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Comparative characterization of phenolic and other polar compounds in Spanish melon cultivars by using high-performance liquid chromatography coupled to electrospray ionization quadrupole-time of flight mass spectrometry

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Abstract

Melon (*Cucumis melo L*.), belonging to the Cucurbitaceae family, is a significant source of phytochemicals which provide human health benefits. High-performance liquid chromatography coupled to electrospray ionization mass spectrometry quadropole-time of flight (HPLC-ESI-QTOF-MS) was used for the comprehensive characterization of 14 extracts from 3 Spanish varieties of melon (Galia, Cantaloupe, and Piel de Sapo). A total of 56 different compounds were tentatively identified, including: amino acids and derivatives, nucleosides, organic acids, phenolic acids and derivatives, esters, flavonoids, lignans, and other polar compounds. Of these, 25 were tentatively characterized for the first time in *C. melo* varieties. Principal-component analysis (PCA) was applied to gain an overview of the distribution of the melon varieties and to clearly separate the different varieties. The result of the PCA for the negative mode was evaluated. The variables most decisive to discriminate among varieties included 12 of the metabolites tentatively identified.

Keywords: *Cucumis melo*, characterization, phenolic compounds, tandem mass spectrometry, PCA.

1. Introduction

Research on fruits and vegetables is increasing because of their benefits to human health. Indeed, a positive correlation has been reported between fruit consumption and the decrease risk of several chronic diseases including obesity, cardiovascular disease, and certain types of cancer (Jansen et al., 2011; Boeing et al., 2012).

Melon is one of the most widely cultivated and consumed fruits in the world. In Spain, more than 800,000 tons were produced in 2011, with a cultivation area exceeding 28,000 Ha (Seoane Spielgelberg, Rábade Rodríguez, & López Pérez, 2013), with Almería (Andalusia) being one of the main producer provinces. Spanish consumption was around 9.05 Kg per person in 2012. Melon (*Cucumis melo L*.), belongs to the family Cucurbitaceae, commonly known as cucurbits or the gourd family. In Spain, the most widely produced *Cucumis melo* groups are Cantalupensis and Inodorus, and the most consumed market varieties are Galia and Cantaloupe, belonging to Cantalupensis group, and Piel de Sapo, belonging to the Inodorus group.

Fruits, together with vegetables, represent the major source of phytochemicals and other compounds such as amino acids and fatty acids (Poiroux-Gonord, Bidel, Fanciullino, Gautier, Lauri-Lopez, & Urban, 2010). In this sense, melon is a significant source of phytochemicals, mainly polyphenols and other antioxidants, which provide potential health benefits, especially aiding the cardiovascular system and (; Maietti et al., 2012). Its chemical composition depends on the cultivar, environmental conditions, and also on the stage of fruit maturity (Villanueva, Tenorio, Esteban, & Mendoza, 2004).

In this context, some melon varieties have been studied in depth, mainly by gas chromatography, providing a thorough knowledge of the volatile composition, which is the major determinant of melon-fruit quality perceived by consumers (Bernillon et al., 2012; Biais et al., 2009; Kourkoutas, Elmore, & Mottram, 2006; Obando-Ulloa, Ruiz, Monforte, & Fernández-Trujillo, 2010; Pang, Chen, Hu, Zhang, & Wu, 2012). By this technique, more than 240 volatile compounds have been identified in Galia or Cantaloupe melons while only 42 compounds have been identified in Piel de Sapo variety (Obando-Ulloa et al., 2010). Other techniques such as

quantitative nuclear magnetic resonance spectroscopy (^{1}H-NMR) or liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have been applied to characterize the polar fraction of different melon varieties (Biais et al., 2009; De Marino, Festa, Zollo, & Iorizzi, 2009; Moing et al., 2011; Villanueva et al., 2004), although the phytochemical composition of the polar fraction of these three typical Spanish varieties has not yet been completely characterized.

In the present work, using HPLC-ESI-Q-TOF-MS, we offer a comprehensive characterization of polar the fraction of 14 extracts from 3 different Spanish melon varieties (Galia, Cantaloupe, and Piel de Sapo), collected in different ripening periods. Furthermore, PCA was applied to the data set to evaluate the compounds responsible for discriminating between the varieties.

2. Experimental

2.1. Chemicals

HPLC-MS acetonitrile and methanol were purchased from Fisher (Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leics, UK). Acetic acid of analytical grade (purity>99.5%) was acquired from Fluka (Switzerland). Distilled water with a resistance of 18.2 MΩ was deionized in a Milli-Q system (Bedford, MA, USA).

2.2. Plant material

Fourteen samples of three different varieties of melon (Galia, Cantaloupe, and Piel de Sapo) were collected in 2011 from May to September from El Ejido Almería, Andalusia (Spain). After the harvest, the samples were washed with distilled water and transported directly to the lab under refrigeration conditions at 4 ºC. The samples were stored at the same temperature until its treatment.

2.3. Sample treatment

Fresh melon samples were peeled and the seeds were removed. Later 1.5 kg of edible portion were crushed, , and placed in the lyophilizer shelf (Christ Alpha 1-2 LD Freeze dryer, Shropshire, UK), which was pre-cooled at -50ºC for 1 h at 1 mbar and then were frozen at -20 ºC until analyzed. Afterwards, 0.5 g of lyophilized melons were extracted using 15 ml of 80:20

(v/v) methanol/H_2O, sonicated for 15 min at 984 × g and the supernatant was collected in a round-bottom flask. Next, the solvent was evaporated by using a rotary evaporator under vacuum at 40ºC, and the dried residue was redissolved in 2 mL of 80:20 (v/v) methanol/H_2O. Finally, the extract was filtered through a 0.2 µm syringe filter and stored at -20ºC until analyzed.

2.4. Chromatographic separation

HPLC analyses were carried out using an Agilent 1200 Series Rapid Resolution LC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a vacuum degasser, a binary pump, an autosampler, and a diode array detector (DAD). The column used for the chromatographic separation was a Zorbax Eclipse Plus C_{18} (1.8 µm, 150 mm × 4.6 mm) (Agilent Technologies, Palo Alto, CA, USA). Acidified water (0.5% acetic acid, v/v) and acetonitrile were used as mobile phases A and B, respectively. The gradient was programmed as follows: 0 min, 5% B;10 min, 30% B; 12 min, 33% B; 16 min, 38% B; 19 min, 50% B; 22 min, 95% B; 24 min 5% B, and finally a conditioning cycle of 10 min with the initial conditions. The flow rate was set at 0.80 mL/min throughout the gradient and the effluent from the HPLC column was split using a T-type splitter before being introduced into the mass spectrometer (split ratio = 1:3) to provide a stable spray and consequently reproducible results. The injection volume in the HPLC system was 10 µL.

2.5. ESI-Q-TOF-MS analyses

HPLC system was coupled to a micrOTOF-Q II mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with electrospray ionization (ESI) operating in the negative mode. The detection was made considering a mass range of 50–1100 *m*/*z* and using a capillary voltage of +4000 V, a dry gas temperature of 210ºC, a dry gas flow of 8.0 l/min, a nebulizer pressure of 2.0 bar, and spectra rate of 1 Hz. Moreover, automatic MS/MS experiments were performed using nitrogen as the collision gas adjusting the collision energy values as follows: m/z 100, 20 eV; *m/z* 500, 25 eV; m/z 1000, 30 eV.

For the necessary mass accuracy to identify compounds, external instrument calibration was used. For this, the calibrant used was sodium acetate clusters consisting of 5 mM sodium hydroxide and water: 2-propanol 1:1 (v/v) with 0.2% of acetic acid. This calibrant was injected

at the beginning of the run using a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL, USA) directly connected to the interface, equipped with a Hamilton syringe (Reno, NV, USA).

The accuracy of the mass data for the molecular ions was controlled by Data Analysis 4.0. Software (Bruker Daltonik) offering a list of possible elemental formulas by using the GenerateMolecularFormula^{TM} Editor. The Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration, and ring-plus double-bond equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (sigma value) for increased confidence in the suggested molecular formula.

2.6. PCA

The data were evaluated with ProfileAnalysis for PCA after Pareto scaling (Bruker Daltonik, Bremen, Germany). The LC–MS data were prepared for PCA using FindMolecularFeature (FMF) peak finder algorithm and advanced bucketing. The LC–MS was integrated from 2-22 min in time and 49-1001 *m/z* in mass.

3. Results and discussion

3.1. Compound identification

The base peak chromatograms of the three varieties of melon resulting from the HPLC-ESI-QTOF-MS method described above are shown in Fig. 1, where the peaks are numbered according to their elution order. The compounds were tentatively identified by interpretation of their MS and MS/MS spectra determined by QTOF-MS and also considering the data from the literature and open-access mass-spectra databases such as Metlin and MassBank. Table 1 summarizes the MS data of the compounds tentatively identified, including experimental and calculated *m/z* for the molecular formula provided, error, sigma value, and the main fragments obtained by MS/MS, as well as the proposed compound for each peak. The compounds characterized may be classified into different groups: amino acids and derivatives, nucleosides, organic acids, phenolic acids and derivatives, esters, flavonoids, lignans, and other polar compounds.

3.1.1. Amino acids and derivatives

Several amino acids and derivatives were tentatively identified in the different melon varieties, most of them previously reported in *Cucumis melo* or Cucurbitaceae family (Bernillon et al., 2012; Gonda et al., 2010; Lamikanra, Chen, Banks, & Hunter, 2000; Moing et al., 2011). In this way, single amino acids such as glutamine (peak 2), tyrosine (peak 13), phenylalanine (peak 19) and tryptophan (peaks 29 or 33) were found in these melon samples. These compounds generally presented a common fragmentation pattern due to the consecutive losses of ammonia and carbon dioxide. These amino acids were found in the three melon varieties except tyrosine, which was found only in the varieties Piel de Sapo and Cantaloupe.

Three dipeptides were also detected, namely, isoleucyl-serine (peak 20) and leucyl-isoleucine (peak 41) in Piel de Sapo, and alanyl-phenylalanine (peak 38) in the variety Galia. Isoleucyl-serine presented different fragments due to the loss of the formaldehyde group from serine (*m/z* 187) and the loss of the serine amino acid (*m/z* 130), while leucyl-isoleucine and alanyl-phenylalanine produced only one product ion due to decarboxylation and dehydration, respectively. Leucyl-isoleucine has previously been characterized in other fruits (Özcan & Şenyuva, 2006). Three amino acid derivatives were also characterized in Galia and Cantaloupe: pyroglutamic acid hexoside (peak 9), hexose-leucine (peaks 14 or 15), and hexose-phenyalanine (peak 22). The fructoside derivatives of leucine and phenylalanine presented a common fragmentation pattern consistent with the cleavage of the hexoside bond which produced fragments at *m/z* 130 and 164, respectively. MS/MS spectrum of peak 9 showed two fragments at *m/z* 128 and 200 due to the losses of the hexoside moiety and the rupture of pyroglutamic acid. It is the first time in which these compounds have been characterized in *C. melo* or Cucurbitaceae family.

In addition to these compounds, two tripeptides were detected only in Piel de Sapo: peak 58 (Asp-leu-leu/Asp-Ile-Ile) and peak 61 (Asp-leu-phe/ Asp-Ile-phe). Peak 58 presented different fragments at *m/z* 340, 323, 279, and 130. This fragmentation pattern matched the dehydration (*m/z* 340) followed by the loss of one amine terminal group (*m/z* 323), further decarboxylation (*m/z* 279), and finally the loss of leucine or isoleucione residue (*m/z* 130). The other tripeptide found in Piel de Sapo (peak 61) had a fragment at *m/z* 357 due to the loss of water and one amine terminal group. The main fragments were compared with the Metlin database.

3.1.2. Nucleosides

Two nucleosides, namely uridine and guanosine, were tentatively identified in the melon samples. Peak 12, found in Piel de Sapo and Galia, was characterized as uridine, showing a fragment at *m/z* 110 consistent with the uracil group (Kang, 2012). This nucleoside has been previously described in other varieties of melon (Cohen et al., 2012). On the other hand, peak 16 was characterized as guanosine and presented a major fragment at *m/z* 150 corresponding to $[M\text{-}H\text{-}pentose]^-$ as well as another at *m/z* 133 due to the loss of the amino group. This nucleoside, which appeared in the three varieties, has been previously characterized in Cucurbitaceae family (Iswaldi, Gómez-Caravaca, Lozano-Sánchez, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2013) but not in melon.

3.1.3. Organic acids

Organic acids, together with sugars and amino acids, are a widely represented group in melon samples that significantly contribute to the overall aroma of melon (Lignou, Parker, Oruna-Concha, & Mottram, 2013). One of the main organic acids that are accumulated in most of the fruits, including melon fruit is citric acid (Cohen et al., 2012). Three citric acid isomers were found in all the varieties (peaks 6, 7, and 8) presenting a fragment at *m/z* 111 due to $[M\text{-}H\text{-}CO_2\text{-}2H_2O]^-$.

Compounds 39 and 40 were characterized as isomers of hydroxybutanoic acid ethyl ester-hexoside. This compound has been previously described in other melon varieties together with its fragment at *m/z* 131 and its presence has been associated with amino acids such as alanine, glutamine, isoleucine, phenylalanine, tryptophan or tyrosine (Bernillon et al., 2012).

Peak 76 was characterized as azelaic acid, a 9-carbon saturated linear dicarboxylic acid found naturally in some plants (Vinholes et al., 2011), especially in seed (Bondia-Pons et al., 2013). Its fragment at *m/z* 125 is due to the loss of one of the acid groups and further dehydration, and it has been found in Cucurbitaceae (Dan & Thakur, 1995) but is the first time that this compound has been reported in *C. melo*.

Peak 82 was characterized as 9,12,13-trihydroxyoctadeca dienoic acid. This organic acid, tentatively identified for the first time in melon, was characterized mainly in the variety Piel de Sapo and also, with lower intensity, in the variety Cantaloupe. This presented two main

fragments at *m/z* 211 and 229 due to the C12-C13 bond cleavage and the subsequent dehydration of the formed product ion. Similarly, peaks 81 and 83 were characterized as trihydroxyoctadeca-dienoic acid isomers, which presented an individual fragment at *m/z* 211 and at *m/z* 171, respectively.

3.1.4. Phenolic acids and derivatives

The predominant group of phenolic antioxidants in the melon samples studied was composed of phenolic acids, which may be further classified into hydroxybenzoic, hydroxycinnamic, and phenolic acid derivatives. Several hydroxycinnamic acids and derivatives were characterized in the samples under study such as ferulic acid (peak 50) and p-coumaric acid hexoside (peak 18). P-coumaric acid-hexoside was found in the three melon varieties and its fragment at *m/z* 164 has been previously described in Cucurbitaceae (Abu-Reidah, Arráez-Román, Quirantes-Piné, Fernández-Arroyo, Segura-Carretero, & Fernández-Gutiérrez, 2012). On the other hand, ferulic acid, which appeared only in the variety Cantaloupe, showed a fragment at *m/z* 134 corresponding to $[M\text{-}H\text{-}CH_3\text{-}CO_2]^-$. Other authors have previously identified ferulic acid derivatives in Escrito melon (Moing et al., 2011).

Concerning hydroxybenzoic acid derivatives, isomers of gentisic acid hexoside (peaks 21, 23 or 24) and hydroxybenzoic acid hexoside (peak 37) were found in the melon samples analyzed. Gentisic acid hexoside isomers presented a fragment at *m/z* 153 matching with the gentisic acid moiety. These isomers were found in Piel de Sapo and Cantaloupe and, although these compounds are widely distributed in the plant kingdom, they are described here in *C. melo* (and Cucurbitaceae) for the first time. Hydroxybenzoic acid hexoside, present only in Cantaloupe, has been previously described in other melon varieties together with its fragmentation pattern (Bernillon et al., 2012).

Furthermore, three phenolic acid derivatives were characterized, namely vanillic acid dihexoside (peak 27), vanillic acid hexoside (peak 31), and coelovirin A and B (peaks 35 or 42). In this group of compounds, the most frequent fragmentation corresponded to the loss of the hexoside moiety as in the case of coelovirin a or b with a fragment at *m/z* 179, as reported in the literature for other plants (Huang, Li, Shi, Mo, Wang, & Yang, 2004), but this is the first time in which it has been reported in Cucurbitaceae. The hexoside and dihexoside derivatives of

vanillic acid both presented a fragment at *m/z* 167 due to the vanillic acid moiety. The dihexoside derivative appeared in the three varieties studied and has been previously characterized in other melon varieties (Bernillon et al., 2012; Moing et al., 2011) while the monohexoside has not been described before in *C. melo*. Cantaloupe was the variety that presented the most compounds from this group, while Piel de Sapo was the poorest in phenolic acid derivatives.

3.1.5. Lignans

Three lignans were characterized in Cantaloupe and one of them also appeared in Piel de Sapo: two citrusin A isomers (peaks 48 and 55) and isolariciresinol hexoside or lariciresinol hexoside (peak 64). The proposed fragmentation pattern of citrusin A is shown in Fig. 2 (a).

Compound 55 showed the precursor ion and a fragment ion at *m/z* 521 and 359, respectively. The fragment ion corresponds to lariciresinol or isolariciresinol after the neutral loss of the hexoside moiety. This compound has been described in some Cucurbitaceae species (Abu-Reidah, Arráez-Román, Quirantes-Piné, Fernández-Arroyo, Segura-Carretero, & Fernández-Gutiérrez, 2012) but not in melon fruit.

3.1.6. Esters

Melon aroma, determined by unique combinations of aroma-active compounds, strongly depends on the variety (Pang et al., 2012). Among other compounds, alcohols take part in the aroma profile, which has been extensively investigated, especially by using gas chromatography. The method applied here enabled the identification of three alcohols in all three varieties.

Peaks 69 and 71 were characterized as isomers of butanedioldiacetate. These compounds presented a fragment at *m/z* 131, which has previously been described in other melon varieties (Moing et al., 2011).

The MS/MS spectrum of compound 77 showed a fragment at *m/z* 127 due to [M-HCOOH]. This compound was characterized as methyl-4-butoxybutanoate, which has been previously described in muskmelon (Xiao, Zhao, Wang, Dong, & Jiao, 2010).

3.1.7. Flavonoids

Flavonoids are the most common and widely distributed group in plant phenolic compounds, which are usually very effective antioxidants. Different flavonoids were found mainly in the

variety Galia. Two of these, eriodictyol rutinoside and hesperidin, had never before been described in the family Cucurbitaceae or the species *C. melo*. Eriodictyol rutinoside (peak 59) showed a fragment at *m/z* 287 due to the loss of the rutinoside group. Hesperidin (Peak 70), the only flavonoid detected in all the three varieties of melon studied, presented the fragmentation pattern depicted in Fig. 2 (b) and it has been characterized in other fruits (Kuroyanagi et al., 2008). Peak 67 has been tentatively identified as diosmetin rutinoside, since this compound and its fragment at *m/z* 299 have been previously reported in Cucurbitaceae (Siciliano, De Tommasi, Morelli, & Braca, 2004).

3.1.8. Other polar compounds

Besides the abovementioned compounds tentatively identified, another 12 polar compounds were tentatively identified from different families found in the study samples.

According to Biais et al. (Biais et al. 2010), the most important properties of melon for organoleptic quality and consumer acceptance are the aroma profile and the sucrose level.In this regard, 3 sucrose isomers (peak 3, 4, and 11), which showed fragments at *m/z* 101, 113, 119, and 161, were characterized on the basis of their previously described fragmentation pattern (Taylor, March, Longerich, & Stadey, 2005). Galia was the variety which contained all the three of the sucrose isomers. In addition, one vitamin (peak 25) was also characterized in Piel de Sapo and Cantaloupe as pantothenic acid. This vitamin (B_5) with an hexose group has been previously described in melon fruit (Bernillon et al., 2012; Moing et al., 2011) and its assignment is consistent with the presence of the fragment at *m/z* 146 due to $[M\text{-}H\text{-}CO\text{-}CO_2]^-$, as described by Gómez-Romero (Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010).

A phenolic compound derivative was characterized in the variety Cantaloupe as cistanoside E (peak 36) on the basis of its MS/MS spectrum, where a fragment at *m/z* 167 appeared due to the cleavage of the bond between the phenolic group and the dihexoside moiety.

Two isomers of benzyl alcohol hexose-pentose (peaks 43 and 45) have previously been described in other melon varieties (Bernillon et al., 2012) as well as their fragment at *m/z* 161 which has been reported in other fruits (Moco, Vervoort, Moco, Bino, De Vos, & Bino, 2007).

Related to these compounds, peak with RT 10.50 was tentatively identified as phenethyl alcohol hexose-pentose with a fragment at *m/z* 149, corresponding, to the pentose group of the hexose-pentose moiety. This compound has never before been described in *C. melo* or Cucurbitaceae but has been characterized in other fruits (Kuang, Xia, Yang, Lian, Zhang, & Li, 2009).

The iridoid serrulatoside was found in the variety Galia (peak 52) and has previously been reported in other plants (Bazylak, Rosiak, & Shi, 1996), although not in *C. melo* or in any other member of Cucurbitaceae. Its fragmentation pattern is shown in Fig. 2 (c).

A cyclohexanone hexoside was found and characterized as roseoside A (peak 47). This compound presented two main fragments, at *m/z* 153 and 161, the first being due to the loss of the cyclohexanone moiety and the second one to the loss of the hexose moiety. This compound has been found in the plant kingdom (Champavier, Comte, Vercauteren, Allais, & Chulia, 1999; Iha, Matsunami, Otsuka, Kawahata, Yamaguchi, & Takeda, 2012) but not previously in the Cucurbitaceae family.

Finally, in the three varieties studied, two hormones were tentatively identified as gibberellin A7 (peak 80) and zeatin hexoside (peak 26). Gibberellin A7 presented fragments at *m/z* 211 and 229, in agreement with the fragmentation pattern recorded in MassBank database. In the case of zeatin hexoside, its fragments at *m/z* 308 and 146 were due to the loss of the hydrocarbon chain and the subsequent loss of the hexoside moiety from the product ion. Both are described in Cucurbitaceae as plant-growth-regulating hormones that play an essential role in the plant growth and development (Pimenta Lange et al., 2013; Sidik, Hashim, Mohamad, & Abdullah, 2012).

3.1.9. Unknown compounds

There were 27 compounds for which there was insufficient evidence to propose a structure.

3.2. Multivariate data analysis

For an overview of the distribution of the melon varieties and to explore chemical markers contributing to the classification, a PCA was performed. Firstly, a peak-finder algorithm was applied to combine ions belonging to one compound, such as common adducts, isotopes, and charge states. In this way, features were determined using LC-MS chromatograms acquired

from each of the 14 melon samples. Then, the LC-MS data were prepared for the statistical analysis by generating a so-called variable or bucket table. The buckets represent pairs of retention-time and *m/z* values in such a way that their intensities are listed in the bucket table for each sample and a PCA was calculated based on this table.

The PCA scores plot (PC1 vs. PC2) shows a clear separation of the three melon varieties (Fig. 3 a). Piel de sapo is separated from the others on the first PC and Galia and Cantaloupe on the second. This plot also revealed that the varieties Cantaloupe and Galia are quite similar in composition but very different with respect to Piel de Sapo.

The loading plot shows how principal components are related to their pairs of retention time and *m/z* value in such a way the pairs that are far away from the central cloud are responsible for the variance within the data set. The orientation of these points in the loadings plot corresponds to the distribution of analysis in scores plot. Therefore, the PCA loadings plor (Fig. 3 b) enabled to identify several compounds as being mainly responsible for the observed sample differentiation among varieties, as shown in bold in the Table 1. Thus, 8 tentatively identified metabolites constituted the major difference between Piel de Sapo and the other two varieties (Galia and Cantaloupe), as reflected in Fig. 3, these were hexoside and dihexoside derivatives of vanillic acid, one of the isomers of citric acid, and butanodioldiacetate, zeatin hexoside, 9,12,13-trihydroxyoctadeca-10,15-dienoic acid, guanosine, and hydroxybutanoic acid ethyl ester-hexoside. These compounds presented a significantly higher intensity in Piel de Sapo samples than in the other varieties, while the isomer of citric acid was found only in this variety, in agreement with previous research demonstrating the variation in the citrate content of the fruit depending on planting dates and cultivars (Vallone et al., 2013).

Although Galia and Cantaloupe showed fewer differences between them, 4 compounds proved to be discriminative. Among these, tryptophan or one of its isomers as well as an isomer of benzyl alcohol hexose-pentose were found in both varieties but not in Piel de Sapo samples, presenting higher intensity in Cantaloupe. On the other hand, pyroglutamic acid hexoside was characterized only in Cantaloupe whereas hesperidin was found in both Galia and Cantaloupe, but with significantly higher intensity in the Galia.

Some of these compounds (vanillic acid dihexoside, hydroxybutanoic acid ethyl ester-hexoside, and benzyl alcohol hexose-pentose), which might be hexoside precursors of volatile

compounds (Kilic, Kollmannsberger, & Nitz, 2005), have previously been described as differentiators of two varieties from the Cantalupensis group (Bernillon et al., 2012).

As previously reported, the chemical composition of melon and other fruits is strongly influenced by the physiological state of the plants and by the environmental parameters as well as by the genotype. Thus, the PCA results in this study revealed genotype to be the parameter which most influences the phytochemical composition, as found by other authors (Liu et al., 2004; Manohar & Murthy, 2012).

4. Conclusions

In the present work, HPLC-ESI-QTOF-MS has been confirmed to be a powerful analytical technique for separating and detecting phenolic and other polar compounds in *Cucumis melo* varieties. This is the first available qualitative characterization of the Spanish varieties Galia, Cantaloupe, and Piel de Sapo, by this technique. With this method, 56 compounds were tentatively identified on the basis of their chromatographic retention, MS data, and MS/MS fragmentation pattern. The most representative groups of compounds tentatively identified were amino acids and derivatives, phenolic acid and derivatives, and organic acids although nucleosides, alcohols, lignans, flavonoids and other polar compounds were also characterized. Of these compounds 25 have been tentatively identified for the first time in *C. melo*. Preliminary PCA has shown a clear separation among varieties, although further investigations are needed to confirm the differentiation among the varieties studied. This work provides a better understanding of the distinction between those different Spanish melon varieties. The importance of knowledge concerning fruit composition is increasing due to their high consumption and our study may help to improve the data in food-composition tables.

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FIGURE CAPTION

Fig. 1. Base-peak chromatogram (BPC) of *C. melo* by HPLC-ESI-TOF-MS in the negative ion mode for (a) Piel de Sapo, (b) Galia and (c) Cantaloupe varieties.

Fig. 2. Fragmentation pattern for (a) citrusin A, (b) hesperidin and (c) serrulatoside.

Fig. 3. Principal Component Analysis of the metabolite composition of three melon varieties: a) PCA scores plot (PC1 vs. PC2). Symbol shape corresponds to different varieties (cross = Piel de Sapo; triangle = Cantaloupe; circle = Galia); b) PCA loading plot. Numbers identify the compounds reported in Table 1 in bold.

Table 1. HPLC-ESI-TOF-MS data of the compounds identified.

Peak[a]	RT (min)	m/z experimental	Molecular formula	m/z calculated	Error (ppm)	mSigma value	MS/MS Fragments[b]	Proposed compound	Variety		
									Piel de Sapo	Galia	Cantaloupe
AMINOACIDS AND DERIVATIVES											
2	1.88	145.0629	C5H9N2O3	145.0619	6.9	6.9	127.0504(100%)	Glutamine	+	+	+
9	**2.85**	**290.0880**	**C11H16NO8**	**290.0881**	**0.4**	**18.5**	**128.0338 (100%) 200.0574 (31.2%)**	**Pyroglutamic acid hexoside**	**-**	**-**	**+**
13	3.66	180.0667	C9H10NO3	181.0666	0.1	12.3	119.0497 (100%) 163.0412 (52.8%)	Tyrosine	+	-	+
14	3.70	292.1393	C12H22NO7	292.1402	3.1	4.3	130.0873(100%)	Hexose-leucine isomer 1	-	+	+
15	4.08	292.1381	C12H22NO7	292.1402	7.1	7.6	130.0871(100%)	Hexose-leucine isomer 2	-	-	+
19	5.47	164.0724	C9H10NO2	164.0717	4.1	3.3	147.0462(100%); 148.0519(15.7%); 103.0555(13%); 151.3955(10.1%)	Phenylalanine	+	+	+
22	5.94	326.1233	C15H20NO7	326.1245	3.7	9.1	164.0717(100%)	hexose-phenylalanine	-	+	+
29	**7.09**	**203.0843**	**C11H11N2O2**	**203.0826**	**8.5**	**9.9**	**116.0503 (100%)**	**Tryptophan isomer 1**	**-**	**+**	**+**
33	7.51	203.0848	C11H11N2O2	203.0826	8.8	38.1	116.0514(100%); 142.0660(41.7%); 117.0564(15.6%)	Tryptophan isomer 2	+	+	+
20	5.65	217.1192	C9H17N2O4	217.1194	0.8	5.7	130.0881 (100%) 187.1106 (45.4%)	Isoleucyl-serine	+	-	-
38	8.18	235.1075	C12H15N2O3	235.1088	5.6	14.8	217.1011 (100%)	Alanyl-phenylalanine	-	+	-
41	8.60	243.1712	C12H23N2O3	243.1714	0.7	13.3	199.1793 (100%)	Leucyl-isoleucine	+	-	-
58	10.86	358.1974	C16H28N3O6	358.1984	2.7	29.6	323.1626(100%); 340.1845 (22.8%); 130.0876(15.6%); 279.1797(12.4%)	Asp-Leu-Leu/ Asp-Ile-Ile[c]	+	-	-
61	11.42	392.1803	C19H26N3O6	392.1827	6.1	12.5	357.1418 (100%)	Asp-Leu-Phe/ Asp-Ille-Phe[c]	+	-	-

NUCLEOSIDES											
12	3.60	243.0624	C9H11N2O6	243.0623	0.6	10.1	110.0260(100%); 152.0364(37%)	Uridine	+	+	-
16	**4.78**	**282.0842**	**C10H12N5O5**	**282.0844**	**0.7**	**3.9**	**150.0426(100%); 133.0158(10.2%)**	**Guanosine**	**+**	**+**	**+**
ORGANIC ACIDS											
6	2.38	191.0207	C6H707	191.0197	1	7.1	111.0091(100%)	Citric acid isomer 1	+	+	+
7	2.75	191.0201	C6H707	191.0197	2.1	2.3	111.0088 (100%)	Citric acid isomer 2	+	+	+
8	**2.85**	**191.0209**	**C6H707**	**191.0197**	**6.2**	**3.5**	**111.0081 (100%)**	**Citric acid isomer 3**	**+**	**-**	**-**
39	**8.35**	**293.1249**	**C12H21O8**	**293.1242**	**2.6**	**5.6**	**131.0710 (100%); 113.0208 (9%)**	**Hydroxybutanoic acid ethyl ester-hexoside isomer 1**	**+**	**+**	**+**
40	8.36	293.1271	C12H21O8	293.1242	10	7.7	131.0707 (100%); 113.0401 (10%)	Hydroxybutanoic acid ethyl ester-hexoside isomer 2	+	+	+
76	13.68	187.0988	C9H15O4	187.0976	6.6	10.8	125.0977 (100%)	Azelaic acid	+	+	+
81	18.21	327.2155	C18H31O5	325.2177	6.6	16.6	211.1382 (52.9%)	Trihydroxyoctadecadienoic acid isomer 1	+	-	-
82	**18.51**	**327.2172**	**C18H31O5**	**327.2177**	**1.7**	**11.8**	**211.1335(79.1%); 229.1455(58.6%) 171.1035(26.5%)**	**9,12,13-Trihydroxyoctadeca dienoic acid**	**+**	**-**	**+**
83	18.58	327.2166	C18H31O5	325.2177	3.5	13.3	171.1035 (52.9%)	Trihydroxyoctadecadienoic acid isomer 2	+	-	-
PHENOLIC ACIDS AND DERIVATIVES											
18	5.59	325.094	C15H17O8	325.0929	3.3	27.3	164.0722(100%)	p-Coumaric acid-hexoside	+	+	+
21	5.72	315.0744	C13H15O9	315.0722	7.2	21.15	152.0107(100%); 153.0191(70.2%); 108.0166(30%)	Gentisic acid-hexosideisomer 1	+	-	-
23	6.04	315.0705	C13H15O9	315.0722	5.3	15	153.0192(100%); 109.0286(15.7%)	Gentisic acid - hexoside isomer 2	-	-	+
24	6.22	315.0707	C13H15O9	315.0722	4.6	7.7	152.0121(100%); 153.0181(86.1%); 108.0230(70.6%)	Gentisic acid-hexoside isomer 3	+	-	-
27	**6.69**	**475.1475**	**C20H27O13**	**475.1457**	**3.8**	**4.8**	**167.0352 (100%)**	**Vanillic acid dihexoside**	**+**	**+**	**+**
31	7.41	329.0877	C14H17O9	329.0878	0.4	13.7	167.0358 (100%)	Vanillic acid hexoside	-	-	+

35	7.86	473.1654	C21H29O12	473.1664	2.3	15	179.0725 (100%)	Coelovirin A/ Coelovirin B isomer 1	-	+	+
37	8.15	299.0755	C13H15O8	299.0772	5.7	19.7	137.0222(100%)	Hydroxybenzoic acid hexoside	-	-	+
42	8.75	473.1656	C21H29O12	473.1664	0.4	19.9	179.0733 (100%)	Coelovirin A/ Coelovirin B isomer 2	-	+	+
50	9.95	193.0509	C10H9O4	193.0506	1.4	3.6	134.0341(100%)	Ferulic acid	-	-	+
							LIGNANS				
48	9.80	537.1990	C26H33O12	537.1978	0.5	24.1	327.1239 (100%); 195.0675 (57.7%); 179.0588 (23.5%); 489.1820 (17.7%)	Citrusin A isomer 1	+	-	+
55	10.62	537.2017	C26H33O12	537.1978	7.4	16.9	327.1245(100%); 195.0669(40.9%); 179.0574(37.9%); 161.0451(27%)	Citrusin A isomer 2	-	-	+
64	11.91	521.2059	C26H33O11	521.2028	5.9	104.7	359.1433(100%)	Isolariciresinol hexoside/lariciresinol hexoside	-	-	+
							ESTERS				
69	12.68	173.0833	C8H13O4	173.0819	7.7	10.8	131.0722(100%)	Butanedioldiacetate Isomer 1	+	+	+
71	**13.15**	**173.083**	**C8H13O4**	**173.0819**	**6**	**15.1**	**131.0712(100%)**	**Butanedioldiacetate isomer 2**	**+**	**-**	**-**
77	13.92	173.1184	C9H17O3	173.1183	0.6	5	127.1130 (55%)	Methyl-4-butoxybutanoate	+	+	+
							FLAVONOIDS				
59	11.12	595.1704	C27H31O15	595.1668	5.9	10.5	287.0561 (100%)	Eriodictyol rutinoside	-	+	-
67	12.56	607.1672	C28H31O15	607.1668	0.6	24.6	299.0597(100%); 145.5485(46.5%)	Diosmetin rutinoside	-	+	-
70	**12.78**	**609.184**	**C28H33O15**	**609.1825**	**2.5**	**8.9**	**301.0734(100%); 145.2260(8,2%)**	**Hesperidin**	**+**	**+**	**+**
							OTHER POLAR COMPOUNDS				
3	2.03	341.1108	C12H21O11	341.1089	8.2	9.7	165.0408(100%); 113.0224(21.2%); 161.0490(8.4%); 101.0218(6	Sucrose isomer 1	+	+	+

							.5%)				
4	2.14	341.1095	C12H21O11	341.1089	1.7	10	165.0417(100%); 113.0245(16.6%); 119.0358(9.4%); 101.0272(8.3%)	Sucrose isomer 2	-	+	-
11	**3.38**	**341.1081**	**C12H21O11**	**341.1089**	**2.5**	**29.2**	**165.0417(100%); 113.0245(25%)**	**Sucrose isomer 3**	**-**	**+**	**-**
25	6.29	218.1039	C9H16NO5	218.1034	2.2	8.6	146.0825(100%)	Pantothenic acid	+	-	+
26	**6.34**	**380.1547**	**C16H22N5O6**	**380.1576**	**7.4**	**21.1**	**146.0835(23.2%); 308.1354(12.1%)**	**Zeatin hexoside**	**+**	**+**	**+**
43	**9.07**	**401.1461**	**C18H25O10**	**401.1453**	**1.9**	**9.8**	**161.0440(100%); 179.0587 (93%)**	**Benzyl alcohol-hexose-pentose isomer 1**	**+**	**+**	**+**
45	9.28	401.1466	C18H25O10	401.1453	3.1	24.4	161.0446(100%); 179.0597(88%)	Benzyl alcohol-hexose-pentose isomer 2	+	+	+
47	9.53	385.1843	C19H29O8	385.1868	6.4	16.1	153.0925 (100%); 161.0505 (33.2%); 113.0225 (23.4%)	Vomifoliol hexoside (roseoside A)	+	+	+
52	10.10	589.2521	C29H41O14	589.2502	3.2	17.8	161.0503 (13.1%); 179.0548 (100%); 367.2113 (70.3%)	Serrulatoside	-	+	+
54	10.50	415.1627	C19H27O10	415.1610	4.3	9.8	149.2253 (100%)	Phenethyl alcohol-hexose-pentose	+	+	+
80	14.67	329.1378	C19H21O5	329.1394	4.9	14.4	211.1339(54.9%); 229.1449(43.6%); 171.1016(11.3%)	Gibberellin A7	+	+	+
36	8.13	475.1832	C21H31O12	475.1821	2.4	32.2	167.0353 (100%)	Cistanoside E	-	-	+

[a] Peak numbers assigned according to the overall elution order; Compounds in bold corresponding to the metabolites which constituted the major difference between the three varieties.

[b] Numbers in brackets show the relative abundance of each MS/MS fragment expressed in percentage

[c] Amino acids are denoted by the three letter code: Aspartic acid, Asp, isoleucine, lle, leeucine, Leu, phenylalanine, Phe.

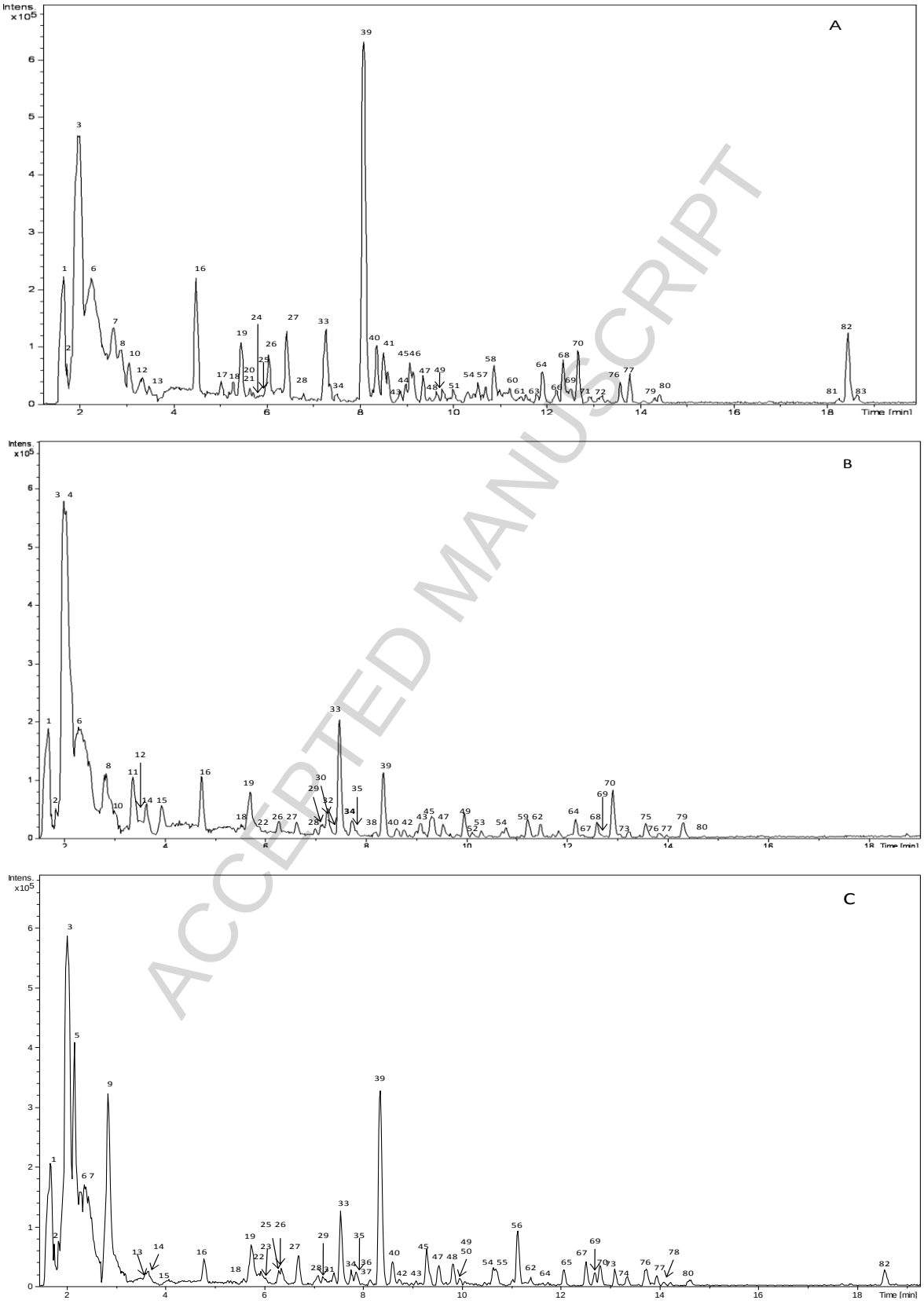


Figure 1. Base-peak chromatogram (BPC) of *C. melo* by HPLC-ESI-TOF-MS in the negative ion mode for (a) Piel de Sapo, (b) Galia and (c) Cantaloupe varieties.

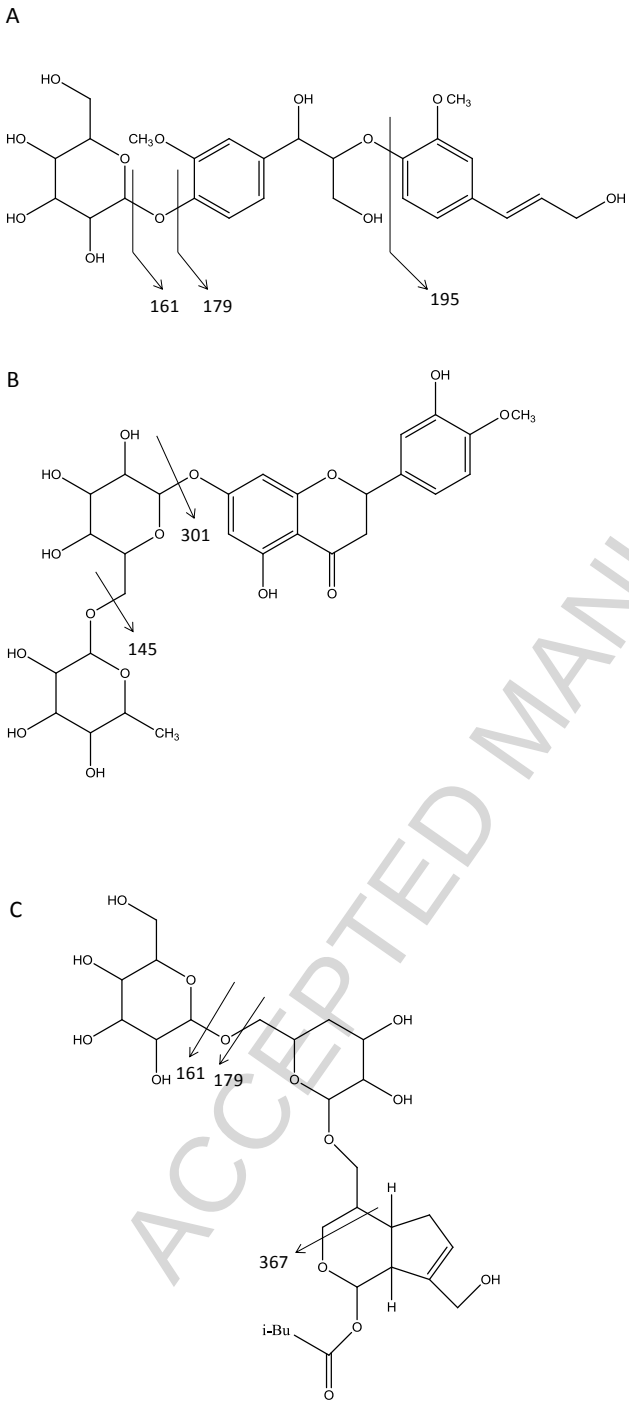
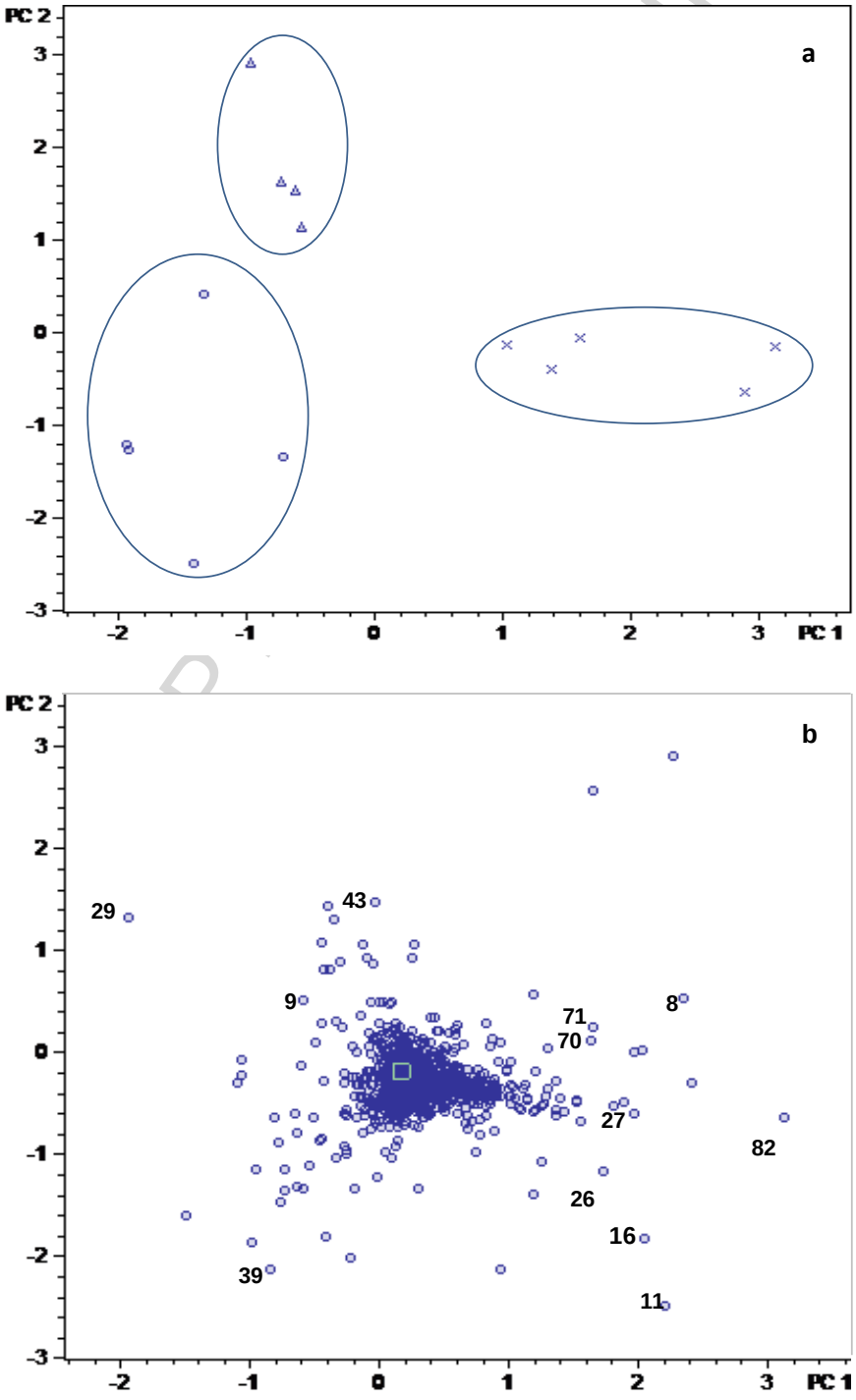
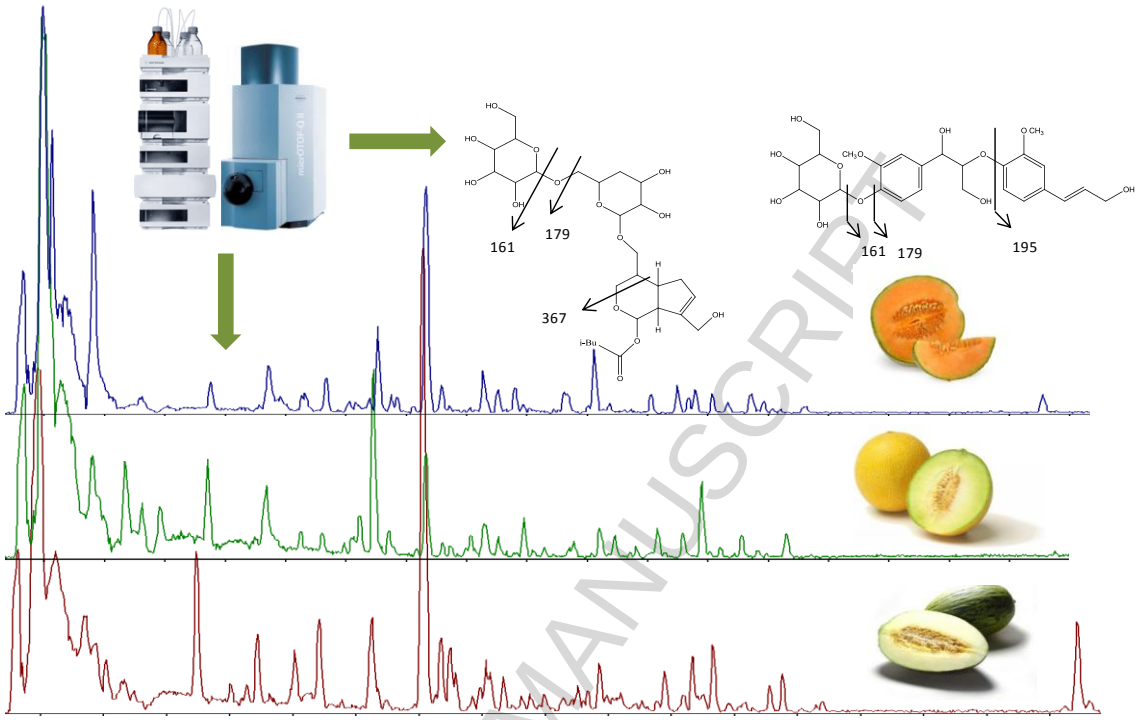


Fig. 2. Fragmentation pattern for (a) citrusin A, (b) hesperidin, and (c) serrulatoside.

Fig. 3. Principal Component Analysis to the composition of the three varieties: a) PCA scores plot (PC1 vs. PC2); Symbol shape corresponds to different varieties (cross = Piel de Sapo; triangle = Cantaloupe; circle = Galia); b) PCA loadings plot. Numbers identify the compounds reported in Table 1 in bold.





Graphical Abstract

HIGHLIGHTS

- - Polar fraction from 3 *Cucumis melo* varieties was analyzed by using HPLC-ESI-QTOF-MS
- 56 compounds have been tentatively identified by MS/MS fragmentation pattern
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- 25 of these compounds were characterized for the first time in *C.melo* varieties.
- PCA showed 12 metabolites which were decisive to discriminate among these varieties.