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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

Hafida Zitouni[a], Marie Laure Fauconnier[b], Lahcen Hssaini[c], Rachida Ouaabou[d], Manuel Viuda-Martos[e], Francisca Hernández[f], Sezai Ercisli[g], Lhoussain Ait Haddou[h], Zerhoune Messaoudi[h], and Hafida Hanine[a]

[a]Laboratory of Bioprocess and Bio-interfaces, Faculty of Science and Technics, University Sultan Moulay Slimane, Beni-Mellal, Morocco; [b]Laboratory of Chemistry of Natural Molecules, Gembloux AgroBiotech, University of Liege, Gembloux, Belgium; [c]National Institute of Agricultural Research, Rabat, Morocco; [d]Licvedde/eriddecv (Research Team of Innovation and Sustainable Development & Expertise in Green Chemistry), Faculty of Science Semlalia, Cadi Ayyad University, Marrakesh, Morocco; [e]Dpto. Tecnología Agroalimentaria, Ipoa. Escuela Politécnica Superior de Orihuela. (Universidad Miguel Hernández), Alicante, Spain; [f]Dpto. Producción Vegetal Y, Cuela Politécnica Superior de Orihuela (Universidad Miguel Hernández de Elche), Alicante, Spain; [g]Agricultural Faculty Department of Horticulture, Ataturk University, Erzurum, Turkey; [h]Department of Arboriculture, Horticulture and Viticulture, National School of Agriculture, Meknes, Morocco

ABSTRACT

This study aims to identify the individual phenolics and volatile compounds, as well as the organic acids of strawberry tree (*Arbutus unedo* L.) genotype fruits. The antioxidant activities were also assessed using three methods (DPPH, ABTS and βeta carotene bleaching assays) significant differences ($p^{\wedge}0.05$) 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. Seventeen phenolics compounds were identified by HPLC in *A. unedo* fruits. Gallocatechol and catechin were the most abundant compounds. Among the volatile compounds identified, hexadecanoic acid was the most abundant in all the genotype 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

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 plant naturally grown as population or solitary tree in countries, such as Morocco, Tunisia, Algeria, Turkey, Syria, Greece, Croatia, France, Portugal, and Spain (Serçe et al., 2010). It is 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-Rodriquez et al., 2011). In Morocco, it is known as "Sasnou" and it is widely used in traditional medicine, such as antiseptics,

CONTACT Hafida Hanine h.hanine@usms.ma Laboratory of Bioprocess and Bio-interfaces, Faculty of Science and Technics, University Sultan Moulay Slimane, BO523, Beni-Mellal, Morocco

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-Rodriquez 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 (Alarcão-E-Silva et al., 2001; Ayaz et al., 2000; Fortalezas et al., 2010; Males et al., 2006; Pallauf et al., 2008; Pawlowska et al., 2006; 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 and reducing platelet aggregation (Gentile et 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 Alarcão-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 solids and organic acids), ii) to evaluate the antioxidant activities of *A. unedo* fruits using three methods (DPPH, ABTS, and βeta 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.

Materials and Methods

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

Table 1. Origins geographic of the different genotypes studied.

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

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.

Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, rutin, βeta carotene, Folin Ciocalteu reagent, ascorbic acid, sodium carbonate (Na_2CO_3) 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-glucoside and quercetin-3-O-galactoside) were obtained from Extrasynthese (Genay, France), the water was distilled and filtered through a Milli-Qapparatus filter.

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 according 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 H_2O, 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.

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 × 7.8 mm) and apre-column (Supelguard 5 cm × 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 ($r^2 \geq 0.999$). The results were expressed as g 100 g^{-1} of dry weight (DW).

Phytochemical Composition

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.

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).

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).

Total Anthocyanins

Total anthocyanin content (TAC) of samples was determined using the pH differential method with some modifications according to Jackobek 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.

$$TAC = (A*MW*DF*1000/\varepsilon*L) \quad (1)$$

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; ε: molar absorptivity coefficient of cyanidin-3-glucoside (26900 L mol^{-1} cm^{-1}).

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.

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):

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

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

IC50 value (mg equivalent to 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.

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 each extract 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 βeta carotene blanching assay was determined according to Barros et al. (2010). βeta 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):

$$AA = 100\ [1- (A_o - A_t)/(A_{oo}- A_{ot})] \quad (3)$$

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.

Extraction and Determination of Polyphenolic Compounds

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°C). After the time, the samples were centrifuged for 10 min, 8000 g at 4°C. 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°C. 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°C until further use.

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.

Volatile Compound Analysis

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 × 0.25 mm i.d. × 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.

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).

Results and Discussion

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 ($p < .001$). 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; Özcan and Hacıseferoğulları (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

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 column, 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)

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-Rodriquez 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 Özcan and Hacıseferoğulları (2007) who reported 5.57 and 4.6, respectively. The low pH value can have a big advantage in 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 Müller 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).

Organic Acids

The results obtained for organic acids content were reported in Table 3. Significant differences ($P < .001$) 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

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

*** denote significant of difference at level 0.001; Data values are means ± SD; Values in bold represent, in each column, 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

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-Rodriquez 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 Alarcão-E-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 another 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-Rodriquez 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.

Phytochemical Composition

Total Phenols

The total phenols content (TPC) of *A. unedo* fruits were reported in Table 4. Significant differences ($p = .044$) 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

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 ± 0.88 cd	**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 column, 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 $p^{\circ}0.05$; **GAE**: Gallic acid equivalent; **RE**: Rutin equivalent; **C-3-GE**: Cyanidin-3-glucoside equivalent

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 light of the fact that modern diets are often lacking of bioactive compounds.

Total Flavonoids

The results of the total flavonoids content were summarized in Table 4. A significant variation was observed (p = .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.

Total Anthocyanins

The total anthocyanins content was reported in Table 4. A statistically significant variation (p = .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).

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 activity 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* fruit grown in Tunisia was 3.2 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 analyzed 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

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 column, 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

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

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***

*** denote significant of difference at level 0.001; Data values are means ± SD; Values in bold represent, in each column, 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

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

Genotype name	EA	C3G	Q3X	RT	Q3GA	Q3G	C3,5DG	C3A
KSB	18.00 ± 0.00d	**0.43 ± 0.01a**	**4.09 ± 0.01^{e}**	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 ± 0.01^{e}**	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***

*** denote significant of difference at level 0.001; Data values are means ± SD; Values in bold represent, in each column, 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.

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

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	**1 H 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

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) analyzed 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 another study, Barros et al. (2010) analyzed the antioxidant activity through 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 (Barros et al., 2010; Fortalezas et al., 2010; Isbilir et al., 2012; Mendes et al., 2011; Morales et al., 2013; Pallauf et al., 2008; Ruiz-Rodriquez et al., 2011; Seker and Toplu, 2010). 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; Liu et al., 2018) which can be explained by the higher composition of strawberry, pomegranate, grape, and apple in polyphenols.

Table 9. Correlation coefficients among biochemical parameters analyzed.

	Ta	Ph	Tss	C ac	M ac	A ac	S ac	Tp	Tf	Ant	Dpph	Abts	B-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***
C3,5 G	,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

* 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.

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

	EADI	EADII	EA	C3G	Q3X	RT	Q3GA	Q3G	C3,5 G	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_5 G	**.789****	**.632***	**.820****	**.962****	-,300	**−.819****	-,124	**−.528***	1	
C3A	**.758****	**.526***	**.743****	**.994****	-,419	**−.846****	-,119	**−.602***	**.986****	1

* 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.

Profile of Polyphenolic Compounds

The results of polyphenolic compounds were summarized in Table 6 and 7 Significant variations were observed at $p < .001$ among all genotypes. Seventeen 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).

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

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**
Beta 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
1 H-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.

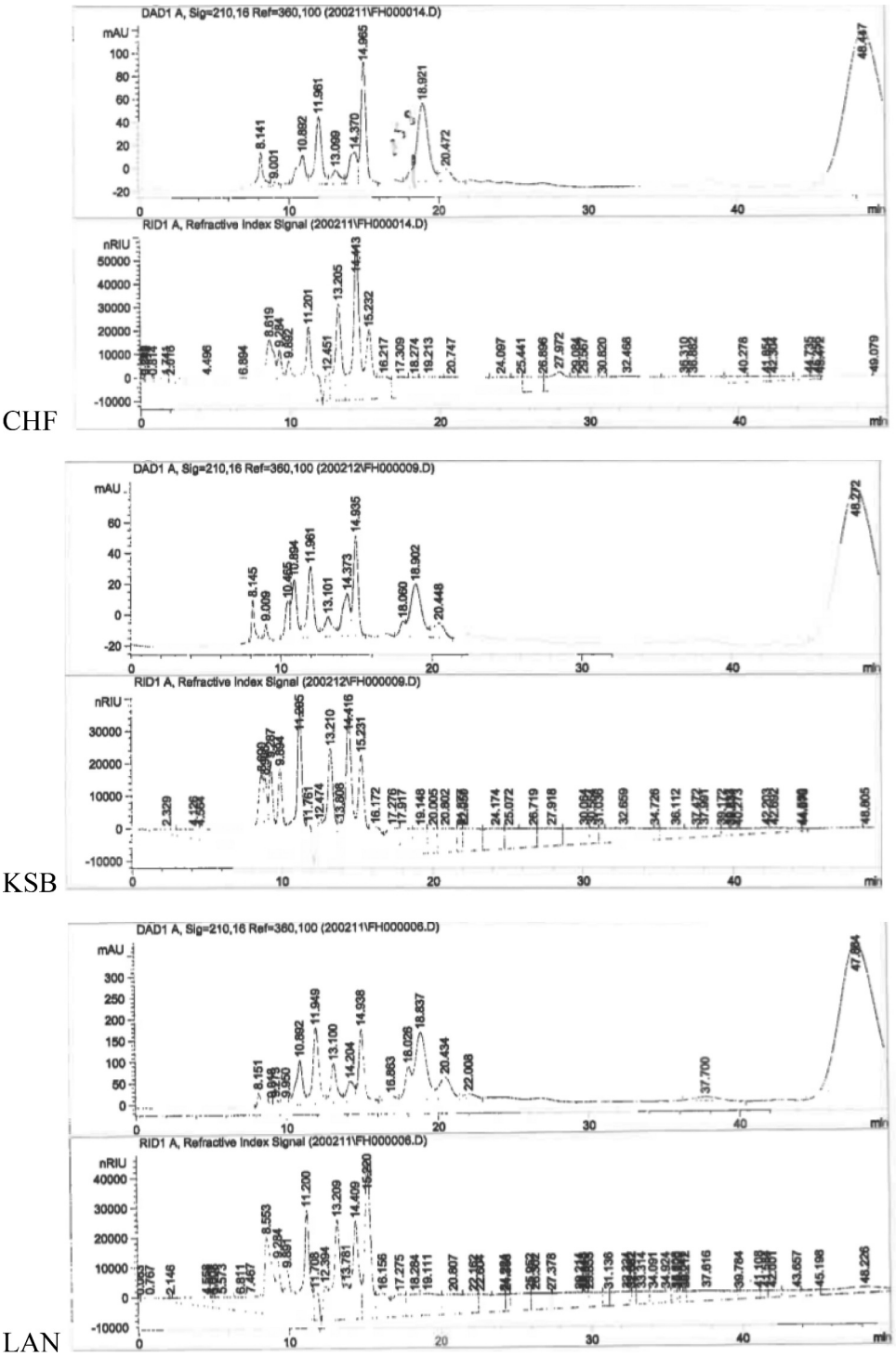


Figure 1. HPLC chromatogram of organic acids profile of the studied strawberry tree genotypes.

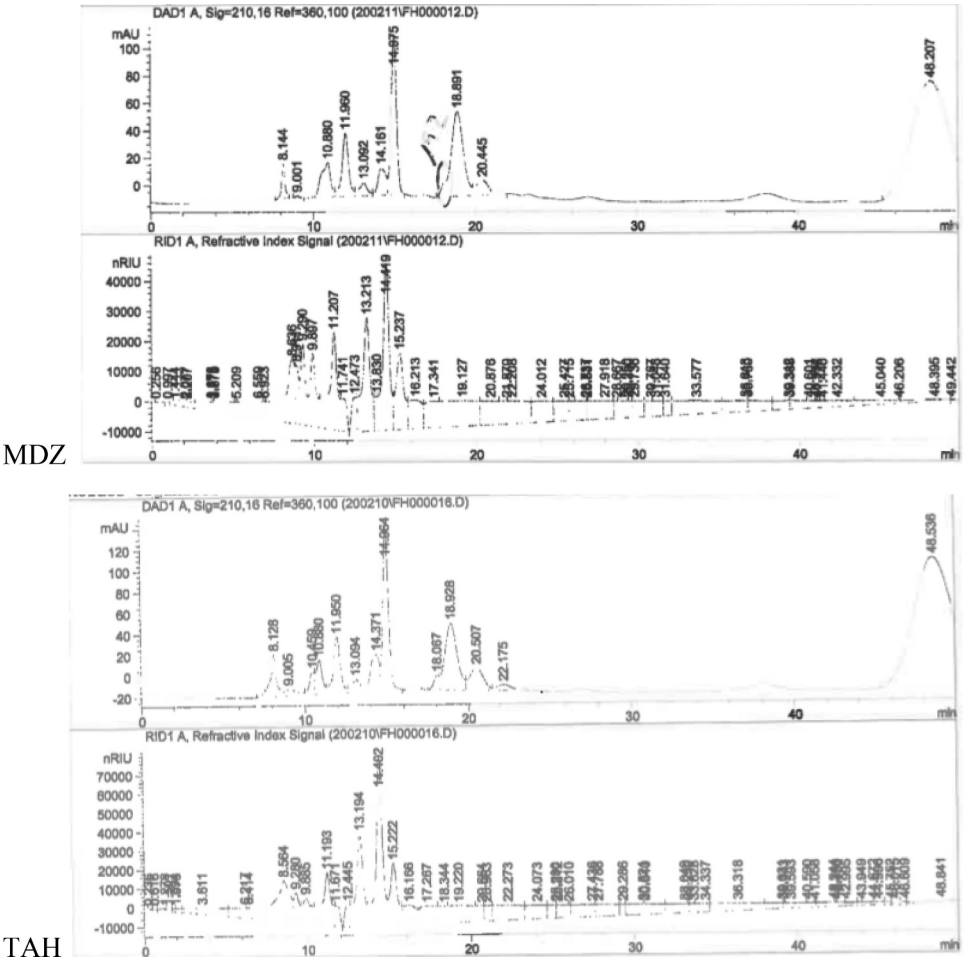
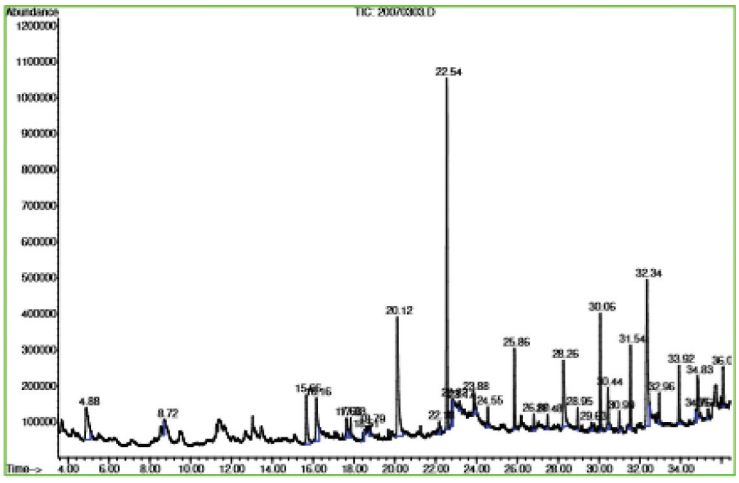


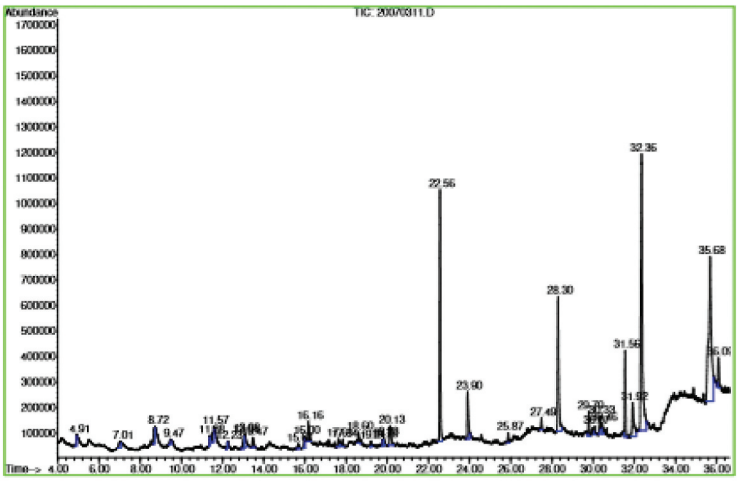
Figure 1. (Continued).

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 exceeded 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; Hakala et al., 2001; Hamilton-Kemp et al., 1996).

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





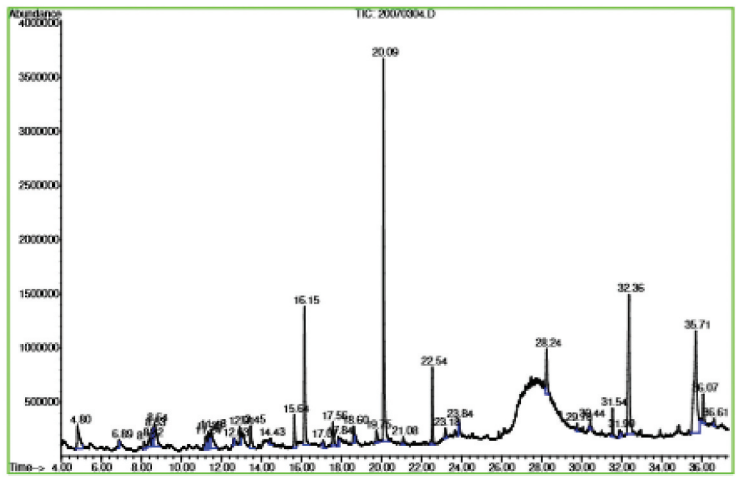
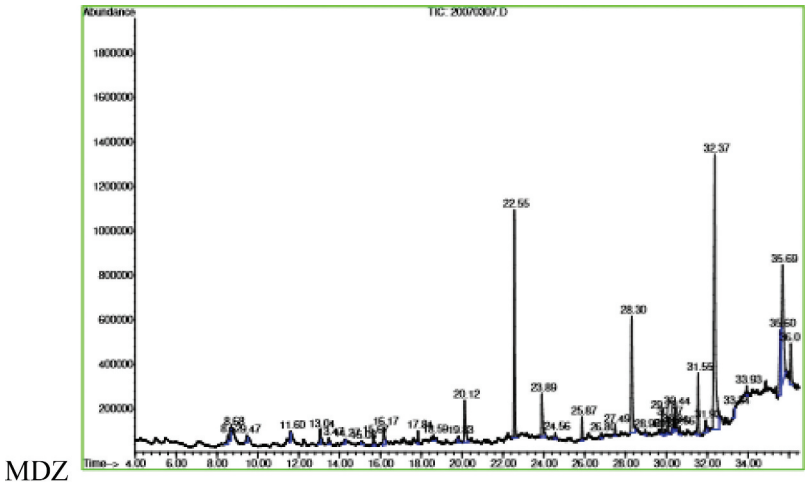


Figure 2. Chromatographic profile of A. unedo fruits of the five genotypes.



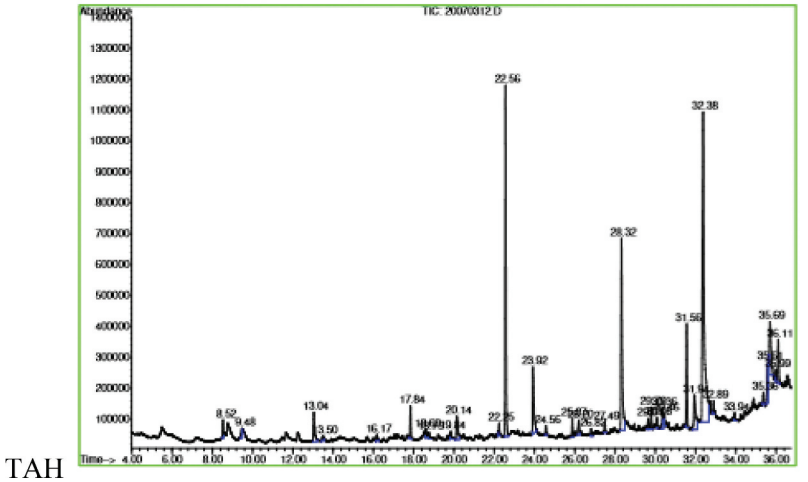


Figure 2. (Continued).

DPPH and total anthocyanins (r = 0.931; p < .05) as well as between malic acid and titrable acidity (r = 0.763; p < .01). Citric acid was also correlated to titrable acidity (r = 0.522; p < .05), pH (r = 0.751; p < .01) and soluble solids (r = −0.949; p < .01). The results reported also, correlations between DPPH and anthocyanins (r = 0.645; p < .01). Moreover, this study revealed correlations between ABTS and ascorbic acid (r = 0.526; p < .05), anthocyanins (r = 0.748; p < .01), and DPPH (r = 0.883; p < .01). In addition, it conveyed correlations between βeta carotene and ascorbic acid (r = 0.514; p < .05), anthocyanins (r = 0.554; p < .05), DPPH (r = 0.950; p < .01), and ABTS (r = 0.864; p < .01). Also, cyanidin-3-glucoside was correlated to anthocyanins (r = 0.680; p < .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 (Anastasiadi et al., 2010; Liu et al., 2008; Serçe et al., 2010; Su and Chien, 2007). The correlation coefficients may

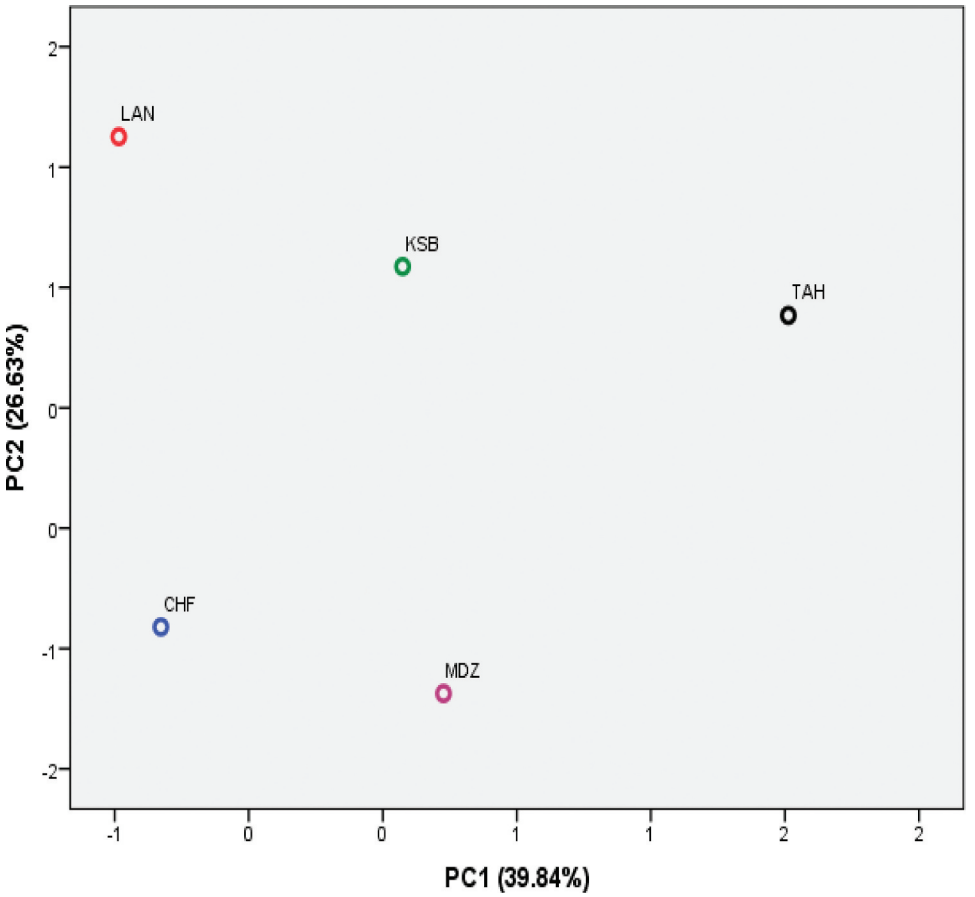


Figure 3. Scatter plot for the first two principal components (PC1/PC2, 66.47% of total variance) for the studied strawberry tree genotypes based on their phenolics, antioxidant potency, organic acid and volatile compounds contents.

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).

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 (Colak, 2019; Gündoğdu et al., 2018). 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 and 3). 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 (Colak, 2019; Gündoğdu et al., 2018).

Conclusion

This study revealed that *A. unedo* fruits can be considered an important source of polyphenols (25–39 mg GAE/g DW). Among the 17 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 valorization.

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.

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ORCID

Hafida Zitouni http://orcid.org/0000-0002-6701-3346
Marie Laure Fauconnier http://orcid.org/0000-0002-2027-3896
Lahcen Hssaini http://orcid.org/0000-0002-6739-3895
Rachida Ouaabou http://orcid.org/0000-0003-0550-848X
Manuel Viuda-Martos http://orcid.org/0000-0001-9801-3819
Francisca Hernández http://orcid.org/0000-0003-3739-8748
Sezai Ercisli http://orcid.org/0000-0001-5006-5687
Hafida Hanine http://orcid.org/0000-0002-9309-5672

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