genotypes indicated an increase in the titratable acidity, pH and citric acid. Our results are in agreement with several studies (Gündoğdu, et al. 2018; Colak, 2019).

**4. Conclusion**

This study revealed that *A. unedo* fruits can be considered an important source of polyphenols (25-39 mg GAE/g DW). Among the seventeen phenolic compounds identiﬁed by HPLC, gallocatechol and catechin were the most abundant compounds. Moreover, four organic acids were identified in *A. unedo* fruits which citric acid was the most dominant. Results showed also that hexadecanoic acid was the most abundant volatile compound in all the studied genotypes. According to results obtained in this study, *A. unedo* fruits are strong radical scavengers that can be considered as good sources of natural antioxidants, the fact that may encourage their daily intakes as an alternative source of bioactive compounds in the local population diet.In view of its biochemical composition, the use of *A. unedo* fruits in some food and medicinal products may be also suggested. This study contributes not only to a better knowledge of these wild fruits but also to their valorisation.

**Author contribution**

Hafida Zitouni and Lahcen Hssaini: Supervision, Conceptualization, Methodology, Software, Formal analysis and Writing Original draft. Hafida Hanine, Lahcen Hssaini, and Messaoudi Zerhoune: Conceptualization and Validation. Marie Laure Fauconnier, Francisca Hernandez, Manuel Viuda-Martos: Formal analysis. Sezai Ercisli: Review and Rachida Ouaabou, Ait Haddou Lhoussain : Data Curation.

**Declaration of Interest**

The authors declare no conflict of interest.

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**References**

Alarcão-E-Silva, M., A. Leitão, H. Azinheira, and M.C.A. Leitão. 2001. The *Arbutus unedo* berry: studies on its color and chemical characteristics at two mature stages. J. Food Compost. 14:27-35.

Anastasiadi, M., H. Pratsinis, D. Kletsas, A.L. Skaltsounis, and S.A. Haroutounian. 2010. Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. Food Res. Inter. 43:805-813.

AOAC. 2002. Official Methods of Analysis. Association of Official Agricultural Chemists. 17th Ed. Gaithersburg, USA. 480 p.

Ayaz, F.A., M. Kucukislamoglu, and M. Reunanen. 2000. Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var. ellipsoidea) fruits. J.Agric. Food Chem. 13:171-177.

Barron, D., and P.X. Etiévant. 1990. The volatile constituents of strawberry jam. Zeitschrift fur lebensmittel-Untersuchung und-Forschung. 191:279-285.

Barros, L., A.M. Carvalho, J.S. Morais, and I.C. Ferreira. 2010. Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties. Food Chem. 120:247-254.

Ben Salem, I., S. Ouesleti⁠b, Y. Mabrouk⁠a, A. Landolsi⁠c, M. Saidi⁠a, and A. Boulilla⁠d. 2018. Exploring the nutraceutical potential and biological activities of *Arbutus unedo* L. (Ericaceae) fruits. Ind. Crop. Prod. 122:726-731.

Bnouham, M., F.Z. Merhfour, A. Legssyer, H. Mekhﬁ, S. Maallem, and A. Ziyyat. 2007. Antihyperglycemic activity of *Arbutus unedo*, *Ammoides pusilla* and *Thymelaea hirsuta*. Pharmazie. 62(8):630-2.

Bouzid, K., F. Toumi Benali, R. Chadli, M. Bouzouina, A. Bouzid, A. Benchohra, and M.M. Dif. 2014. Extraction, Identification and Quantitative HPLC Analysis of Flavonoids From Fruit Extracts of *Arbutus unedo* L from Tiaret Area (Western Algeria). Eurp. J. Mol. Biotech. 6(4):160-169.

Celikel, G., L. Demirsoy, and H. Demirsoy. 2008. The strawberry tree (*Arbutus unedo* L.) selection in Turkey. Sci. Hortic. 118:115-119.

Colak, A.M. 2019. Morphological and Biochemical Diversity in Fruits of *Arbutus unedo* L. from East Aegean Region in Turkey. Erwerbs-Obstbau. 61:379-383.

Dorman, H.J.D., and R. Hiltunen. 2004. Fe (II) reductive and free radical scavenging properties of summer savory (*Satureja hortensis* L.) extract and subfractions. Food Chem. 88:193-199.

Doukani, K., and M. Hadjer. 2015. Physico-chemical and nutritional characterization of *Arbutus unedo* L. from the region of Tiaret (Algeria). Int. J. Humanities, Arts, Medicine and Sci. 3(8):1-1.

Doukani, K., and S. Tabak. 2015. Profil Physicochimique du fruit "Lendj" (*Arbutus unedo* L.). Revue «Nature & Technologie». B-Sciences Agronomiques et Biologiques. 12:53-66.

Fonseca, D.F.S., Â.C. Salvador, S.A.O. Santos, C. Vilela, C.S.R. Freire, A.J.D. Silvestre, and S.M. Rocha. 2015. Bioactive phytochemicals from wild *Arbutus unedo* L. berries from different locations in Portugal: quantification of lipophilic components. Int. J. Mol. Sci. 16:14194-14209.

Fortalezas, S., L. Tavares, R. Pimpão, M. Tyagi, V. Pontes, P.M. Alves, G. McDougall, D. Stewart, R.B. Ferreira, and C.N. Santos. 2010. Antioxidant properties and neuroprotective capacity of strawberry tree fruit (*Arbutus unedo*). Nutrients. 2:214-229.

Ganhão, R., M. Estévez, P. Kylli, M. Heinonen, and D. Morcuende. 2010. Characterization of selected wild Mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. J.Agric. Food Chem. 58:854-861.

Garcia-Alonso, M., S. de Pascual-Teresa, C. Santos-Buelga, and J.C. Rivas-Gonzalo. 2004. Evaluation of the antioxidant properties of fruits. Food Chem. 84:13-18.

Genskowsky, E., L.A. Puente, J.A. Pérez-Álvarez, J. Fernández-López, L.A. Muñoz, and M. Viuda-Martos. 2016. Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [Aristotelia chilensis (Molina) Stuntz] a Chilean blackberry. J. Sci. Food. Agric. 96:4235-4242.

Gentile, C., M. Allegra, F. Angileri, A. M. Pintaudi, M. A. Livrea, and L. Tesoriere. 2012. “Polymeric proanthocyanidins from Sicilian pistachio (*Pistacia vera* L.) nut extract inhibit lipopolysaccharide-induced inflammatory response in RAW 264.7 cells”. Eur. J. Nutr. 51(3):353-63.

Gil, M.,  [F.A. Tomás-Barberán](https://www.researchgate.net/profile/Francisco_Tomas-Barberan?_sg%5B0%5D=y31p0bhGgRiQeglJmnB2wEJ_y_Q74ccnH1TznNxzmkxSkd3NP27m0l9OrbW9PWulsGVghQU.BFXnnuBup_RBNFbzyxOwUbKGfRj63mM9ZoyXO0vI3UWRphXEvFP5kwRRK8Dt3f3FE4mO9PrOxORkYwxV7VYSVA&_sg%5B1%5D=1iMNlRjRloHrbzGw0VqXQ8LA73WjyEowSwEhCm9ObJBIMX4P3f9XGabEmZGcggfDLuKzlic.l5k8cP7r5o2UNYwkt7Pcal6opDHMnKxidT0uXUG42YY26rse5z-3jRmmL-ctTi_TxNoNF5r2IMepq1jbwgVt-w), B. [Hess-Pierce](https://www.researchgate.net/scientific-contributions/33551535_Betty_Hess-Pierce?_sg%5B0%5D=y31p0bhGgRiQeglJmnB2wEJ_y_Q74ccnH1TznNxzmkxSkd3NP27m0l9OrbW9PWulsGVghQU.BFXnnuBup_RBNFbzyxOwUbKGfRj63mM9ZoyXO0vI3UWRphXEvFP5kwRRK8Dt3f3FE4mO9PrOxORkYwxV7VYSVA&_sg%5B1%5D=1iMNlRjRloHrbzGw0VqXQ8LA73WjyEowSwEhCm9ObJBIMX4P3f9XGabEmZGcggfDLuKzlic.l5k8cP7r5o2UNYwkt7Pcal6opDHMnKxidT0uXUG42YY26rse5z-3jRmmL-ctTi_TxNoNF5r2IMepq1jbwgVt-w), and A. [Kade](https://www.researchgate.net/profile/Adel_Kader?_sg%5B0%5D=y31p0bhGgRiQeglJmnB2wEJ_y_Q74ccnH1TznNxzmkxSkd3NP27m0l9OrbW9PWulsGVghQU.BFXnnuBup_RBNFbzyxOwUbKGfRj63mM9ZoyXO0vI3UWRphXEvFP5kwRRK8Dt3f3FE4mO9PrOxORkYwxV7VYSVA&_sg%5B1%5D=1iMNlRjRloHrbzGw0VqXQ8LA73WjyEowSwEhCm9ObJBIMX4P3f9XGabEmZGcggfDLuKzlic.l5k8cP7r5o2UNYwkt7Pcal6opDHMnKxidT0uXUG42YY26rse5z-3jRmmL-ctTi_TxNoNF5r2IMepq1jbwgVt-w). 2000. Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing, J.Agric. Food Chem. 48(10):4581-9.

Giusti, M., and R.E. Wrolstad. 2001. Characterization and measurement of 1041 anthocyanins by UV–visible spectroscopy, current protocols. Food Chem. Anal. 1042:F1.2.1-F1.2.13.

González, E.A., A.T. Agrasar, L.M. Castro, I.O. Fernández, and N.P. Guerra. 2011. Solid state fermentation of red raspberry (*Rubus ideaus* L) and Arbutus berry (*Arbutus unedo* L) and characterization of their distillates. Food Research Int. 44:1419-1426.

Gündoğdu, M., S. Ercisli, I. Canan, E. Orman, M. Sameeullah, M. Naeem, and R. Ben Aye. 2018. Diversity in phenolic compound, biochemical and pomological characteristics of *Arbutus unedo* fruits. Folia Hort. 30(1):139-146.

Hakala, M., M. Ahro, J. Kauppinen, H. Kallio. 2001. Determination of strawberry volatiles with low resolution gas phase FT-IP analyzer. European Food Research and Technology. 212:505-510.

Hamilton-Kemp, T.R., D.D. Archbold, J.H. Loughrin, R.W. Collins, M.E. Byers. 1996. Metabolism of Natural Volatile Compounds by Strawberry Fruit. Journal of Agricultural and Food Chemistry. 44(9):2802-2805.

Hernández, F., L. Noguera-Artiaga, F. Burló, A. Wojdyło, A.A. Carbonell-Barrachina, and P. Legua. 2016. Physico-chemical, nutritional, and volatile composition and sensory profile of Spanish jujube (Ziziphus jujuba Mill.) fruits. J.Sci. Food Agric. 96(8):2682-2691.

Huberson, M. Evolution du pH Pendant la Fermentation Alcoolique de Moûts de Raisins: Modélisation et Interprétation Métabolique. 2008. Ph.D. Thesis, Institut National Polytechnique de Toulouse, Toulouse, France, p. 121.

Isbilir, S.S., H.H. Orak, H. Yagar, and N. Ekinci. 2012. Determination of antioxydant activities of strawberry tree (*Arbutus unedo* L.) flowers and fruits at different ripenning stages. Acta Sci. Pol., Hortorum Cultus. 11(3): 223-237.

Jackobek, L., M. Šeruga, I. Novak, and M. Medvidovic-Kosanovic. 2007. Flavonols, Phenolic acids and Antioxidant Activity Of Some Red Fruits. Deutsche Leben smittel Rundschau. 103:369-378.

Jurica, K., I. Brcic-Karaconji, R. Jurisic-Grubesic, and D. Vitali-Cepoa. 2017. The nutritional and antioxidant properties of strawberry tree (*Arbutus unedo* L.) fruit. Food Safety and Quality Congress with international participation. New Achievements and Future Challenges.

Lamaison, J.L., and A. Carnat. 1990. Teneurs en principaux flavonoids des fleurs de Crataegeus monogyna Jacq et de Crataegeus laevigata (Poiret D. C) en fonction de la vegetation. Pharm. Acta Helv. 65(11):315-320.

[Liu](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20Q%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), Q., [G-Y. Tang](https://www.ncbi.nlm.nih.gov/pubmed/?term=Tang%20GY%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [C-N. Zhao](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zhao%20CN%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [X-L. Feng](https://www.ncbi.nlm.nih.gov/pubmed/?term=Feng%20XL%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [X-Y. Xu](https://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20XY%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [S-Y. Cao](https://www.ncbi.nlm.nih.gov/pubmed/?term=Cao%20SY%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [X. Meng](https://www.ncbi.nlm.nih.gov/pubmed/?term=Meng%20X%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [S. Li](https://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20S%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), [R-Y. Gan](https://www.ncbi.nlm.nih.gov/pubmed/?term=Gan%20RY%5BAuthor%5D&cauthor=true&cauthor_uid=30249027), and [H-B. Li](https://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20HB%5BAuthor%5D&cauthor=true&cauthor_uid=30249027). 2018. Comparison of Antioxidant Activities of Different Grape Varieties, [Molecules](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6222363/). 23(10):24-32.

Liu, X., M. Zhao, J. Wang, B. Yang, and Y. Jiang. 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Anal. J. Food Compos. 21:219-228.

Maccarrone, E., G. Cuffari, A. Passerini, and P. Rapisarda. 1990. The anthocyanins of *Arbutus unedo* L. fruits (Ericaceae). Ann. Chim. 80:171-176.

Males, Z., Plazibat, M., Vundac, V.B., and Zunta, I., 2006. Qualitative and quantitative analysis of ﬂavonoids of the strawberry tree – *Arbutus unedo* L. (Ericaceae). Acta Pharm. 56, 245–250.

Maragò, E., P. Iacopini, F. Camangi, C. Scattino, A. Ranieri, A. Stefani and L. Sebastiani. 2015. Phenolic profile and antioxidant activity in apple juice and pomace: effects of different storage conditions, Fruits. 70:213-223.

Mendes, L., V. de Freitas, P. Baptista, and P. Carvalho. 2011. Comparative antihemolytic and radical scavenging activities of strawberry tree (*Arbutus unedo* L.) leaf and fruit. Food Chem. Toxicol. 49:2285-2291.

Mennen, L.I., D. Sapinho, A. De Bree, N. Arnault, S. Bertrais, P. Galan and S. Hercberg. 2004. Consumption of foods rich in flavonoids is related to a decreased cardiovascular risk in apparently healthy French women. Journal of nutrition. 134(4):923-926.

Messaid, H. 2008. Optimisation du Processus D’immersion-Réhydratation du Système Dates Sèche-Jus D’Orange. Master’s Thesis, Université M’hamed Bouguara, Boumerdés, Algeria, p. 74.

Morales, P., I.C.F.R. Ferreira, A.M. Carvalho, V. Fernández-Ruiz, M.C. Sánchez-Mata, M. Câmara, R. Morales, and J. Tardio. 2013. Wild edible fruits as a potential source of phytochemicals with capacity to inhibit lipid peroxidation. Eur. J. Lip. Sci. Technol. 115:176-185.

Müller, L., S. Gnoyke, A.M. Popken, and V. Böhm. 2010. Antioxidant capacity and related parameters of different fruit formulations. Food. Sci. Technol. 43:992-999.

Norman, P.E., P. Tongoona, and P.E. Shanahan. 2011. Determination of interrelationships among agrmorphological traits of yams (Discorea spp.) using correlation and factor analyses. J. Appl. Biosci. 45: 3059–3070.

Oliveira, I.; de Pinho, P.G.; Malheiro, R.; Baptista, P.; Pereira, J.A. 2011, Volatile profile of *Arbutus unedo* L. fruits through ripening stage. *Food Chem*, 128(3), 667-673.

Özcan, M.M., and H. Hacıseferoğulları. 2007. The strawberry tree (*Arbutus unedo* L.) fruits: Chemical composition, physical properties and mineral contents. J. Food. Eng. 44:307-315.

Pallauf, K., J.C. Rivas-Gonzalo, M.D. Castillo, M.P. Cano, and S. Pascual-Teresa. 2008. Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. J. Food. Compos. Analys. 21:273-281.

Pandey, K.B., and S.I. Rizvi. 2009. “Plant polyphenols as dietary antioxidants in human health and disease”. Oxid Med Cell Longev. 2(5):270-278.

Pawlowska, A.M., M. De-Leo, and A. Braca. 2006. Phenolics of *Arbutus unedo* L. (Ericaceae) fruits: Identiﬁcation of anthocyanins and gallic acid derivatives. J.Agric. Food Chem. 54:10234-10238.

[Pimpão](https://www.researchgate.net/profile/Rui_Carlos_Pimpao?_sg%5B0%5D=yffFBcslxC3flgXVnZeuxyTrIP5EkoYLdCSmyrN_y27q7V7TNMzUFUd8Ehtts7mbQuqSTqM.KWRDPEGFkBbEq6dHnp-L0ZocwN6-RRB10E2c_enaG38FKMbKCIF81oEKIrNKCTYdJA2R7YGKEp5Z9i8VxRLGZw&_sg%5B1%5D=m_AqnWouQBAZmCjKYfhw4WPQX_arcN0AW3DLSuBu2CSt5dXNHhngQzWKWaM4dzkTr8ihQoM.-5xPB_o8oulcfbOtI7ms_RHn1voR7gAnd52Rfohb8WRSfTfz4FfA_vinf79V1wxE19G3gS8Wccl6ghCLBWJyPg), R.C.S., T. [Dew](https://www.researchgate.net/scientific-contributions/57236136-Tristan-P-Dew?_sg%5B0%5D=yffFBcslxC3flgXVnZeuxyTrIP5EkoYLdCSmyrN_y27q7V7TNMzUFUd8Ehtts7mbQuqSTqM.KWRDPEGFkBbEq6dHnp-L0ZocwN6-RRB10E2c_enaG38FKMbKCIF81oEKIrNKCTYdJA2R7YGKEp5Z9i8VxRLGZw&_sg%5B1%5D=m_AqnWouQBAZmCjKYfhw4WPQX_arcN0AW3DLSuBu2CSt5dXNHhngQzWKWaM4dzkTr8ihQoM.-5xPB_o8oulcfbOtI7ms_RHn1voR7gAnd52Rfohb8WRSfTfz4FfA_vinf79V1wxE19G3gS8Wccl6ghCLBWJyPg),  [P.B. Oliveira](https://www.researchgate.net/profile/Pedro_Bras_De_Oliveira?_sg%5B0%5D=yffFBcslxC3flgXVnZeuxyTrIP5EkoYLdCSmyrN_y27q7V7TNMzUFUd8Ehtts7mbQuqSTqM.KWRDPEGFkBbEq6dHnp-L0ZocwN6-RRB10E2c_enaG38FKMbKCIF81oEKIrNKCTYdJA2R7YGKEp5Z9i8VxRLGZw&_sg%5B1%5D=m_AqnWouQBAZmCjKYfhw4WPQX_arcN0AW3DLSuBu2CSt5dXNHhngQzWKWaM4dzkTr8ihQoM.-5xPB_o8oulcfbOtI7ms_RHn1voR7gAnd52Rfohb8WRSfTfz4FfA_vinf79V1wxE19G3gS8Wccl6ghCLBWJyPg), and [C.N. Santos](https://www.researchgate.net/profile/Claudia_Santos9?_sg%5B0%5D=yffFBcslxC3flgXVnZeuxyTrIP5EkoYLdCSmyrN_y27q7V7TNMzUFUd8Ehtts7mbQuqSTqM.KWRDPEGFkBbEq6dHnp-L0ZocwN6-RRB10E2c_enaG38FKMbKCIF81oEKIrNKCTYdJA2R7YGKEp5Z9i8VxRLGZw&_sg%5B1%5D=m_AqnWouQBAZmCjKYfhw4WPQX_arcN0AW3DLSuBu2CSt5dXNHhngQzWKWaM4dzkTr8ihQoM.-5xPB_o8oulcfbOtI7ms_RHn1voR7gAnd52Rfohb8WRSfTfz4FfA_vinf79V1wxE19G3gS8Wccl6ghCLBWJyPg). 2013. Analysis of Phenolic Compounds in Portuguese Wild and Commercial Berries after Multienzyme Hydrolysis. J.Agric. Food Chem. 61(17).

Podgornik, M., I. Vuk, I. Vrhovnik, and D. Bandelj. 2010. Scientia Horticulturae Asurvey and morphological evaluationof ﬁg (*Ficus Carica* L.) genetic resources from Slovenia. Sci Hortic. 125(3):380-389.

Rodríguez, M.L., J.M. Estrela, and ÁL. Ortega. 2013. Natural polyphenols and apoptosis induction in cancer therapy. J. Carcinog Mutagn. S6:004.

Ruiz-Rodriquez, B.M., P. Morales, V. Fernandz-Ruiz, M.C. Sánchez-Mata, M. Cámara, C. Díez-Marqués, M. Pardo-De-Santayana, M. Molina, and J. Tardío. 2011. Valorization of wild strawberrytree fruits (*Arbutus unedo* L) through nutritional assessment and natural production data .Food. Research. Inter. 44: 1244-1253.

Santini, A., R. Romano, G. Meca, A. Raiola, and A. Ritieni. 2014. Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. J. Food. Research. 3(4): 0887-0895.

Schempp, H., S. Christof, U. Mayr, and D. Treutter. 2015. Phenolic compounds in juices of apple cultivars and their relation to antioxidant activity. J. Applied Botany and Food Quality. 89:11-20.

Seker, M., and C. Toplu. 2010. Determination and comparison of chemical characteristics of *Arbutus unedo* L. and *Arbutus andrachnae* L. (family Ericaceae) fruits. J. Med. Food. 13(4), 1013-1018.

Serçe, S., M. Özgen, A.A. Torun, and S. Ercişli. 2010. Chemical composition, antioxidant activities and total phenolic content of *Arbutus andrachne* L. (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey. J. Food. Compos. 23: 619-623.

Shehzad, A., S. Ul. Islam, E.A. Al-Suhaimi, and Y., S. Lee. 2018. Pleiotropic Effects of Bioactive Phytochemicals (Polyphenols and Terpenes), 47-88p.

Su, M.S., and P.J. Chien. 2007. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (Vaccinium ashei) fluid products as affected by fermentation. Food Chem. 104:182-187.

Sulusoglu, M., and ES. Cavusoglu. 2011. *Arbutus unedo* L. (strawberry tree) selection in Turkey Samanli mountain locations. J. Med. Plants. Res. 5(15):3545-3551.

Tavares, L., S. Fortalezas, C. Carrilho, G. Mc Dougall, D. Stewart, R.B. Ferreira, and C.N. Santos. 2010. Antioxidant and antiproliferative properties of strawberry tree tissues. J. Berry Res. 1:3-12.

Vidrih, R., J. Hribar, Z. Prgomet, and N. Poklar-Ulrih. 2013. The physicochemical properties of strawberry tree (*Arbutus unedo* L.) fruits. Croat. J. Food. Sci. Technol. 5(1):29–33.

|  |  |  |  |
| --- | --- | --- | --- |
| Geographical origin | Code | Zone | Altitude (m) |
| Chefchaouen | CHF | Rif | 534 |
| Moulay Driss Zerhoun | MDZ | Middle Atlas | 820 |
| Laanoucer | LAN | Middle Atlas | 1700 |
| El Ksiba | KSB | Middle Atlas | 1360 |
| Tahnaout | TAH | High Atlas | 1200 |

Table 1. Origins geographic of the different genotypes studied

Table 2. Physicochemical parameters of *A. unedo* genotypes

|  |  |  |  |
| --- | --- | --- | --- |
| Genotype name | TA (g malic acid/  100 g FW) | pH | TSS (%) |
| KSB | 0.72 ± 0.02ab | **2.44 ± 0.03a** | **18.53 ± 0.50d** |
| CHF | 0.81 ± 0.01b | 3.76 ± 0.01c | 16.63 ± 0.40b |
| MDZ | **0.65 ± 0.01a** | 3.71 ± 0.01c | 16.83 ± 0.29bc |
| LAN | 0.97 ± 0.01c | **3.92 ± 0.02d** | **14.83 ± 0.29a** |
| TAH | **1.01 ± 0.10c** | 2.99 ± 0.10b | 17.53 ± 0.45c |
| Mean | 0.83 | 3.36 | 16.87 |
| Std. Deviation | 0.15 | 0.58 | 1.30 |
| ANOVA  Mean square | 0.07\*\*\* | 1.19\*\*\* | 5.56\*\*\* |

\*\*\* denote significant of difference at level 0.001; Data values are means ± SD; Values in bold represent, in each colunm, the minimum and the maximum for each variable; Different letters (a-d) in the columns represent statistically significant differences among genotypes according to Duncan's multi-range test at p˂0.05; **TA :** Titratable acidity; **TSS:** Total soluble solids, FW ( Fresh weight)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genotype name | Citric acid | Malic acid | Ascorbic acid | Succinic acid |
| KSB | **1.74 ± 0.31a** | **1.53 ± 0.29a** | **0.28 ± 0.07a** | 0.60 ± 0.13a |
| CHF | 3.22 ± 0.11b | 2.36 ± 0.08c | 0.71 ± 0.09b | **0.49 ± 0.03a** |
| MDZ | 2.76 ± 0.10b | 1.88 ± 0.08b | 0.95 ± 0.07c | 0.77 ± 0.05b |
| LAN | **5.32 ± 0.40c** | 2.32 ± 0.15c | 0.68 ± 0.04b | **4.66 ± 0.12d** |
| TAH | 2.80 ± 0.15b | **2.87 ± 0.12d** | **1.00 ± 0.01c** | 1.11 ± 0.01c |
| Mean | 3.17 | 2.19 | 0.72 | 1.52 |
| Std. Deviation | 1.24 | 0.49 | 0.27 | 1.64 |
| ANOVA  Mean square | 5.24\*\*\* | 0.78\*\*\* | 0.24\*\*\* | 9.38\*\*\* |

Table 3. Composition of organic acid and ascorbic acid of *A. unedo* genotypes (g/100 g DW) ( Dry weight)

\*\*\* denote significant of difference at level 0.001; Data values are means ± SD; Values in bold represent, in each colunm, the minimum and the maximum for each variable; Different letters (a-d) in the columns represent statistically significant differences among genotypes according to Duncan's multi-range test at p˂0.05

Table 4. Phytochemical composition at genotypes site

|  |  |  |  |
| --- | --- | --- | --- |
| Genotype name | Total phenols  (mg GAE/g DW) | Total flavonoids  (mg RE/g DW) | Total anthocyanins  (mg C-3-GE/100 g DW) |
| KSB | **25.37 ± 5.60a** | **3.30 ± 0.60a** | **0.15 ± 0.09a** |
| CHF | 28.71 ± 7.34a | 4.49 ± 0.87ab | 0.30 ± 0.14a |
| MDZ | 34.72 ± 6.53ab | 6.09 ± 0.88cd | **0.64 ± 0.20b** |
| LAN | **39.06 ± 2.44b** | 5.07 ± 1.04bc | 0.18 ± 0.09a |
| TAH | 27.07 ± 0.96a | **7.07 ± 0.67d** | 0.43 ± 0.23ab |
| Mean | 30.98 | 5.20 | 0.34 |
| Std. deviation | 6.88 | 1.51 | 0.23 |
| ANOVA  Mean square | 98.39\* | 6.31\*\* | 0.12\* |

\* denote significant of difference at level 0.05; \*\* denote significant of difference at level 0.01; Data values are means ± SD; Values in bold represent, in each colunm, the minimum and the maximum for each variable; Different letters (a-d) in the columns represent statistically significant differences among genotypes according to Duncan's multi-range test at p˂0.05 ; **GAE :** Gallic acid equivalent ; **RE :** Rutin equivalent ; **C-3-GE :** Cyanidin-3-glucoside equivalent

Table 5. Free radical scavenging activity (DPPH and ABTS) and βeta carotene (mean ± SD) in mg ascorbic acid equivalent/g DW (Dry Weight) of *A. unedo* genotypes.

|  |  |  |  |
| --- | --- | --- | --- |
| Genotype name | DPPH | ABTS | β-CAROTENE |
| KSB | 5.75 ± 2.00ab | 4.83 ± 1.88ab | 3.50 ± 0.75ab |
| CHF | 4.50 ± 2.41ab | 3.33 ± 1.13a | 2.83 ± 0.76a |
| MDZ | **21.08 ± 5.55c** | **19.58 ± 4.49c** | **13.00 ± 4.34c** |
| LAN | **3.33 ± 1.51a** | **2.25 ± 0.90a** | **1.08 ± 0.38a** |
| TAH | 10.00 ± 3.77b | 9.08 ± 3.01b | 7.50 ± 3.12b |
| Mean | 8.93 | 7.82 | 5.58 |
| Std. deviation | 7.29 | 6.92 | 4.87 |
| ANOVA Mean square | 157.43\*\*\* | 150.03\*\*\* | 68.12\*\*\* |

\*\*\* denote significant of difference at level 0.001; Data values are means ± SD; Values in bold represent, in each colunm, the minimum and the maximum for each variable; Different letters (a-c) in the columns represent statistically significant differences among genotypes according to Duncan's multi-range test at p˂0.05

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype name | GA | PC | GC | GAD | CAT | CA | SA | EADI | EADII |
| KSB | 21.88 ± 0.01c | 3.14 ± 0.01c | 45.23 ± 0.05c | 10.15 ± 0.01d | 33.60 ± 0.03c | 14.50 ± 0.00d | 7.40 ± 0.01c | 18.59 ± 0.01d | 15.96 ± 0.01c |
| CHF | 6.09 ± 0.00b | 2.57 ± 0.01b | **16.15 ± 0.03a** | **4.98 ± 0.00a** | **49.36 ± 0.01e** | **5.55 ± 0.00a** | **4.27 ± 0.00a** | 13.32 ± 0.01b | **8.97 ± 0.01a** |
| MDZ | **4.56 ± 0.02a** | **1.84 ± 0.00a** | 17.11 ± 0.07b | 7.36 ± 0.01c | 38.98 ± 0.05d | 12.10 ± 0.01b | 6.17 ± 0.01b | 17.22 ± 0.05c | 9.40 ± 0.04b |
| LAN | 35.83 ± 0.02d | 4.18 ± 0.03d | 58.79 ± 0.33d | 7.30 ± 0.01b | **22.09 ± 0.08a** | 12.48 ± 0.02c | **7.94 ± 0.02e** | **8.05 ± 0.03a** | 9.40 ± 0.10b |
| TAH | **36.93 ± 0.02e** | **5.90 ± 0.01e** | **65.31 ± 0.04e** | **14.54 ± 0.02e** | 24.68 ± 0.08b | **27.42 ± 0.02e** | 7.80 ± 0.01d | **25.06 ± 0.04e** | **21.39 ± 0.02d** |
| Mean | 21.06 | 3.53 | 40.52 | 8.87 | 33.74 | 14.41 | 6.72 | 16.45 | 13.02 |
| Std. deviation | 14.40 | 1.46 | 21.27 | 3.39 | 10.24 | 7.42 | 1.42 | 5.85 | 5.10 |
| ANOVA  mean square | 725.36\*\*\* | 7.49\*\*\* | 1584.06\*\*\* | 40.19\*\*\* | 327.11\*\*\* | 192.58\*\*\* | 7.06\*\*\* | 119.70\*\*\* | 90.92\*\*\* |

Table 6. Polyphenolic compounds at genotypes site (mean ± SD)

\*\*\* denote significant of difference at level 0.001 ; Data values are means ± SD; Values in bold represent, in each colunm, the minimum and the maximum for each variable; Different letters (a-e) in the columns represent statistically significant differences among genotypes according to Duncan's multi-range test at p˂0.05 ; GA: Gallic acid; PC: Protocatechuic; GC: Gallocatechol ; GAD: Gallic acid derivative; CAT: Catechin; CA: Chlorogenic acid; SA: Syringic acid; EADI: Ellagic acid derivative I; EADII: Ellagic acid derivative II

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype name | EA | C3G | Q3X | RT | Q3GA | Q3G | C3,5DG | C3A |
| KSB | 18.00 ± 0.00d | **0.43 ± 0.01a** | **4.09 ± 0.01e** | 1.06 ± 0.01c | **3.46 ± 0.02d** | **2.89 ± 0.00d** | n.d | n.d |
| CHF | **8.42 ± 0.01a** | 2.27 ± 0.00c | 2.11 ± 0.01b | 1.17 ± 0.00d | **1.66 ± 0.00a** | **2.11 ± 0.01a** | **0.61 ± 0.00a** | **0.36 ± 0.01a** |
| MDZ | 14.34 ± 0.02c | 5.68 ± 0.01d | **1.43 ± 0.01a** | 0.96 ± 0.00b | 3.02 ± 0.01c | 2.12 ± 0.01a | 1.59 ± 0.02b | 1.07 ± 0.00b |
| LAN | 10.27 ± 0.05b | 0.57 ± 0.02b | 2.72 ± 0.03c | **1.26 ± 0.01e** | 3.03 ± 0.04c | 2.54 ± 0.02c | n.d | n.d |
| TAH | **33.73 ± 0.02e** | **7.21 ± 0.01e** | 2.81 ± 0.03d | **0.90 ± 0.02a** | 2.73 ± 0.02b | 2.27 ± 0.01b | **3.30 ± 0.02c** | **1.64 ± 0.01c** |
| Mean | 16.95 | 3.23 | 2.63 | 1.07 | 2.78 | 2.39 | 1.10 | 0.61 |
| Std.deviation | 9.34 | 2.84 | 0.91 | 0.14 | 0.63 | 0.30 | 1.29 | 0.67 |
| ANOVA mean square | 305.06\*\*\* | 28.25\*\*\* | 2.91\*\*\* | 0.06\*\*\* | 1.38\*\*\* | 0.33\*\*\* | 5.82\*\*\* | 1.55\*\*\* |

Table 7 . Polyphenolic compounds at genotypes site (mean ± SD) (continued).

\*\*\* denote significant of difference at level 0.001 ; Data values are means ± SD; Values in bold represent, in each colunm, the minimum and the maximum for each variable; Different letters (a-e) in the columns represent statistically significant differences among genotypes according to Duncan's multi-range test at p˂0.05 ; EA: Ellagic acid; C3G: Cyanidin-3-glucoside; Q3X: Quercetin-3-xyloside; RT: Rutin; Q3GA: Quercetin-3-galactoside; Q3G: Quercetin-3-glucoside; C3,5D: Cyanidin-3,5-diglucoside; C3A : Cyanidin-3-arabinoside.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Retention time  (min) | Compounds (%) | Genotype name | | | | |
| **CHF** | **MDZ** | **LAN** | **KSB** | **TAH** |
| 4.89 | **Furfural** | 10.88 | n.d. | 7.28 | 0.58 | n.d. |
| 8.52 | **Phenol** | 5.15 | 1.55 | 1.92 | 1.71 | 1.04 |
| 9.51 | **Limonene** | n.d. | 1.29 | n.d. | 0.48 | 2.69 |
| 12.26 | **Benzene (2 Methyl 2 propenyl)** | n.d. | n.d. | n.d. | 0.46 | n.d. |
| 13.47 | **N-ethyl-1.3-dithioisoindoline** | n.d. | 0.98 | 4.09 | n.d. | n.d. |
| 17.12 | **1H Indole** | n.d. | n.d. | 1.35 | n.d. | n.d. |
| 21.08 | **Phenol. 2-methoxy-4-(1-propenyl)-** | n.d. | n.d. | 1.21 | n.d. | n.d. |
| 23.88 | **Dodecanoic acid** | 3.43 | 6.36 | 2.84 | 4.49 | 6.09 |
| 25.86 | **Dodecanoic acid. trimethylsilyl ester** | 8.00 | 2.43 | n.d. | 0.71 | 1.15 |
| 27.50 | **3-Dodecene. (E)-** | n.d. | n.d. | n.d. | 0.66 | 0.65 |
| 28.26 | **Tetradecanoid acid** | 11.69 | 16.81 | 6.90 | 12.97 | 18.04 |
| 29.79 | **Neophytadiene** | n.d. | 2.14 | n.d. | 1.72 | 1.37 |
| 30.06 | **Tetradecanoic acid. trimethylsilyl ester** | 11.31 | 1.79 | n.d. | 0.55 | 0.66 |
| 31.54 | **Hexadecanoic acid. methyl ester** | 8.27 | 6.15 | 3.06 | 5.39 | 6.21 |
| 33.84 | **Hexadecanoic acid. trimethyl silyester** | 6.08 | n.d. | n.d. | n.d. | n.d. |
| 32.89 | **Hexadecanoic acid. ethyl ester** | n.d. | n.d. | n.d. | n.d. | 0.67 |
| 30.38 | **Pentadecanoic acid** | n.d. | 0.70 | n.d. | 1.56 | 1.89 |
| 32.35 | **Hexadecanoid acid** | 27.68 | 52.18 | 29.51 | 32.20 | 41.68 |
| 30.46 | **1.2-Benzenedicarboxylic acid. Bis (2-methylpropyl) ester** | n.d. | 2.09 | n.d. | 0.46 | n.d. |
| 31.95 | **Oxacycloheptadecan-2-one** | n.d. | n.d. | n.d. | 4.77 | 4.89 |
| 35.59 | **9.12-Octadecadienoic acid (Z.Z)-** | 1.71 | n.d. | n.d. | n.d. | 3.77 |
| 35.71 | **9-Octadecenoic acid (Z** | 1.18 | n.d. | 37.60 | 28.89 | 6.31 |
| 36.09 | **Octadecanoid acid** | 4.63 | 5.54 | 4.26 | 2.87 | n.d. |
| 35.36 | **Octadecanoic acid. methyl ester** | n.d. | n.d. | n.d. | n.d. | 0.72 |
| 40.03 | **Hexanedioic acid. Bis (2-ethylhexyl) ester** | n.d. | n.d. | n.d. | n.d. | 2.15 |

Table 8 . Main relative volatile composition (%) characteristics of each genotype from different geographical origin

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | TA | pH | TSS | C AC | M AC | A AC | S AC | TP | TF | ANT | DPPH | ABTS | β-CAR | GA | PC | GC | GAD | CAT | CA | SA |
| TA | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| pH | ,073 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| TSS | -,376 | **-.857\*\*** | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C AC | **.522\*** | **.751\*\*** | **-.949\*\*** | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| M AC | **.763\*\*** | ,290 | -,355 | ,430 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A AC | ,271 | ,478 | -,318 | ,236 | **.694\*\*** | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S AC | **.537\*** | ,468 | **-.799\*\*** | **.898\*\*** | ,216 | ,004 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| TP | ,061 | **.631\*** | **-.641\*** | **.637\*** | ,015 | ,221 | **.600\*** | 1 |  |  |  |  |  |  |  |  |  |  |  |  |
| TF | ,383 | ,252 | -,180 | ,125 | **.557\*** | **.831\*\*** | ,056 | ,129 | 1 |  |  |  |  |  |  |  |  |  |  |  |
| ANT | -,160 | ,210 | ,066 | -,181 | ,124 | **.638\*** | -,315 | ,336 | **.521\*** | 1 |  |  |  |  |  |  |  |  |  |  |
| DPPH | **-.517\*** | ,110 | ,185 | -,313 | -,160 | ,499 | -,348 | ,141 | ,439 | **.645\*\*** | 1 |  |  |  |  |  |  |  |  |  |
| ABTS | -,506 | ,086 | ,204 | -,320 | -,134 | **.526\*** | -,372 | ,198 | ,385 | **.748\*\*** | **.883\*\*** | 1 |  |  |  |  |  |  |  |  |
| β-CAR | -,470 | ,021 | ,297 | -,375 | -,068 | **.514\*** | -,419 | -,056 | ,438 | **.554\*** | **.950\*\*** | **.864\*\*** | 1 |  |  |  |  |  |  |  |
| GA | **.812\*\*** | -,257 | -,160 | ,368 | ,441 | -,002 | **.608\*** | ,054 | ,217 | -,349 | -,414 | -,412 | -,364 | 1 |  |  |  |  |  |  |
| PC | **.872\*\*** | -,282 | -,013 | ,203 | .**686\*\*** | ,241 | ,330 | -,125 | ,416 | -,177 | -,324 | -,318 | -,225 | **.908\*\*** | 1 |  |  |  |  |  |
| GC | **.761\*\*** | -,366 | -,048 | ,257 | ,388 | -,030 | **.526\*** | -,010 | ,210 | -,331 | -,369 | -,364 | -,313 | **.992\*\*** | **.907\*\*** | 1 |  |  |  |  |
| GAD | ,437 | **-.675\*\*** | ,489 | -,352 | ,333 | ,192 | -,128 | -,356 | ,407 | ,062 | ,084 | ,108 | ,192 | **.662\*\*** | **.786\*\*** | **.736\*\*** | 1 |  |  |  |
| CAT | **-.633\*** | ,179 | ,218 | -,384 | -,275 | -,082 | **-.674\*\*** | -,224 | -,297 | ,183 | ,144 | ,137 | ,134 | **-.925\*\*** | **-.756\*\*** | **-.926\*\*** | **-.628\*** | 1 |  |  |
| CA | **.521\*** | -,510 | ,330 | -,201 | ,453 | ,356 | -,011 | -,233 | **.545\*** | ,140 | ,141 | ,165 | ,241 | **.700\*\*** | **.829\*\*** | **.757\*\*** | **.978\*\*** | **-.694\*\*** | 1 |  |
| SA | ,452 | -,390 | -,004 | ,164 | ,058 | -,072 | **.525\*** | ,118 | ,184 | -,190 | -,077 | -,065 | -,069 | **.854\*\*** | **.658\*\*** | **.886\*\*** | **.687\*\*** | **-.961\*\*** | **.707\*\*** | 1 |
| EADI | ,026 | **-.681\*\*** | **.761\*\*** | **-.732\*\*** | ,192 | ,280 | **-.653\*\*** | **-.552\*** | ,388 | ,327 | ,364 | ,393 | ,482 | ,124 | ,396 | ,221 | **.819\*\*** | -,091 | **.763\*\*** | ,201 |
| EADII | ,409 | **-.772\*\*** | **.609\*** | -,468 | ,314 | ,071 | -,272 | -,506 | ,284 | -,028 | -,035 | -,016 | ,091 | **.590\*** | **.757\*\*** | **.669\*\*** | **.968\*\*** | -,478 | **.909\*\*** | **.549\*** |
| EA | ,410 | **-.599\*** | ,503 | -,397 | ,428 | ,348 | -,253 | -,386 | **.514\*** | ,193 | ,183 | ,209 | ,302 | **.541\*** | **.747\*\*** | **.612\*** | **.975\*\*** | -,496 | **.968\*\*** | **.534\*** |
| C3G | ,113 | ,012 | ,205 | -,258 | ,507 | **.849\*\*** | -,380 | -,109 | **.795\*\*** | **.680\*\*** | **.642\*\*** | **.669\*\*** | **.716\*\*** | -,024 | ,289 | ,010 | **.516\*** | -,050 | **.605\*** | ,009 |
| Q3X | ,192 | **-.801\*\*** | ,458 | -,284 | -,269 | **-.750\*\*** | ,042 | -,389 | -,484 | **-.617\*** | **-.557\*** | **-.559\*** | -,512 | **.536\*** | ,390 | **.592\*** | ,455 | -,395 | ,305 | **.532\*** |
| RT | ,166 | ,469 | **-.629\*** | **.668\*\*** | -,116 | -,467 | **.625\*** | ,372 | -,493 | **-.529\*** | **-.656\*\*** | **-.689\*\*** | **-.738\*\*** | ,054 | -,190 | -,039 | **-.683\*\*** | ,043 | **-.671\*\*** | -,151 |
| Q3GA | -,146 | -,484 | ,221 | -,156 | -,497 | -,315 | ,241 | ,093 | -,091 | -,082 | ,198 | ,216 | ,154 | ,385 | ,104 | ,452 | ,421 | **-.626\*** | ,382 | **.797\*\*** |
| Q3G | -,003 | **-.648\*\*** | ,290 | -,153 | **-.520\*** | **-.823\*\*** | ,228 | -,149 | **-.566\*** | **-.599\*** | -,435 | -,439 | -,451 | ,449 | ,157 | ,496 | ,251 | -,440 | ,121 | **.600\*** |
| C3,5G | ,318 | -,125 | ,251 | -,246 | **.625\*** | **.783\*\*** | -,326 | -,203 | **.784\*\*** | **.539\*** | ,458 | ,483 | **.560\*** | ,197 | **.521\*** | ,232 | **.685\*\*** | -,195 | **.759\*\*** | ,143 |
| C3A | ,188 | -,053 | ,235 | -,265 | **.551\*** | **.825\*\*** | -,367 | -,150 | **.792\*\*** | **.631\*** | **.579\*** | **.608\*** | **.666\*\*** | ,064 | ,382 | ,100 | **.594\*** | -,111 | **.676\*\*** | ,068 |

Table 9 . Correlation coefﬁcients among biochemical parameters analyzed

\* Correlation is significant at the 0.05 level; \*\* Correlation is significant at the 0.01 level; **TP**: Total phenols; **TF**: Total flavonoids; **ANT**: Anthocyanins; **βCAR:** β-carotene; **TA:** Titrable acidity; **TSS:** Total soluble solids ; **GA:** Gallic acid; **PC:** Protocatechuic; **GC:** Gallocatechol ; **GAD:** Gallic acid derivative; **CAT:** Catechin; **CA:** Chlorogenic acid; **SA:** Syringic acid; **EADI:** Ellagic acid derivative I; **EADII:** Ellagic acid derivative II; **EA:** Ellagic acid; **C3G:** Cyanidin-3-glucoside; **Q3X:** Quercetin-3-xyloside; **RT:** Rutin; **Q3GA:** Quercetin-3-galactoside; **Q3G:** Quercetin-3-glucoside; **C3,5D:** Cyanidin-3,5-diglucoside; **C3A:** Cyanidin-3-arabinoside.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | EADI | EADII | EA | C3G | Q3X | RT | Q3GA | Q3G | C3,5G | C3A |
| EADI | 1 |  |  |  |  |  |  |  |  |  |
| EADII | **.853\*\*** | 1 |  |  |  |  |  |  |  |  |
| EA | **.893\*\*** | .**953\*\*** | 1 |  |  |  |  |  |  |  |
| C3G | **.716\*\*** | ,440 | .674\*\* | 1 |  |  |  |  |  |  |
| Q3X | ,186 | **.549\*** | ,289 | -,501 | 1 |  |  |  |  |  |
| RT | **-.925\*\*** | **-.648\*\*** | **-.780\*\*** | **-.840\*\*** | ,103 | 1 |  |  |  |  |
| Q3GA | ,153 | ,291 | ,261 | -,132 | ,485 | -,219 | 1 |  |  |  |
| Q3G | -,062 | ,280 | ,046 | **-.655\*\*** | **.913\*\*** | ,237 | **.704\*\*** | 1 |  |  |
| C3\_5G | **.789\*\*** | **.632\*** | **.820\*\*** | **.962\*\*** | -,300 | **-.819\*\*** | -,124 | **-.528\*** | 1 |  |
| C3A | **.758\*\*** | **.526\*** | **.743\*\*** | **.994\*\*** | -,419 | **-.846\*\*** | -,119 | **-.602\*** | **.986\*\*** | 1 |

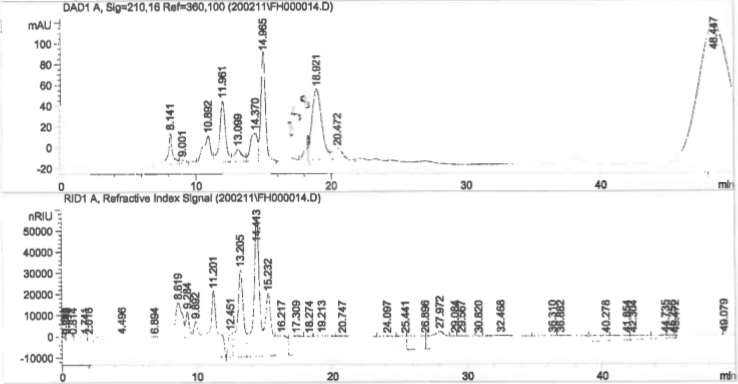
Table 10 . Correlation coefﬁcients among biochemical parameters analyzed (continued).

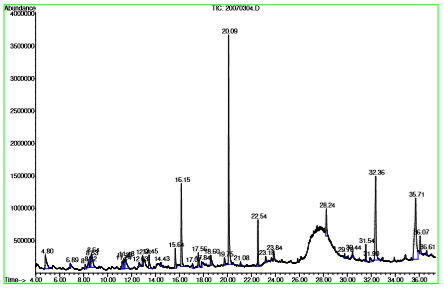
\* Correlation is significant at the 0.05 level; \*\* Correlation is significant at the 0.01 level; **EADI:** Ellagic acid derivative I; **EADII:** Ellagic acid derivative II; **EA:** Ellagic acid; **C3G:** Cyanidin-3-glucoside; **Q3X:** Quercetin-3-xyloside; **RT:** Rutin; **Q3GA:** Quercetin-3-galactoside; **Q3G:** Quercetin-3-glucoside; **C3,5D:** Cyanidin-3,5-diglucoside; **C3A:** Cyanidin-3-arabinoside.

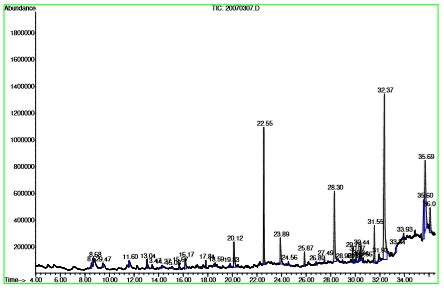
Table 11 . Eigenvectors of principal component axes from PCA analysis of studied variables

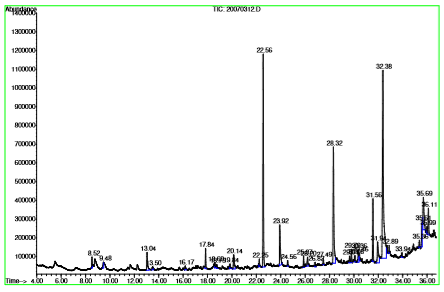
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component Matrix | | | | |
|  | **Principal component** | | | |
| **1** | **2** | **3** | **4** |
| Titratable acidity | ,173 | **,642** | ,494 | **,560** |
| pH | -,470 | -,203 | **,845** | -,154 |
| Total soluble solids | **,610** | -,148 | **-,779** | -,017 |
| Citric acid | **-,553** | ,355 | **,748** | ,090 |
| Malic acid | ,315 | ,108 | **,648** | **,685** |
| Ascorbic acid | ,438 | -,397 | **,797** | ,122 |
| Succinic acid | -,438 | **,660** | **,592** | -,146 |
| Total phenols | **-,505** | ,084 | **,750** | -,419 |
| Flavonoids | **,620** | -,165 | **,764** | ,067 |
| Anthocyanins | ,474 | **-,718** | ,433 | -,269 |
| DPPH | ,459 | **-,626** | ,249 | **-,578** |
| ABTS | ,479 | **-,616** | ,242 | **-,577** |
| Βeta carotene | **,584** | **-,622** | ,221 | -,472 |
| Gallic acid | ,292 | **,907** | ,267 | ,141 |
| Protocatechuic | **,527** | **,679** | ,293 | ,418 |
| Gallocatechol | ,378 | **,901** | ,187 | ,099 |
| Gallic acid derivative | **,894** | ,443 | -,034 | ,058 |
| Catechin | -,316 | **-,839** | -,383 | ,221 |
| Chlorogenic acid | **,886** | ,427 | ,173 | ,051 |
| Syringic acid | ,389 | **,827** | ,162 | -,373 |
| Ellagic acid derivative I | **,958** | -,117 | -,241 | ,098 |
| Ellagic acid derivative II | **,861** | ,402 | -,216 | ,223 |
| Ellagic acid | **,955** | ,258 | ,027 | ,147 |
| Cyanidin-3-Glucoside | **,797** | -,419 | ,430 | ,066 |
| Quercetin-3-Xyloside | ,113 | **,741** | **-,660** | ,048 |
| Rutin | **-,932** | ,318 | ,055 | ,163 |
| Quercetin-3-Galactoside | ,262 | **,514** | -,169 | **-,799** |
| Quercetin-3-Glucoside | -,089 | **,744** | **-,586** | -,309 |
| Cyanidin-3,5-Diglucoside | **,871** | -,211 | ,384 | ,223 |
| Cyanidin-3-Arabinoside | **,840** | -,339 | ,408 | ,120 |
| Phenol | **-,626** | -,452 | -,174 | **,612** |
| Dodecanoic acid | **,854** | -,399 | ,058 | -,327 |
| Tetradecanoic acid | **,879** | -,468 | -,068 | -,060 |
| Hexadecanoic acid | **,618** | -,465 | ,306 | **-,556** |
| Hexadecanoic acid, methyl ester | ,176 | **-,778** | -,301 | **,523** |
| Neophytadiene | **,676** | -,253 | -,282 | **-,632** |
| Tetradecanoic acid, trimethylsilyl ester | -,415 | **-,621** | -,150 | **,647** |
| Limonene | **,968** | -,048 | ,243 | ,031 |
| Pentadecanoic acid | **,897** | ,232 | -,369 | -,078 |
| Furfural | **-,801** | -,068 | ,146 | **,577** |
| Hexadecanoic acid, trimethylsilyl ester | -,463 | **-,509** | -,154 | **,709** |
| N-ethyl-1,3-dithioisoindoline | **-,537** | ,486 | **,610** | -,320 |
| 1H-Indole | **-,551** | **,630** | **,529** | -,145 |
| 9,12-Octadecadienoic acid (Z,Z)- | **,643** | -,016 | ,197 | **,740** |
| 9-Octadecenoic acid (Z)- | -,381 | **,878** | -,120 | -,266 |
| Octadecanoic acid | **-,760** | -,499 | ,083 | -,409 |
| Dodecanoic acid, trimethylsilyl ester | -,332 | **-,723** | -,130 | **,592** |
| Benzene, (2-methyl-2-propenyl)- | ,041 | ,328 | **-,899** | -,286 |
| 3-Dodecene, (E)- | **,719** | ,444 | **-,526** | ,098 |
| Oxacycloheptadecan-2-one | **,732** | ,442 | **-,507** | ,109 |
| Hexanedioic acid, bis (2-ethylhexyl) ester | **,846** | ,215 | ,265 | ,411 |
| Phenol, 2-methoxy-4-(1-propenyl)- | **-,551** | **,630** | **,529** | -,145 |
| 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester | ,127 | **-,664** | ,261 | **-,690** |
| Hexadecanoic acid, ethyl ester | **,846** | ,215 | ,265 | ,411 |
| Octadecanoic acid, methyl ester | **,846** | ,215 | ,265 | ,411 |
| % of Variance | **39,849** | **26,631** | **18,248** | **15,271** |
| Cumulative % | **39,849** | **66,481** | **84,729** | **100,000** |

Eigenvalues higher than |0.5| are marked in bold.









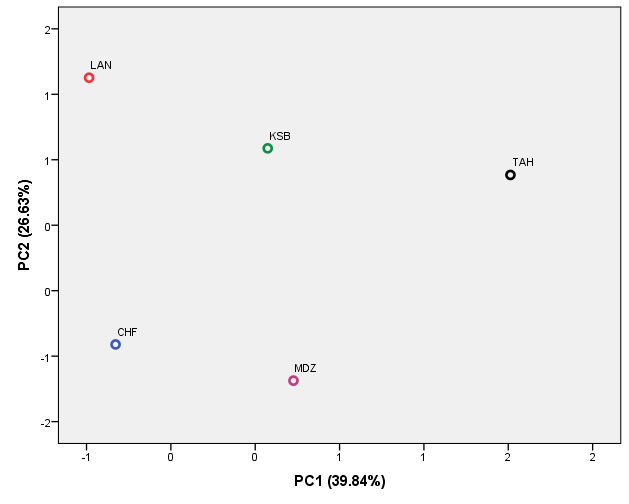


Figure 1. Scatter plot for the ﬁrst two principal components (PC1/PC2, 66.47% of total variance) for the studied *A. unedo* genotypes based on their phenolics compounds, antioxidant activity, organic acids and volatile compounds