Oxygenated heterocyclic compounds to differentiate Citrus spp. essential oils through metabolomic strategies

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Oxygenated heterocyclic compounds to differentiate *Citrus* spp. essential oils through metabolomic strategies

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Abstract

This study aimed to characterize and discriminate 44 authenticated commercial samples of citrus essential oils (EO) from seven species (bergamot, lemon, bigarade, orange, mandarin, grapefruit, lime) by analyzing the non-volatile oxygenated heterocyclic compounds (OHC) by UHPLC/TOF-HRMS, multivariate data analysis (PCA, PLS-DA) and metabolomic strategies; the OHC fraction includes coumarins, furocoumarins, and polymethoxylated flavonoids. Two different approaches were adopted: i) targeted profiling based on quantifying 18 furocoumarins and coumarins, some of which are regulated by law, and ii) targeted fingerprinting based on 140 OHCs reported in citrus essential oils, from which 38 discriminant markers were defined. This approach correctly discriminated the *Citrus* species; its "sensitivity" to relatively low adulteration rate (10%) was highly satisfactory.

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The proposed method is complementary to that of analyzing the citrus EO volatile part by GC techniques.

Keywords:

Citrus spp; Metabolomic strategy, Oxygenated heterocyclic compounds; UHPLC/TOF-MS; Multivariate data analysis, discriminant markers

1. **INTRODUCTION**

Citrus fruits are among the most widely produced crops; their essential oils (EOs), mainly obtained by cold pressing their peel, are widely used as natural flavoring materials. These products have distinctive organoleptic properties and present numerous applications in different areas, including beverages, confectionery, pharmaceuticals, cosmetics, and perfumes.

More than 200 components have been identified in *Citrus* essential oils; most are contained in one of two fractions (Mondello, Zappia, Dugo, & Dugo, 2002; Tranchida, Bonaccorsi, Dugo, Mondello, & Dugo, 2012; Dugo, Cotroneo, Verzera, & Bonaccorsi, 2002; Dugo, Cotroneo, Bonaccorsi, & Restuccia, 2011)*:*

i) the volatile fraction, accounting for 85% to 99% of the EO. This fraction contains mono- and sesquiterpene hydrocarbons and their related oxygen derivatives, but also linear hydrocarbons, aldehydes, alcohols, ketones, and esters (Shaw, 1979). A group of nitrogen trace-components, significantly contributing to the characteristic olfactory note, has also been identified in these EOs (Thomas & Bassols, 1992).

ii) the non-volatile residue, ranging from 1 to 10 % of the whole EO, consisting chiefly of hydrocarbons, fatty acids, and waxes, together with the so-called oxygenated heterocyclic components (OHCs) (~~figure~~ Figure 1a). This fraction mainly contains furocoumarins (Fcs), coumarins (Cs) and polymethoxylated flavonoids (PMFs), naturally present in citrus fruit and differently distributed in citrus products; they strongly influence their organoleptic and biological properties, e.g. naringine affects taste. Moreover, the OHC qualitative and quantitative composition is diagnostic of each citrus EO, and plays an important role in their characterization and authentication for the flavor and fragrance fields (Dugo, Mondello, d'Alcontres, Cavazza, & Dugo, 1997).

Furocoumarins are known to be phototoxic, and have been shown to induce mutagenesis, carcinogenesis, and photodermatitis (Bonamonte, Foti, Lionetti, Rigano, & Angelini, 2010); their toxicity has led the European Community to limit the use of photosensitizing furocoumarins in cosmetic products (CEE, 2010). The International Fragrance Association (IFRA) recommends that the total content of bergapten should not exceed 15 ppm in finished consumer products to be applied to UV-exposed skin areas. The Opinion of the Scientific Committee on Cosmetic products and Non-Food Products intended for consumers (SCCNFP/0392/00, September 25th, 2001) proposes permitting the use of furocoumarin-containing essential oils in all types of cosmetic product provided that the total concentration of furocoumarin-like substances in the finished product does not exceed 1 ppm. The European Commission recently proposed authorizing a total of 5 ppm of any combination of 6 markers (i.e. bergapten (5-MOP), bergamottin, byacangelicol, epoxybergamottin, isopimpinellin and oxypeucedanin) in any type of leave-on finished consumer product. In parallel, it also recommended a less rigorous restriction for rinse-off finished products, i.e. 50 ppm, a level defined by the difference in skin exposure (SCCNFP, 2001). Conversely, polymethoxylated flavones possess antioxidant, anti-mutagenic, and antitumoral activities, and are considered to be potentially chemopreventive (Weber et al., 2006).

The potentially-phototoxic furocoumarins, and more in general the OHCs, have been the object of several studies, aimed not only at their characterization in different citrus EOs, but also at their detection and quantitation. An important contribution to the identification and definition of the OHC composition in these matrices was made by the Messina University group under P. and G. Dugo and Mondello; they recently reviewed in detail approaches and methods to analyze both citrus EOs and related OHCs (Mondello et al., 2002; Tranchida et al., 2012; Dugo & McHale, 2002; Dugo & Russo, 2011) (the list of articles concerning OHCs in each citrus EO investigated, and cited in the book chapter references is included in ~~Supporting~~

~~Information~~ Table ~~SM~~1 and Supporting Information Table SM2-SM3). The usual method to analyze OHCs in citrus EOs is reverse-phase HPLC, combined with different detectors (UV, fluorescence, and mass spectrometry) (Tranchida et al., 2012; Dugo & Russo, 2011; Frérot & Decorzant, 2004; Macmaster et al., 2012; Russo et al., 2015).

The need to develop analytical methods focusing on OHC distribution to authenticate and detect adulteration of citrus EOs was first pointed out in 1971, by Stanley (Stanley & Jurd, 1971). Recent advances in LC and MS, in particular the introduction of UHPLC coupled with high resolution time-of-flight mass spectrometry (HR-TOF-MS), have opened new perspectives for phytochemical analysis, in particular for quality control and authentication of raw plant materials, or of products containing them (Eugster et al., 2014; Masson, Liberto, Brevard, Bicchi, & Rubiolo, 2014). These techniques make it possible to acquire a significant set of exact masses from a group of complex samples, but more sophisticated strategies are required to manage the huge amount of data generated. The metabolomics approaches (mainly fingerprinting and profiling) have recently been shown to provide useful and effective support to the analysis of matrices of vegetable origin, enabling the potential of these analytical platforms to be fully exploited (Masson et al., 2014; Mehl et al., 2014; Marti et al., 2015; Farag, El-Ahmady, Elian, & Wessjohann, 2013). Metabolite fingerprinting is in general an untargeted method that, combined with appropriate statistical data processing, provides a mean of comparing the profiles of a group of samples, in order to detect quali- and quantitative metabolite differences between them, or to classify and/or discriminate among them. Fingerprinting is a rapid high-throughput screening approach, whose main goal is not the identification of each metabolite. Metabolite profiling is a targeted method, in which samples are compared on the basis of the quali-quantitative distribution of a selected number of known metabolites (markers).(Halket et al., 2005).

This approach was recently applied to characterize, differentiate, and check the authenticity of cold-pressed lemon EOs. Mehl *et al.* evaluated the potential of volatile and non-volatile fraction composition in classifying 64 lemon EOs from Argentina, Spain and Italy, using GC-FID/MS and FT-MIR for the volatiles, and FT-MIR, ^{1}H-NMR and UHPLC/TOF-MS for the non-volatile residues (Mehl et al., 2014). The data blocks resulting from the above techniques were sequentially processed by unsupervised and supervised multivariate statistical analyses, affording both the discrimination of the investigated EOs in terms of geographic origin and production process, and the definition of important classes of discriminant variables. Marti *et al* successfully applied a metabolomic approach, based on UHPLC/TOF-MS profiling and ^{1}H-NMR fingerprinting, to highlight metabolite differences suitable for detecting adulteration within a set of lemon EO samples of different geographic origins (Marti et al., 2015). The combination of a specially-developed analytical protocol and multivariate analysis was able to detect significant chemical variations between Argentine and Italian samples, and identified discriminating markers belonging to the furocoumarin, flavonoid, terpenoid, and fatty acid groups.

In general, citrus EO analyses for quality control are run by GC or enantioselective-GC, combined with MS or IRMS of the volatile fraction, and take no account of OHCs. Specific analysis are carried out by HPLC-DAD or MS when furocoumarins monitoring is required because of regulations. Within a project aiming to explore the potential of metabolomics approaches when applied to phytochemical analysis (Masson et al. 2014), this study investigated the possibility of discriminating and authenticating cold-pressed citrus EOs from seven different species, by analyzing the non-volatile OHC fraction by UHPLC/TOF-HRMS. The species considered were bergamot (*Citrus bergamia* Risso), lemon (*Citrus limon* (L.) Osbeck.), bigarade (*Citrus x aurantium* L.), orange (*Citrus sinensis* (L.) Osbeck.), mandarin (*Citrus reticulata* Blanco), grapefruit (*Citrus paradisi* Macfad), and lime (*Citrus aurantifolia*

(Christm.) Swingle*)*. Two approaches were applied: i) the first based on metabolite profiling on 18 furocoumarins and coumarins in the citrus EOs investigated, ii) the second based on metabolite targeted fingerprinting of the 140 OHC compounds reported in the literature, thus considering a full set of compounds (OHC patterns), unlike what often happens, where few targeted compounds are investigated. For both approaches, unsupervised multivariate data analysis was applied in order to examining the sample distribution and or groupings of samples. A supervised method was then used, in particular in the second approach for classifying samples according to their species, and using an orthogonal data treatment to extract markers responsible of the species differentiation.

2.**MATERIAL AND METHODS**

2.1Chemicals and samples

Acetonitrile (ACN), methanol (MeOH), tetrahydrofuran (THF), formic acid, and ammonium formate (LC/MS grade) were from Biosolve (Dieuze, France). Distilled water was purified 'in-house' using an ELGA MilliQ system VeoliaWater STI (Le Plessis-Robinson, France). Leucine-Enkephalin was from Waters (Milford, USA). A mixture of 15 FCs (bergapten, psoralen, xanthotoxin, bergamottin, epoxybergamottin, byakangelicol, byakangelicin isopimpinellin, imperatorin, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, heraclenin, phellopterin, 8-geranyloxypsoralen) was from Chromadex (Irvine, USA). citropten and angelicin were from Sigma-Aldrich (Saint Louis, USA) and herniarin was from Extrasynthese (Genay, France)

Fifty six samples of citrus EOs were analyzed. These included different products: i) 44 commercial citrus EOs (cold-pressed oil) distributed as follows: bergamot (15 samples), lemon (5), bigarade (3), orange (6), mandarin (3), grapefruit (8) lime (4), (2 distilled and 2 expressed EOs) and ii) 12 in-house extracts from fruit peel samples, in particular 2 samples from bergamot, 3 from lemon, 2 from bigarade, 2 from orange, 2 from mandarin, and 1 from grapefruit. The 44 investigated citrus EOs have been supplied from Robertet quality control laboratory, and were selected after their unequivocal authenticity was proved through physico-chemical parameters, GC-FID (or MS), and when needed enantioselective GC analysis. .

2.2 Sample preparation

Commercial samples were processed as follows. Approximately 100 mg of exactly weighed EOs were diluted with ACN/MeOH (50:50) in a 10mL volumetric flask. All samples were then diluted, to obtain at least four different concentrations, in the linear concentration range necessary to apply the extrapolative dilution approach, depending on the targeted compounds. All these dilutions were then analyzed by UHPLC/TOF-MS.

The citrus peel specimens (100 g) were extracted by maceration in 500mL of an ACN/MeOH (50:50) mixture for 24 hours at room temperature. The extracts were then filtered and diluted to differing extents, depending on the linearity range, to overcome the matrix effect by using an extrapolative dilution approach.

Five EO test samples were prepared in-laboratory as test samples, using the referenced EO samples. In particular, sample 1, labeled orange, consisted of a sweet orange EO spiked with 10 % of bigarade EO, and four bergamot EOs, % spiked respectively with 10% of bigarade (sample 2), grapefruit (sample 3), lemon (sample 4), and lime (sample 5).

2.3Analysis conditions

UHPLC conditions

The UHPLC analyses were carried out on a Waters ACQUITY® UPLC H-class system (Waters Milford, USA). The chromatographic separation was performed at 45°C on an ACQUITY® UPLC HSS T3 (C18 stationary phase) 1.8 µm, 2.1 x 100 mm (Waters Milford, USA). Solvent A was a mixture of H_2O:MeOH:THF (85:10:5) with 0.1% of formic acid and 5mM of ammonium formate solution, while Solvent B was MeOH:ACN:THF (65:30:5) with 0.1% of formic acid and 5mM of ammonium formate. The following gradient profile was applied: from 100% of solvent A in isocratic mode for 1 min, then to 20 % of solvent B in 4 min, to 50 % of solvent B in 3 min, to 90 % of solvent B in 2 min, and finally to 100% of solvent B in 2 min. A 3 min re-equilibration time was applied. Flow rate: 0.45 mL/min, injection volume: 1µL. All samples were analyzed in a continuous sequence, injecting a reference sample every ten injections.

HR-TOF-MS conditions

Analyses were carried out on a Waters XEVO® G2 ToF. ESI source conditions: capillary voltage: 0.7 kV, cone voltage: 30 V, extraction cone: 4V, source temperature: 120°C, desolvation temperature: 400°C, gas flow: 10L/hr, desolvation gas flow: 1200L/hr. The analyses were carried out in positive and negative modes. The mass axis was calibrated with sodium formate in the range 50 to 1200 Da. Mass Lockspray: Leucine Enkephalin was used to correct masses. Two masses were checked in each mode (positive mode: 278.1141; 556.2771; negative mode: 236.1035; 554.2615). The acquisitions were carried out in MS^E mode using argon as collision gas to obtain analyte fragmentation. MS^E mode enables to acquire simultaneously, mass spectra at both low and high collision energy, the former to obtain parent ion, the latter to produce fragment ions.

2.4 Data processing

Quantitative analysis

Quantitation was carried out using the POSI±IVE software package (Waters, Milfors USA). This enables targeted compounds to be quantified in two main steps, provided that a database including molecular formula, retention times, and fragments of the investigated analyte(s) is at first built up. In particular: 1) each target compound is identified in the chromatographic profiles, based on its molecular formula; retention time, and fragments obtained with the MS^E mode; 2) the identified analytes are quantified. The reliability parameters of the analytical method were in accordance with the IUPAC guidelines (Thompson, Ellison, & Wood, 2002). In particular, the following performance parameters were determined: linearity, repeatability and intermediate precision, limit of quantification (LOQ) and limit of detection (LOD). The repeatability and the intermediate precision expressed through the relative standard deviation (RSD%) were determined for each compound at two different concentrations (low and high). Repeatability was measured on six injections at the two concentrations in the same analytical sequence, while intermediate precision was determined similarly, but on separate analytical sequences, run on six different days. LOQs were determined experimentally by calculating the lowest concentration at which the repeatability (RSD%) was acceptable. LOD was calculated experimentally, through ion ratios between parent ion and two daughter ions. The tolerance of the abundance ratios indicated by IOFI (IOFI, 2010) was used to measure the concentration at which it was still possible to identify the targeted compounds with certainty (i.e. the LOD). The reliability of the method parameters was evaluated on standard solutions, because of the difficulty of obtaining citrus EOs free of Fcs and Cs; this choice also made it possible to avoid running a calibration for each species of citrus EO investigated.

Statistical elaboration

The resulting normalized data set was processed using the package MarkerlynxTM; (Waters, Milford, USA) based on the determination of Exact Mass Retention Time pairs (EMRT). Data alignment was achieved by ApexTrack® algorithm included in the MasslynxTM package. The EMRT pairs were then treated through a different statistical treatment using a pareto scaling. These variables were first processed by unsupervised methods:

- Principal Component Analysis (PCA) for evaluating the distribution of the samples. These methods were evaluated through the R2X (cum) i.e. the Cumulative sum of square of the entire X explained by all extracted components.

- Hierarchical cluster analysis (HCA) to study the distances between pairs of samples in order to highlight groupings between them through the Euclidean distance algorithm using single linkage clustering. When distances between samples are relatively small this implies that samples are similar.

A supervised method was then used (Partial Least Square-Discriminant Analysis (PLS-DA)) to classify samples according to the deriving-from species for further species prediction of unknown samples. These statistical methods were evaluated through the goodness of fit R2Y (cum) (cumulative explained variance) and Q2Y (cum) (cumulative predictive ability), obtained with a seven fold cross validation procedure and the specificity and sensitivity of each groups studied.

Specific data treatment, i.e. Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA), was then applied to extract the EMRT pairs responsible for the group separation; when applied to species discrimination. Because of the high numbers of groups studied, this approach was necessary for selecting the main specific and significant markers responsible of the species differentiation, by reducing the numbers of correlate variables.

3. RESULTS AND DISCUSSION

This study mainly focused on OHCs, with the aim of characterizing and authenticating the species of the investigated citrus EOs; it consisted of the UHPLC method development and validation, followed by a comparison of two different approaches applied in parallel to locate the main OHC markers characterizing citrus EOs. The study was carried out on 44 authenticated samples of citrus EOs and 12 in-house extracts obtained directly from the fruit peel used as reference samples for comparison.

The first approach was a targeted profiling, where 18 furocoumarins and coumarins were quantified in 44 samples by UHPLC/HR-TOF-MS with the POSI±IVE software, through the parent and daughter ions obtained in MS^E mode; unlike strategies applied in other studies (Mehl et al., 2014; Marti et al., 2015), the target OHCs were here selected from the start.

The second approach was based on fingerprinting, in which UHPLC/HR-TOF-MS data were compared through metabolomics-derived multivariate processing, based on 140 OHCs reported in the literature and stored in an in-house built database (see 3.2.1.). The discrimination ability of this approach was verified through five in-laboratory prepared EOs, one orange and four bergamot samples differently spiked; bergamot EO was chosen because of its high commercial value.

3.1 Quantitative targeted analysis of coumarins and furocoumarins (Cs and Fcs)

3.1.1 UHPLC-HS-ToF-MS method development

As already shown by Dugrand et al. (2013), Mehl et al. (2014) and Marti et al. (2015), UHPLC with a 1.8µm C18 column enabled to obtain a reliable OHC separation in a reasonable time (about 13.5 min.) of the citrus EO extracts investigated. Figure 1b reports the chromatographic profiles of the target ions adopted to quantify the 18 OHCs under study, Table 1 the quantification data while Table SM1 (supplementary material files) reports the Exact Mass Retention Time (EMRT) values, including those of the diagnostic MS^E fragments of the 18 Fcs and Cs. This information was obtained after optimization of the analytical conditions, by injecting each individual standard into the UHPLC/TOF-MS system, and was used to build the database required by the Posi±ive software. Table SM1 also lists the parameters showing the reliability of the analytical method, applied to each target compound in a solvent standard solution; linearity was determined for each compound, and the correlation line calculated in the appropriate linear range. Repeatability and intermediate precision are here expressed as Relative Standard Deviation % (RSD%) for each target analyte at low and high concentrations, for the above validation periods (see paragraph 2.4). The results show very good repeatability and satisfactory intermediate precision, which in any case never exceeded 7 and 8% respectively in agreement to Horwitz curve. The calibration curves for the target analytes presented good (r2 > 0.99) within the range of 0.1-10 mg/L. LOQ and LOD were in agreement with those reported in the literature for these compounds (Dugo et al., 2011).

The 18 Fcs and Cs analyzed by UHPLC/TOF-MS were affected by a significant matrix effect. This effect caused the reduction or suppression of the signal of the ion(s) of the target compounds, mainly occurring in the ESI mode (Hajslova & Zrostlikova, 2003). The matrix effect was minimized by applying the Extrapolative Dilution Approach (EDA) (Kruve, Leito, & Herodes, 2009), Each sample was therefore diluted at different extent to take into account the matrix effect for each quantified compound to establish a curve with at least 4 points. These experiments showed that Fcs and Cs behave differently, as expected because of the lipophylicity of the matrix that may affect the less polar compounds such as bergamottin (Figure SM1).

3.1.2 Quantitation on commercial samples

The 18 FCs and Cs markers were quantified in the 44 samples of commercial citrus EOs and in the 12 in-house extracts of citrus peel oils. The concentration range of each target compound is summarized in table 1. These results first show that the concentration ranges of all markers in each species are in line with those reported in the literature

Bergamot, lemon, and lime contained large amounts of both Fcs and Cs. Bigarade and grapefruit contained intermediate amounts of the target compounds, while in mandarin and orange they were very low. Distilled lime was almost free from FCs and Cs due to the processing.

The six markers proposed for future restriction (bergamottin, bergapten, byakangelicol, epoxybergamottin, isopimpinelin, and oxypeucedanin) were found in different amounts in the investigated EOs of all species. Bergamottin and bergapten were the two major components in all Eos, while byakangelicol was only detected in expressed lime and lemon EOs. The three other Fcs were found in different concentrations in most EOs. The coumarin citropten was present in significant amounts in bergamot, lemon and expressed lime EOs. The other eleven

Fcs and Cs were found at different concentrations in expressed lime, lemon, and bergamot EOs (Table 1). In any case, the concentration of all markers in each species are well in line with those reported in the literature.

Analysis of the 18 target Fcs and Cs was not sufficient to achieve unequivocal discrimination of citrus EOs in terms of species, both because a) some of them occur in all or most of the investigated EOs, e.g. bergamottin (present in 7 species) and bergapten (6 species), and/or b) the same Fc(s) or C(s) is present in very similar concentrations in different EOs, and is thus not diagnostic of a given species. This limitation may also be due to the wide variability of EO composition within each species, which is known to be related to the crop period (early, mid, or late season), crop year (impact of climatic and environmental factors), origin (effect of soil and climate) and the industrial processes to which they are subjected. The dataset for the 44 samples investigated with a targeted approach based on the 18 compounds previously quantified was thus submitted to unsupervised multivariate statistical analysis, i.e. Principal Component Analysis (PCA). The PCA results (~~figure~~ Figure SM2) show an EO clustering in function of the species not well defined, with several outliers (orange samples) and/or with non-diagnostic dispersions (mandarin, lemon, and lime). These results confirm that quantitative analysis as such of the 18 Fcs and Cs is diagnostic of the regulated compounds, but that it is not sufficient for a species discrimination approach.

3.2 Untargeted OHC fingerprinting approach

This strategy could be adopted because of the potential of UHPLC/TOF-MS, which can successfully support comprehensive metabolite studies to discriminate complex matrices of vegetable origin. The fingerprinting approach was applied to the same set of analysis results previously used in order to select OHCs that can discriminate the EOs of each *Citrus* species.

3.2.1 Building up the reference database and component identification

A dedicated database containing 140 OHCs, previously identified in the seven investigated *Citrus* spp. EOs and extracts, was built up in the Masslynx Software package. Data were retrieved from the original articles in which the compounds were first described. The resulting database included exact mass, molecular formula, RN from CAS, and structure, and was used to characterize and then identify discriminant markers on the basis of their molecular formula or exact mass.

3.2.2 *Characterization and tentative identification of OHCs by UHPLC/TOF-MS.*

Each peak resulting from UHPLC/ToF-MS analysis was characterized through its Exact Mass-Retention Time pair (EMRT). ESI in positive mode resulted to be very effective for the OHC compounds investigated while the results in negative ionization were less interesting. Data elaboration was therefore focused on positive ESI.

The following procedure was adopted for tentative identification:

a) the EMRT of each eluting component was matched with the citrus OHC database, by its exact mass or elemental composition, using mass accuracy as diagnostic parameter for identification. The output indicated components having similar exact masses (or elemental compositions) in both ionization modes (positive and negative).

b) the resulting tentative identification was verified by comparing the exact masses of the experimental fragments of each EMRT pair, obtained at high collision energy in MS^E mode, to those of the theoretical fragmentation proposed by the Mass Fragment software. The probability of a tentative identification being correct therefore markedly increases if, but only if, experimental and theoretical fragmentations agree. When authentic standards are not commercially available, the reported identifications (Table 2) must be considered as tentative/putative, in agreement with the international committee recommendations (Sumner et al., 2007; Creek et al., 2014-Schymanski et al., 2014).

3.2.3 Statistical data treatment

EO statistical discrimination - 850 EMRTs were first extracted from the dataset resulting from the analysis of the 44 samples investigated, and then submitted to HCA and PCA by pareto pre-processing scaling. The high number of EMRTs compared to the number of target compounds is due to the large number of isomers eluting at different retention times, corresponding to the 140 selected exact masses. The resulting HCA (~~figure~~ Figure SM3a) shows a better hierarchical classification of the samples according to their species, with all the samples well grouped according to their species. The PCA score plots (~~figure~~ Figure SM3b) obtained showed a good cumulative explained variance R2[X] (cum) = 0.78 on five principal components. Samples were clustered in three different groups: 1) the first (1) includes lemon, the two lime sub-groups, and bergamot EOs, 2) the second (2) comprises mandarin and orange, 3) the third (3) cluster includes bigarade and grapefruit, at top right. Group discrimination appears to follow the organoleptic characteristics of the fruits. The difficulty in species discrimination is also due to the considerable redundant data contained in these samples, probably deriving from the large number of isomers, which provide the same information but, at the same time, interfere with species characterization. A supervised statistical data treatment was run using a PLS-DA, with the aim of a better species classification in prediction. Similar clustering to previous PCA was observed (data not shown).

It was therefore necessary to consider more dimensions other than the first two, in order to improve the effectiveness of this approach with a R2[Y] (cum) = 0.94 and a predictive ability Q2[Y] (cum) = 0.58 on 8 principal components.

This procedure made it possible to distinguish: bergamot, orange and lemon EOs, grapefruit from bigarade, and orange from mandarin EOs from the score plots (~~figure~~ Figure 2).

Definition of discriminant markers by OPLS-DA

Loading plots corresponding to the PLS-DA data treatments can be used for the extraction and selection of discriminant markers. In this study seven different species of citrus were evaluated, the use of the loading plot could lead the loss of minor compounds which can be essential for their discrimination. OPLS-DA was then applied in order to reduce the numbers of correlated variables and as a consequence highlighting the contribution of minor compounds and to extract the best discriminant OHCs markers for each species. The effective discriminant markers were defined after viewing the whole set of data, through the so-called trend views, i.e. the results of the comparison of the respective abundance (normalized area) of a single EMRT pair in all investigated samples. The trend views of each EMRT can define potential discriminant markers, by comparing their abundance in the samples investigated. Figure 3 reports the trend views of (a) bergamottin (EMRT = 339.1593, 11.35), and (b) meranzin isomer 3 (EMRT = 261.1120, 10.60) showing their diagnostic distribution in the different citrus oil species investigated. This process indicated 38 EMRTs as discriminants for the EOs of the seven investigated citrus species (8 groups). Table 2 reports exact mass (m/z), retention time (RT min), supposed elemental composition, accuracy (ppm), normalized i-FIT, tentative identification, species, fragment ions and related elemental formula, with the corresponding accuracy (ppm), of the 38 discriminant markers resulting from OPLS-DA. Normalized i-FIT provides a measure of the likelihood that the theoretical isotope pattern of an elemental composition matches peaks in a measured spectrum. Thirty four components were tentatively identified and four are still unknown.

The discriminant markers resulting from OPLS-DA included most of the Fcs and Cs adopted for the above profiling (section 3.1) together with other minor OHCs (mainly polymethoxylated flavonoids) tentatively identified by UHPLC-TOF-MS, that play key roles in differentiating the EO samples according to their species. From these results, an interesting connection emerges between the three clusters defined by multivariate analysis (see above) and the structural groups of phenolic discriminant markers: i) mandarin and orange EOs are characterized by PMFs, ii) lemon and lime EOs contain mainly Fcs and Cs, iii) bigarade and grapefruit EOs consist of Cs and PMFs, and iv) bergamot EOs combine PMFs with a high Fcs content.

The grapefruit sample that appeared to be an outlier in the above statistical treatment was characterized by a significant meranzin hydrate content (EMRT=279.1603,10.88), indicating an evolution of this sample compared to the other ones in this group, in which meranzin isomer 2 (EMRT=261.1124,7.91) was predominant.

Discrimination between Citrus species originating the EOs and adulterated samples. A new dataset was created to discriminate the *Citrus* species by the above 38 discriminant markers. The aim was to increase statistical parameters (essentially predictive ability), in order to minimize the possibility of false positives. More than 130 EMRTs (resulting from the extraction of the exact mass of the 38 selected markers in the 44 samples) were extracted from the UHPLC/TOF-MS dataset and submitted to statistical analysis. The resulting PLS-DA provided a better explained variance R2[Y](cum) =0.94 and higher predictive ability Q2[Y](cum) = 0.81. The specificity remained high (100%), while sensitivities for mandarin, grapefruit, and bergamot were 67, 88 and 93% respectively (Ffigure 4).

Five reconstituted EOs were then analyzed following this procedure, in order to evaluate the ability of the above strategy to detect adulterated samples, and to assign EOs to citrus species. Sample 1, labeled orange, corresponded to a sweet orange EO spiked with 10 % of bigarade EO. The other four samples consisted of bergamot EO spiked respectively with 10 % bigarade (sample 2), grapefruit (sample 3), lemon (sample 4), or lime (sample 5) EOs; these were labeled as bergamot samples. The spiking percentage was deliberately kept low to evaluate the sensitivity of this approach to adulteration.

The PLS-DA score plot with 1-3 PCs shows that samples 1, 2 and 4 are clearly plotted out of their groups, while samples 3 and 5 are not unequivocally discriminated, but they result outliers of the bergamot group. (Figure 4) These results show that the OHC fraction can be used for sample authentication, through appropriate processing of the available data, because it is sensitive “to a small adulteration (10%)”. In any case, a larger number of samples would be necessary to characterize each single species, in view of obtaining a more refined and robust statistical model.

In conclusion, modern HPLC and MS techniques (UHPLC/TOF-HRMS), in combination with advanced statistical tools, offer additional possibilities in phytochemical analysis, which can successfully be applied in quality control, to authenticate natural raw ingredients. The discrimination of the species originating citrus EOs was discussed on the basis of the composition of the OHC fraction, i.e. a group of low-volatility phenolic compounds, by two approaches:

1) the first one is based on quantifying 18 furocoumarins and coumarins, and produces diagnostic results and quantitative information concerning components regulated by law, although it can sometimes give false positives;

2) the second one is based on a strategy from metabolomics that, thanks to appropriate and more elaborate data processing, defined 38 discriminant OHC markers (34 of them tentatively

identified) out of the 140 quoted in the literature and stored in the database; this approach correctly discriminated the *Citrus* species and was also sensitive enough to relatively low adulteration (10%).

The commercial nature of the 44 samples considered in the present study explains the wide variability of the EO composition within each species (crop period and year, origin, climatic conditions, and industrial processes) and means that the results are satisfactory, and that their robustness can be increased when data from a much larger number of commercial and authenticated samples would be available.

This method may be considered as a further complementary tool to the well-known and effective approaches based on volatile analysis, involving GC-MS and Enantioselective-GC-(MS) or GC-IRMS techniques; at the same time it provides information on compounds (Fcs and Cs) limited by current and future regulations.

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The authors thank Dr. Anne Chartogne of Waters Company for her support and expertise.

List of the acronyms

5-MOP: 5-MethoxyPsoralen
ACN: Acetonitrile
Cs: Coumarins
EDA: Extrapolative Dilution Approach
EMRT pair: Exact Mass-Retention Time pair
EO: Essential oil
ESI: Electrospray ionization
Fcs: Furocoumarins
FID: Flame ionization detector
FT-MIR: Fourier transform-middle infrared spectrometry
GC: Gaz Chromatophy
HCA: Hierarchical Cluster Analysis
HCV: High Capillary voltage
HPLC: High Performance Liquid Chromatography
HSS: High Strenght Silica

IFRA: International Fragrance Associatione
LC-MS: coupling Liquid Chromatography-Mass Spectrometry
LCV: Low Capillary Voltage
LOD: Limit of Detection
LOQ: Limit of Quantification
MeOH: Methanol
MS: Mass Spectrometer
NMR: Nuclear Magnetic Resonance
OHC: Oxygenated Heterocyclic compounds
OPLS-DA: Orthogonal Partial Least Square – Discriminant analysis
Pc: Principal component
PCA: Principal Component Analysis
PLS-DA: Partial Least Square – Discriminant analysis
PMF: Polymethoxylated flavonoids
Q2[Y](cum): Cumulative predictive ability
R2[Y](cum): Cumulative explained variance
RSD: Residual Standard Deviation
S-plot: Scatter-plot
THF: TetraHydroFuran
TOF-HRMS: Time Of Flight- High Resolution mass spectrometer
UHPLC: Ultra High Performance Liquid Chromatography

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FIGURE CAPTIONS

Figure 1: a) General structure of coumarins, furocoumarins, and polymethoxylated flavones; b) Chromatographic patterns of the 18 target compounds.

Figure 2: PLS-DA scores plot with the 850 EMRTs resulting from the investigated citrus EOs. score plots obtained on 1-4 PCs R2[Y](1)= 0.16, R2[Y](4) = 0.12, Q2[Y](1) = 0.13, Q2[Y](4) = 0.05 Ellipse Hotelling's T2 (95%)

Figure 3: Trend views of two selected discriminant markers: a. (EMRT = 339.1593, 11.35) Bergamottin; b. (EMRT = 261.1120, 10.60) Meranzin isomer 3

Figure 4: PLS-DA score plot with 1-3 PCs of the discrimination of deliberately-adulterated citrus EO samples of orange (1) and bergamot (samples 2-5)

Supplementary material:

Table SM1. EMRT of the target Fcs and Cs, and analytical reliability parameters

Table SM2 . Experimental and literature quantitative ranges of the 18 Fcs and Cs investigated with a full reference list related to the investigated compounds

Table SM3. List of the 38 discriminant markers selected by OPLS-DA with a full reference list related to the investigated compounds

Figure SM1 a) Chromatographic patterns of the extracted mass of bergamottin at different dilutions of a bergamot sample b) plot of regression line associated to the EDA approach

Figure SM2 ~~a) HCA of the investigated citrus EOs, taking the 18 Fcs and Cs b)~~ PCA scores plot of the investigated citrus EOs, taking the 18 Fcs and Cs as variables. R2[X](1)=0.22; R2[X](2)=0.15, Ellipse Hotelling's T2 (95%)

Figure SM3 a) HCA of the investigated citrus EOs, taking the 140 OHC compounds b) PCA scores plot with the 850 EMRTs resulting from the investigated citrus EOs.R2[X](1)= 0.34, R2[X](2) = 0.18, Ellipse Hotelling's T2 (95%)

Table 1. Experimental and literature reported quantitative ranges of the 18 Fcs and Cs investigated. The list of all references related to the investigated compounds is given in Supporting Information Table SM2

Species	Markers	Concentration range (ppm)	Mediane value	Literature data (ppm)	References
Bergamot *Citrus bergamia*	Bergamottin Bergapten Citropten Epoxybergamottin Herniarin Oxypeucedanin Oxypeucedanin hydr. Isopimpinelin Isoimperatorin Psoralen 8-Geranyloxypsoralen	15000-50000 1000-3500 1000-4000 5-60 10-400 1-200 1-50 traces traces traces traces	17621 1925 1650 41 49 1 2	500-28000 1100-9000 1000-8000 10-100 50-100 150-650 10-200 traces 20-550 traces	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Russo et al., 2015)
Lemon *Citrus limon*	8-Geranyloxypsoralen Bergamottin Byakangelicol Citropten Oxypeucedanin Bergapten Byakangelicin Heraclenin Herniarin Isoimperatorin Oxypeucedanin hydr. Phellopterin Epoxybergamottin Imperatorin Isopimpinelin	200-1000 1000-10000 50-1500 300-1500 10-2000 10-150 1-50 10-200 20-300 10-100 20-100 20-200 traces traces traces	293 3363 363 564 365 25 31 13 25 24 80 44	200-3500 1000-4000 200-1300 300-3500 900-2100 10-100 20-100 40-200 traces 20-200 10-300 90-650 traces 40-200 traces	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Russo et al., 2015)
Bigarade *Citrus aurantium*	Bergamottin Epoxybergamottin Bergapten Citropten 8-Geranyloxypsoralen Isopimpinelin Phellopterin Psoralen	50-1500 1000-4000 100-400 1-10 1-10 traces traces traces	122 1934 237 4 5	60-150 800-3500 500-1000 traces 0-120 0-50 traces	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Dugo et al., 2011a), (Russo et al., 2015)
Orange *Citrus sinensis*	Bergamottin Epoxybergamottin Bergapten 8-Geranyloxypsoralen Citropten	10-50 10-100 1-150 traces traces	31 55 2	 traces traces	(Dugrand et al., 2013)
Mandarin *Citrus deliciosa*	Bergamottin Citropten	1-10 1-10	2 2		
Grapefruit	Bergamottin	1500-5000	2785	900-2000	(Dugo &

Citrus X paradisi	Epoxybergamottin Bergapten Citropten 8-Geranyloxypsoralen Isopimpinelin	2000-10000 50-200 10-50 1-15 traces	3741 111 17 5	9500-12000 100-150 20-100 0-60	Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012) (Russo et al., 2015)
Lime ***Citrus aurantifolia***	8-Geranyloxypsoralen Bergamottin Bergapten Citropten Herniarin Isopimpinelin Byakangelicin Byakangelicol Epoxybergamottin Heraclenin Imperatorin Isoimperatorin Oxypeucedanin Oxypeucedanin hydr. Phellopterin Psoralen Xanthotoxin	4000-5000 20000-80000 1500-2000 4500-5500 1500-2000 4000-5000 20-50 100-200 10-20 80-100 250-300 150-200 550-600 500-1000 350-400 15-30 600-750	4290 47844 1975 4914 1802 4302 34 175 8 94 262 205 565 779 384 24 663	500-6500 10000- 45000 200-3500 2000-17000 500-5000 3000-8000 80-1000 traces 200-800 100-400 1000-10000 200-1700 10-100 10-40 10-60	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Bonaccorsi et al., 2011) (Russo et al., 2015)

Table 2. List of the 38 discriminant markers selected by OPLS-DA. The list of all references related to the investigated compounds is given in Supporting Information Table SM3

N°	Ionized exact mass (m/z)	Exact mass (m/z)	Tr (min)	Supposed Elemental Composition	Accuracy (ppm)	i-FIT Norm	Tentative identification	References	Grp	Fragments	Formula	Accuracy (ppm)	Identification confidence*
1	177.0557	176.0479	5.38	$C_{10}H_8O_3$	3.16	0.45	Herniarin	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Russo et al., 2015)	Lime	121.0655	C_8H_9O	1.32	1
2	317.1037	316.09587	6.56	$C_{17}H_{16}O_6$	3.83	3.37	Byakangelicol	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Russo et al., 2015)	Lemon	233.0415	$C_{12}H_9O_5$	5.14	1
3	217.0502	216.0424	6.67	$C_{12}H_8O_4$	0.65	1.77	Isobergapten		Expr. lime	202.0263 174.0323	$C_{11}H_6O_4$ $C_{10}H_6O_3$	1.53 3.48	3
4	207.0660	206.0582	7.37	$C_{11}H_{10}O_4$	1.41	0.01	Citropten	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al.,	Bergamot Lemon Lime	192.0423 121.0650	$C_6H_{10}O_7$ C_8H_9O	1.82 2.81	1

								2009) (Russo et al., 2015)					
5	247.0607	246.0529	7.61	$C_{13}H_{10}O_5$	0.31	0.01	Isopimpinelin	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Bonaccorsi et al., 2011) (Russo et al., 2015)	Lime	232.0362 217.0148	$C_{12}H_8O_5$ $C_{11}H_5O_5$	4.19 5.08	1
6	261.1123	260.1045	7.79	$C_{15}H_{16}O_4$	1.38	0.62	Meranzin isomer 1	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2011a), (Russo et al., 2015)	Bigarade Grapefruit	189.0550 131.0486	$C_{11}H_9O_3$ C_9H_7O	0.89 8.32	3
7	217.0501	216.0423	7.86	$C_{12}H_8O_4$	0.19	0.04	Bergapten	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Dugo et al., 2011a), (Bonaccorsi et al., 2011)	Bergamot Lime Bigarade Lemon Grapefruit	202.0250 174.0311	$C_{11}H_6O_4$ $C_{10}H_6O_3$	7.96 3.41	1

								(Russo et al., 2015)					
8	261.1123	260.1045	7.91	$C_{15}H_{16}O_4$	1.38	6.88	Meranzin isomer 2	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2011a), (Dugo et al., 2009), , (Russo et al., 2015)	Bigarade Grapefruit	189.0541 131.0498	$C_{11}H_9O_3$ C_9H_7O	5.65 0.84	3
9	373.1280	372.1202	8.61	$C_{20}H_{20}O_7$	1.89	3.56	Pentamethoxyflavone isomer 1	(Weber et al., 2006), (Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Mondello, Dugo, & d'Alcontres, 1993), (Dugo et al., 2011b), (Russo et al., 2015)	Orange Bergamot Mandarin	358.1013 343.0732 312.0978	$C_{19}H_{18}O_7$ $C_{18}H_{15}O_7$ $C_{18}H_{16}O_5$	11.04 6.64 6.32	3
10	355.1534	354.1456	8.63	$C_{21}H_{22}O_5$	3.17	5.79	Aurantiumal	(Dugo & McHale, 2002)	Grapefruit	163.0414	$C_9H_7O_3$	11.54	3
11	287.0919	286.0841	8.85	$C_{16}H_{14}O_5$	0.08	0.13	Oxypeucedanin	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al.,	Lemon Lime	203.0351 147.0448	$C_{11}H_7O_4$ $C_9H_7O_2$	3.28 1.33	1

								1999a), (Russo et al., 2012), (Dugo et al., 2009) (Russo et al., 2015)					
12	403.1385	402.1307	8.92	$C_{21}H_{22}O_8$	1.91	4.28	Hexamethoxyflavone isomer 1	(Weber et al., 2006), (Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Mondello et al., 1993), (Dugo et al., 2011b), (Russo et al., 2015)	Orange Mandarin Grapefruit	373.0907 355.0771	$C_{19}H_{17}O_8$ $C_{19}H_{15}O_7$	4.40 13.17	3
13	403.1378	402.1300	9.36	$C_{20}H_{22}O_8$	3.65	4.56	Hexamethoxyflavone isomer 2	(Weber et al., 2006), (Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Dugo et al., 2011a), (Mondello et al., 1993), (Li, Lo, Wang, & Ho, 2010) (Russo et al., 2015)	Orange Mandarin Bigarade Grapefruit	373.0894 355.0772	$C_{19}H_{17}O_8$ $C_{19}H_{15}O_7$	7.89 12.89	3
14	343.1167	342.1089	9.40	$C_{19}H_{18}O_6$	4.2	2.51	Tetra-O-methylscutellarein	(Weber et al., 2006), (Dugo & Russo, 2011),	Orange Mandarin	313.0670 282.0853	$C_{17}H_{13}O_6$ $C_{17}H_{14}O_4$	13.45 13.85	3

								(Dugo et al., 1999a), (Russo et al., 2012), (Mondello et al., 1993), (Dugo et al., 2011b), (Li et al., 2010), (Russo et al., 2015)					
15	359.1121	358.1043	9.57	$C_{19}H_{18}O_7$	2.66	1.36	Hydroxytetramethoxyflavone isomer 1	(Russo et al., 2012), (Dugo et al., 2011b)	Bergamot Orange Mandarin	344.0842 298.0835	$C_{18}H_{16}O_7$ $C_{17}H_{14}O_5$	15.70 2.09	3
16	433.1489	432.1411	9.58	$C_{22}H_{24}O_9$	2.16	5.05	Heptametoxyflavone	(Weber et al., 2006), (Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Mondello et al., 1993) (Russo et al., 2015)	Orange Mandarin Grapefruit	403.0963	$C_{20}H_9O_9$	16.39	3
17	387.1802	386.1724	9.69	$C_{22}H_{26}O_6$	1.39	11.08	unknown 1		Dist. lime				4
18	271.0962	270.0884	9.83	$C_{16}H_{14}O_4$	2.99	0.27	Imperatorin	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009),	Expr. lime lemon	203.0342	$C_{11}H_7O_4$	1.15	1

								(Russo et al., 2015)					
19	359.1123	358.1045	9.86	$C_{19}H_{18}O_7$	2.1	0.12	Hydroxytetramethoxyflavone isomer 2	(Russo et al., 2012), (Dugo et al., 2011b)	Bergamot Orange Mandarin Grapefruit	344.0849 315.0858	$C_{18}H_{16}O_7$ $C_{17}H_{15}O_6$	3.40 3.37	3
20	373.1276	372.1198	9.90	$C_{20}H_{20}O_7$	2.96	4.79	Pentamethoxyflavone isomer 2	(Weber et al., 2006), (Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Dugo et al., 2011a), (Mondello et al., 1993), (Russo et al., 2015)	Mandarin Orange Bigarade Grapefruit	343.0749	$C_{18}H_{15}O_7$	20.04	3
21	389.1227	388.1149	10.02	$C_{20}H_{20}O_8$	2.36	3.41	Hydroxypentamethoxyflavone	(Weber et al., 2006), (Dugo & Russo, 2011), (Russo et al., 2012), (Dugo et al., 2011b), (Russo et al., 2015)	Mandarin Orange Bigarade Bergamot Grapefruit	359.1052 342.0840	$C_{19}H_{19}O_7$ $C_{18}H_{14}O_7$	21.93 29.37	3
22	315.1590	314.1511	10.03	$C_{19}H_{22}O_4$	2.26	0.03	Epoxyaurapten	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Russo et al., 2015)	Grapefruit	297.1488 163.0396 153.1279 135.1175	$C_{19}H_{21}O_3$ $C_9H_7O_3$ $C_{10}H_{17}O$ $C_{10}H_{15}$	0.91 0.50 0.26 0.92	3

23	301.1072	300.0994	10.06	$C_{17}H_{16}O_5$	1.24	3.25	Phellopterin	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Russo et al., 2015)	Lemon Expr. Lime	233.045 218.0168	$C_{12}H_9O_5$ $C_{11}H_6O_5$	0.007 21.66	1
24	403.1381	402.1303	10.10	$C_{21}H_{22}O_8$	2.9	6.32	Hexamethoxyflavone isomer 3	(Weber et al., 2006), (Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2011a), (Mondello et al., 1993), (Russo et al., 2015)	Mandarin Orange Grapefruit Bigarade	373.0908 355.0762	$C_{19}H_{17}O_8$ $C_{19}H_{15}O_7$	4.13 15.71	3
25	245.1174	244.1096	10.19	$C_{15}H_{16}O_3$	1.41	0.07	Unknown 2		Bigarade Grapefruit	189.0546 131.0492	$C_{11}H_9O_3$ C_9H_7O	3.01 3.73	4
26	329.1020	328.0942	10.19	$C_{18}H_{16}O_6$	1.49	5.1	Hydroxytrimethoxyflavone		Bergamot	268.0751	$C_{16}H_{12}O_4$	5.75	3
27	301.1074	300.0996	10.22	$C_{17}H_{16}O_5$	0.58	0.39	Phellopterin isomer	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al.,	Expr. lime	233.0452	$C_{12}H_9O_5$.	0.86	3

								2012), (Dugo et al., 2009), (Russo et al., 2015)					
28	355.1539	354.1461	10.39	$C_{21}H_{22}O_5$	1.76	1.67	Epoxybergamottin	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2011a), (Dugo et al., 1999b), (Russo et al., 2015)	Grapefruit Bigarade	203.0346 153.1268	$C_{11}H_7O_4$ $C_{10}H_7O$	0.82 7.45	1
29	261.1120	260.1042	10.60	$C_{15}H_{16}O_4$	2.53	0.63	Meranzin isomer 3	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2011a), (Russo et al., 2015)	Bigarade Grapefruit	243.0999	$C_{15}H_{15}O_9$	9.13	3
30	355.1542	354.1464	10.97	$C_{21}H_{22}O_5$	0.91	5.34	Cnidicin isomer	(Dugo et al., 2009), (Russo et al., 2012), (Russo et al., 2015)	Lemon Expr.lime Grapefruit	219.0325 173.0279	$C_{11}H_7O_5$ $C_{10}H_5O_3$	14.39 23.29	3
31	339.1592	338.1514	11.04	$C_{21}H_{22}O_4$	1.21	1.02	8-Geranyloxypsoralen	(Dugo & Russo, 2011), (Frérot & Decorzant,	Lemon Expr.lime	203.0338 147.0447	$C_{11}H_7O_4$ $C_9H_7O_2$	3.12 0.65	1

								2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Russo et al., 2015)					
32	299.1640	298.1562	11.18	$C_{19}H_{23}O_3$	2.4	3.57	Aurapten	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Russo et al., 2015)	Grapefruit	163.0396	$C_9H_7O_3$	0.5	3
33	369.1699	368.1621	11.26	$C_{22}H_{24}O_5$	0.74	4.42	5-Geranyloxy-8-methoxypsoralen	(Dugo & Russo, 2011), (Russo et al., 2012), (Dugo et al., 2009), (Russo et al., 2015)	Expr.lime	233.0464 173.0233	$C_{12}H_9O_5$ $C_{10}H_5O_3$	6.01 3.25	3
34	339.1593	338.1515	11.35	$C_{21}H_{22}O_4$	0.91	4.30	Bergamottin	(Dugo & Russo, 2011), (Frérot & Decorzant, 2004), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Russo et al., 2015)	Bergamot Lemon Lime Grapefruit	203.0359 147.0450	$C_{11}H_7O_4$ $C_9H_7O_2$	7.22 2.69	1
35	261.1124	260.1046	11.36	$C_{15}H_{16}O_4$	0.99	2.07	Meranzin isomer 4	(Dugo & Russo, 2011), (Dugo et al.,	Grapefruit	189.0542 131.0476	$C_{11}H_9O_3$ C_9H_7O	5.13 15.95	3

								1999a), (Russo et al., 2012), (Russo et al., 2015)					
36	323.1051	322.0973	11.41				Unknown 3		Dist. Lime	251.0506	$C_{19}H_7O$	3.62	
37	329.1746	328.1668	11.41	$C_{20}H_{24}O_4$	2.01	0.11	5-geranyloxy-7-methoxycoumarin	(Dugo & Russo, 2011), (Dugo et al., 1999a), (Russo et al., 2012), (Dugo et al., 2009), (Russo et al., 2015)	Lemon Lime Bergamot	251.0481 193.0506	$C_{19}H_7O$ $C_{10}H_9O_4$	6.33 2.67	3
38	279.1596	278.1518	12.51	$C_{16}H_{22}O_4$	0.03	1.12	Unknown 4		Dist. lime	163.0396	$C_9H_7O_3$	0.5	4

*An identification confidence according to the request of the CAWG (2007) is indicated: Level 1: Identified Compound (A minimum of two independent and orthogonal data (such as retention time and mass spectrum) compared directly relative to an authentic reference standard Level 2: Putatively Annotated Compound (Compound identified by analysis of spectral data and/or similarity to data in a public database) Level 3: Putatively Characterized Compound Class Level 4: Unknown Compound: unidentified or unclassified but characterized by spectral data.

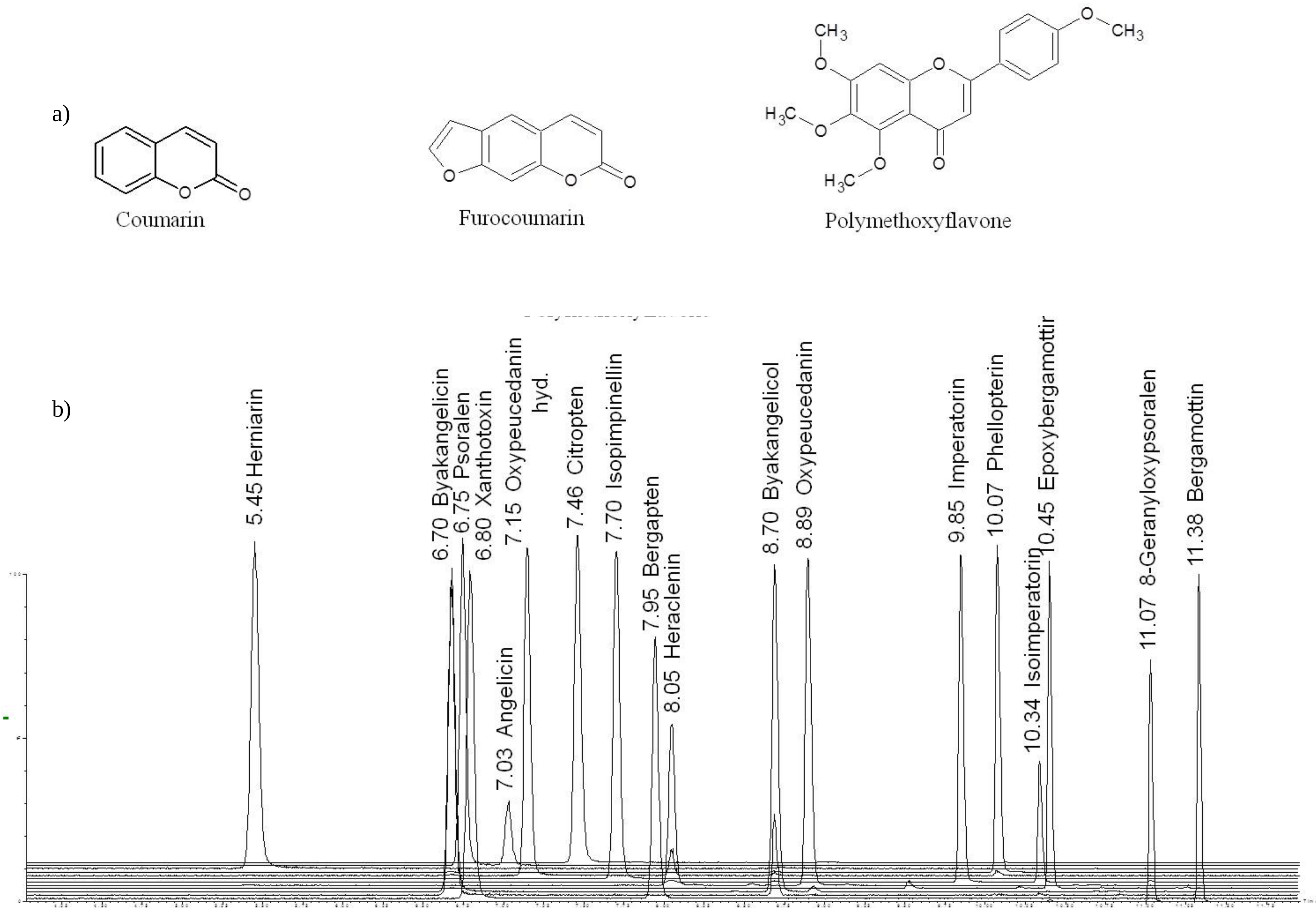

a)
Coumarin
Furocoumarin
Polymethoxyflavone
b)
5.45 Herniarin
6.70 Byakangelicin
6.75 Psoralen
6.80 Xanthotoxin
7.03 Angelicin
7.15 Oxypeucedanin hyd.
7.46 Citropten
7.70 Isopimpinellin
7.95 Bergapten
8.05 Heraclenin
8.70 Byakangelicol
8.89 Oxypeucedanin
9.85 Imperatorin
10.07 Phellopterin
10.34 Isoimperatorin
10.45 Epoxybergamottin
11.07 8-Geranyloxypsoralen
11.38 Bergamottin

Figure 1

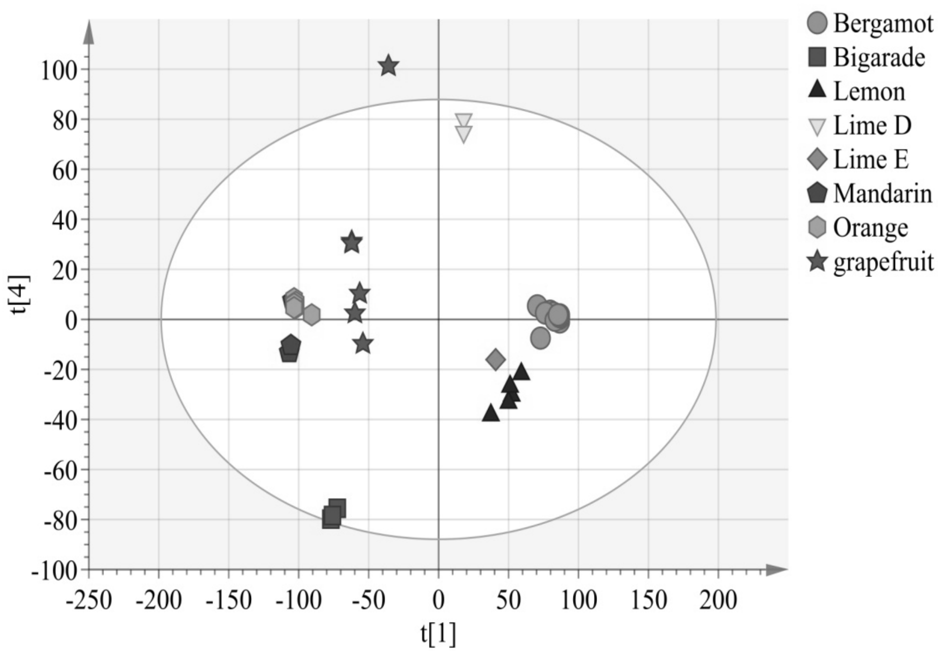
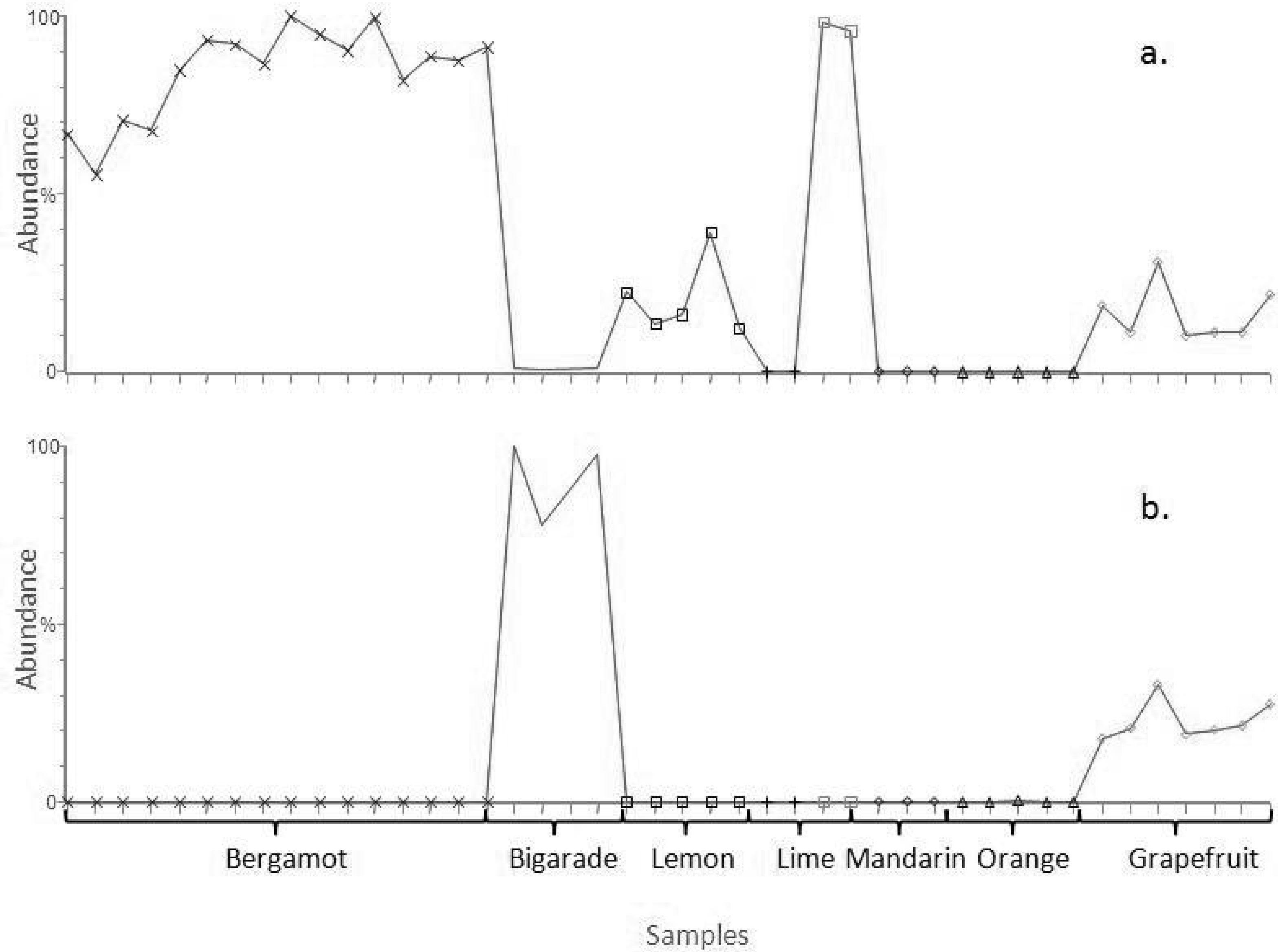
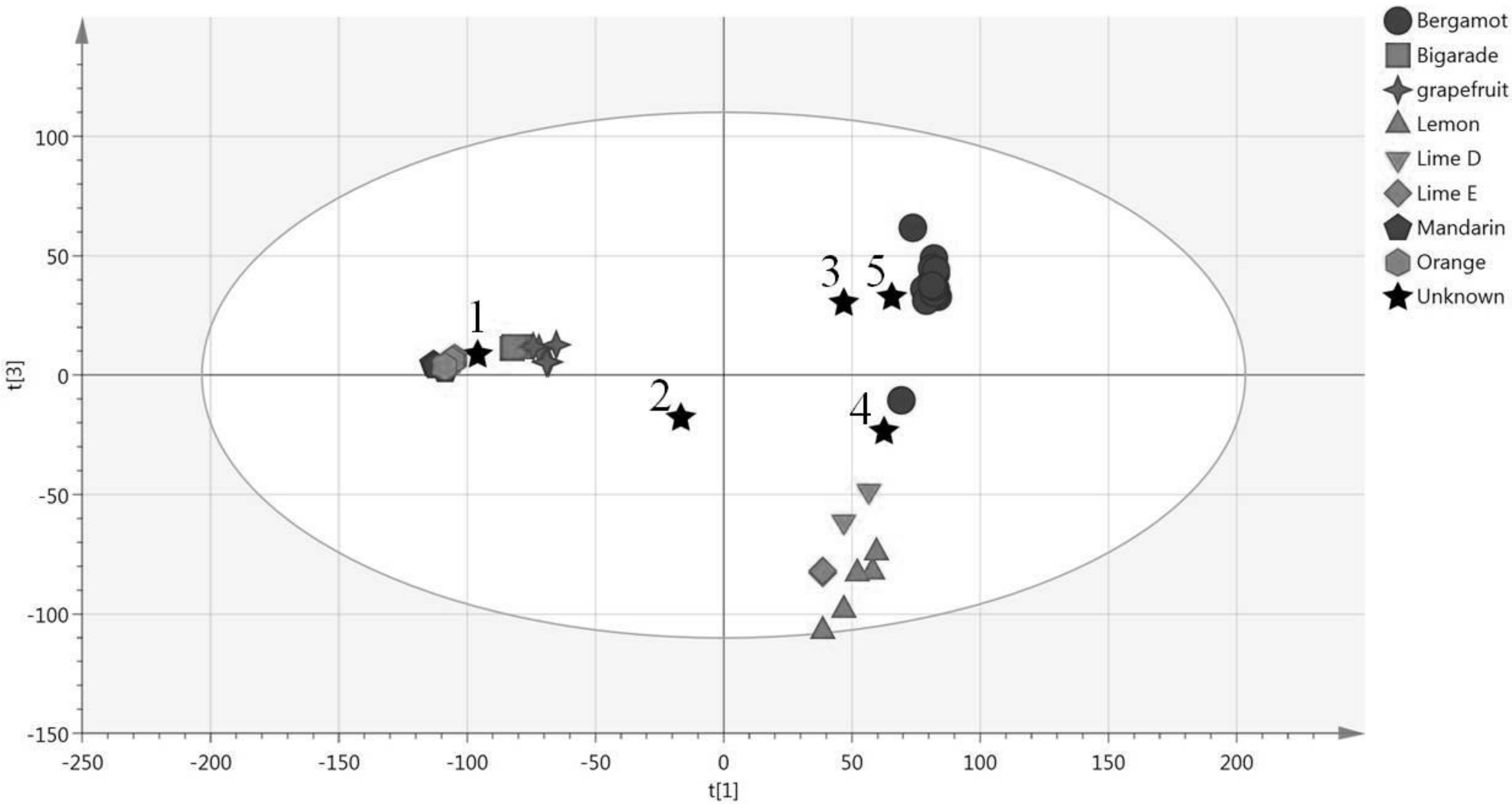

Bergamot
Bigarade
Lemon
Lime D
Lime E
Mandarin
Orange
grapefruit
t[4]
t[1]
100
80
60
40
20
0
-20
-40
-60
-80
-100
-250
-200
-150
-100
-50
0
50
100
150
200

Figure 2



100
Abundance %
0
a.
100
Abundance %
0
b.
Bergamot
Bigarade
Lemon
Lime
Mandarin
Orange
Grapefruit
Samples

Figure 3



Bergamot
Bigarade
grapefruit
Lemon
Lime D
Lime E
Mandarin
Orange
Unknown
1
2
3
4
5
t[3]
t[1]
100
50
0
-50
-100
-150
-250
-200
-150
-100
-50
0
50
100
150
200

Figure 4

Oxygenated heterocyclic compounds to differentiate *Citrus* spp. essential oils through metabolomic strategies

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Table SM1. EMRT of the target Fcs and Cs, and analytical reliability parameters. [a]: high concentration, [b]: low concentration

OHC	Rt (min)	Exact mass [M+H]+	Fragments	R^2	Regression Equation	Linearity range (mg/L)	Repeatability RSD %	Int. Prec. RSD %	LOQ (mg/L)	LOD (mg/L)
Herniarin	5.38	177.0552	133.0655 121.0668	0.9999	245.41x-6.00975	0.1 - 5	3.52[a] 1.24[b]	2.09[a] 3.86[b]	0.050	0.010
Psoralen	6.95	187.0317	131.0502 115.055	0.9926	394.291x + 62.2351	0.2 - 10	1.26 5.04	3.71 4.34	0.106	0.011
Byakangelicin	6.96	335.1131	233.0449 231.0294	0.9986	199.154x - 29.2627	0.1 - 5	2.89 4.34	4.47 4.33	0.053	0.011
Angelicin	6.98	187.0317	131.0491 115.0545	0.9992	71.4831x+1.5915	0.2 - 10	2.48 3.59	4.43 3.79	0.100	0.011
Xanthotoxin	7.01	217.0501	202.0266 174.0317	0.9989	3594.12x + 169.371	0.2 - 10	2.35 3.52	3.92 3.41	0.102	0.010
Oxypeucedanin Hydrate	7.36	305.1025	203.0349 147.048	0.9999	619.863x – 8.66233	0.1 - 5	4.14 6.22	3.70 4.47	0.050	0.010
Citropten	7.39	207.0657	192.0423 121.0645	0.9948	1469.87x+3.45565	0.1 - 5	3.37 3.12	3.47 4.66	0.050	0.010
Isopimpinellin	7.89	247.0606	232.0378 217.0127	0.9980	4650.62x + 555.133	0.2 - 10	6.52 3.34	3.69 4.14	0.101	0.010
Bergapten	8.10	217.0501	202.0267 174.0318	0.9979	2381.62x + 165.362	0.2 - 10	5.78 3.76	3.82 5.08	0.103	0.011
Heraclenin	8.23	287.0919	203.0334 147.043	0.9985	1123.57x + 61.5232	0.2 - 10	5.50 4.05	3.86 4.08	0.101	0.050
Byakangelicol	8.86	317.1025	233.0445 231.0289	0.9976	1389.17x + 62.9711	0.2 - 10	5.21 3.77	4.09 5.55	0.105	0.052

Oxypeucedanin	9.04	287.0919	203.0349 147.0468	0.9978	29.5409x + 0.88207	0.2 - 10	5.41 3.31	3.74 5.00	0.105	0.053
Imperatorin	9.93	271.097	203.0344 147.0461	0.9963	2369.8x + 160.304	0.2 - 10	6.03 3.55	3.78 4.51	0.101	0.010
8-Geranyl-oxypsoralen	11.07	339.1596	203.0342 147.0439	0.9983	622.295x - 52.3108	0.2 - 10	3.21 3.89	5.34 4.75	0.101	0.050
Phellopterin	10.13	301.1076	233.0444 218.0211	0.9948	3183.21x + 286.096	0.2 - 10	6.50 3.27	3.53 5.45	0.102	0.010
Isoimperatorin	10.4	271.097	203.0344 147.0461	0.9958	488.205x + 47.188	0.2 - 10	7.61 3.49	4.16 5.55	0.102	0.010
Epoxybergamottin	10.45	355.1545	203.0342 153.1291	0.9992	113.838 x - 7.97378	0.2 - 10	3.71 4.46	4.44 5.02	0.105	0.053
Bergamottin	11.38	339.1596	203.0352 147.0449	0.9988	513.205x - 40.1257	0.2 - 10	3.21 4.25	4.57 4.83	0.106	0.011

Table SM2. Experimental and literature quantitative ranges of the 18 Fcs and Cs investigated with a full reference list related to the investigated compounds

Species	Markers	Concentration range (ppm)	Mediane value	Litterature data (ppm)	References
Bergamot ***Citrus bergamia*** **Risso**	Bergamottin Bergapten Citropten Epoxybergamottin Herniarin Oxypeucedanin Oxypeucedanin hydr. Isopimpinelin Isoimperatorin Psoralen 8-Geranyloxypsoralen	15000-50000 1000-3500 1000-4000 5-60 10-400 1-200 1-50 traces traces traces traces	17621 1925 1650 41 49 1 2	500-28000 1100-9000 1000-8000 10-100 50-100 150-650 10-200 traces 20-550 traces	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo, Mondello, Proteggente, & Dugo, 1999), (Di Giacomo & Calvarano, 1978), (Analytical Methods Committee, 1987), (Costa et al., 2010), (Dugo et al., 1999b), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Gionfriddo, Postorino, & Bovalo, 1997), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi, Dugo, McNair, & Dugo, 2000), (Vernin, Bianchini, & Siouffi, 1979), (Mondello, d'Alcontres, del Duce, & Crispo, 1993), (Dellacassa, Lorenzo, Moyna, Verzera, & Cavazza, 1997), (Calvarano, Ferlazzo, & Di Giacomo, 1979), (Porcaro & Shubiack, 1974), (Cieri, 1969), (Madsen & Latz, 1970) (Russo et al., 2015)

Lemon ***Citrus limon* (L.) Osbeck**	8-Geranyloxypsoralen Bergamottin Byakangelicol Citropten Oxypeucedanin Bergapten Byakangelicin Heraclenin Herniarin Isoimperatorin Oxypeucedanin hydr. Phellopterin Epoxybergamottin Imperatorin Isopimpinelin	200-1000 1000-10000 50-1500 300-1500 10-2000 10-150 1-50 10-200 20-300 10-100 20-100 20-200 traces traces traces	293 3363 363 564 365 25 31 13 25 24 80 44	200-3500 1000-4000 200-1300 300-3500 900-2100 10-100 20-100 40-200 traces 20-200 10-300 90-650 traces 40-200 traces	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (Bonaccorsi et al., 2000), (Calabro & Curro, 1976), (Shu, Walradt, & Taylor, 1975), (McHale & Sheridan, 1988), (Marin, Marchisio, Janinet, & le Bon, 2000), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Dugo, Mondello, Cogliandro, Cavazza, & Dugo, 1998), (Fisher & Trama, 1979), (Glandian, Corneteau, Drouet, & Rouzet, 1978) (Russo et al., 2015)
Bigarade ***Citrus x aurantium* L.**	Bergamottin Epoxybergamottin Bergapten Citropten 8-Geranyloxypsoralen Isopimpinelin Phellopterin Psoralen	50-1500 1000-4000 100-400 1-10 1-10 traces traces traces	122 1934 237 4 5	60-150 800-3500 500-1000 traces 0-120 0-50 traces	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Shu et al., 1975), (Fisher & Trama, 1979), (Dugo, Mondello, Cogliandro, Verzera, & Dugo,

					1996), (Dugo et al., 2011a), (Chouchi, Barth, Reverchon, & Della Porta, 1996), (Boelens & Sindreu, 1986) (Russo et al., 2015)
Orange ***Citrus sinensis*** **(L.) Osbeck**	Bergamottin Epoxybergamottin Bergapten 8-Geranyloxypsoralen Citropten	10-50 10-100 1-150 traces traces	31 55 2	traces traces	(Dugrand et al., 2013)
Mandarin ***Citrus reticulata*** **Blanco**	Bergamottin Citropten	1-10 1-10	2 2		
Grapefruit ***Citrus paradisi*** **Macfad**	Bergamottin Epoxybergamottin Bergapten Citropten 8-Geranyloxypsoralen Isopimpinelin	1500-5000 2000-10000 50-200 10-50 1-15 traces	2785 3741 111 17 5	900-2000 9500-12000 100-150 20-100 0-60	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (McHale & Sheridan, 1989), (Shu et al., 1975), (Fisher & Trama, 1979), (Dugo et al., 1996), (Feger, Brandauer, Gabris, & Ziegler, 2006), (Tatum & Berry, 1979) (Russo et al., 2015)
Lime ***Citrus aurantifolia*** **(Christm.)Swingle**	8-Geranyloxypsoralen Bergamottin Bergapten Citropten Herniarin Isopimpinelin Byakangelicin Byakangelicol Epoxybergamottin Heraclenin Imperatorin Isoimperatorin	4000-5000 20000-80000 1500-2000 4500-5500 1500-2000 4000-5000 20-50 100-200 10-20 80-100 250-300 150-200 550-600 500-1000 350-400 15-30 600-750	4290 47844 1975 4914 1802 4302 34 175 8 94 262 205 565 779 384 24 663	500-6500 10000-45000 200-3500 2000-17000 500-5000 3000-8000 80-1000 traces 200-800 100-400 1000-10000 200-1700 10-100 10-40 10-60	(Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Shu et al., 1975), (Cieri, 1969), (Madsen & Latz, 1970), (Feger et al., 2006),

	Oxypeucedanin Oxypeucedanin hydr. Phellopterin Psoralen Xanthotoxin				(Bonaccorsi et al., 2011), (Dugo, Mondello, Lamonica, & Dugo, 1997), (Stanley & Vanier, 1967), (Nigg et al., 1993) (Russo et al., 2015)

Table SM3. List of the 38 discriminant markers selected by OPLS-DA with a full reference list related to the investigated compounds

N°	Ionized exact mass (m/z)	Exact mass (m/z)	Tr (min)	Supposed Elemental Composition	Accuracy (ppm)	Tentative identification	References	Grp	Fragments	Formula	Accuracy
1	177.0557	176.0479	5.38	$C_{10}H_8O_3$	3.16	Herniarin	(Dugo et al., 1999a), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al., 1997) (Russo et al., 2015)	Lime	121.0655	C_8H_9O	1.32
2	317.1037	316.09587	6.56	$C_{17}H_{16}O_6$	3.83	Byakangelicol	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (McHale & Sheridan, 1988), (Marin et al., 2000), (Ziegler & Spiteller, 1992), (Dellacassa et al.,	Lemon	233.0415	$C_{12}H_9O_5$	5.14

							1997), (Dugo et al., 1998) (Russo et al., 2015) (Glandian et al., 1978)				
3	217.0502	216.0424	6.67	$C_{12}H_8O_4$	0.65	Isobergapten		Expr.d lime	202.0263 174.0323	$C_{11}H_6O_4$ $C_{10}H_6O_3$	1.53 3.48
4	207.0660	206.0582	7.37	$C_{11}H_{10}O_4$	1.41	Citropten	(Dugo et al., 1999a), (Dugo et al., 1999) (Costa et al., 2010), (Dugo et al., 2009), (Russo et al., 2012), (Gionfriddo et al., 1997), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Calabro & Curro, 1976), (Vernin et al., 1979), (Mondello et al., 1993), (Dellacassa et al., 1997), (Calvarano et al., 1979), (Cieri, 1969), (McHale & Sheridan, 1988), (Marin et al., 2000),	Bergamot Lemon Lime	192.0423 121.0650	$C_6H_{10}O_7$ C_8H_9O	1.82 2.81

							(Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Dugo et al., 1998), (Madsen & Latz, 1970), (Glandian et al., 1978), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al., 1997), (Stanley & Vanier, 1967), (Nigg et al., 1993) (Russo et al., 2015)				
5	247.0607	246.0529	7.61	$C_{13}H_{10}O_5$	0.31	Isopimpinelin	(Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Cieri, 1969), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al., 1997), (Stanley & Vanier, 1967), (Nigg et al., 1993)	Lime	232.0362 217.0148	$C_{12}H_8O_5$ $C_{11}H_5O_5$	4.19 5.08

							(Russo et al., 2015)				
6	261.1123	260.1045	7.79	$C_{15}H_{16}O_4$	1.38	Meranzin isomer 1	(Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Dugo et al., 1996), (Dugo et al., 2011a), (Chouchi et al., 1996), (Feger et al., 2006), (Tatum & Berry, 1979), *(Mencherini et al., 2013), (Boelens & Sindreu, 1986), (Boelens & Jimenez, 1989)* (Russo et al., 2015)	Bigarade Grapefruit	189.0550 131.0486	$C_{11}H_9O_3$ C_9H_7O	0.89 8.32
7	217.0501	216.0423	7.86	$C_{12}H_8O_4$	0.19	Bergapten	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 1999), (Di Giacomo & Calvarano, 1978), (Analytical	Bergamot Lime Bigarade Lemon Grapefruit	202.0250 174.0311	$C_{11}H_6O_4$ $C_{10}H_6O_3$	7.96 3.41

							Methods Committee, 1987), (Costa et al., 2010), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Gionfriddo et al., 1997), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Vernin et al., 1979), (Mondello et al., 1993), (Dellacassa et al., 1997), (Calvarano et al., 1979), (Shu et al., 1975), (Porcaro & Shubiack, 1974), (Cieri, 1969), (Marin et al., 2000), (Fisher & Trama, 1979), (Glandian et al., 1978), (Dugo et al., 1996), (Dugo et al., 2011a),					

							(Chouchi et al., 1996) (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al., 1997) (Stanley & Vanier, 1967), (Nigg et al., 1993) (Russo et al., 2015)				
8	261.1123	260.1045	7.91	$C_{15}H_{16}O_4$	1.38	Meranzin isomer 2	(Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Dugo et al., 1996), (Dugo et al., 2011a), (Chouchi et al., 1996), (Feger et al., 2006), (Tatum & Berry, 1979), *(Mencherini et al., 2013)*, *(Boelens & Sindreu, 1986), (Boelens & Jimenez, 1989)* (Russo et al.,	Bigarade Grapefruit	189.0541 131.0498	$C_{11}H_9O_3$ C_9H_7O	5.65 0.84

							2015)				
9	373.1280	372.1202	8.61	$C_{20}H_{20}O_7$	1.89	Sinensetin isomer 1	(Dugo et al., 1999a), (Costa et al., 2010), (Dugo et al., 1999b), (Russo et al., 2012), (Bonaccorsi et al., 2000), *(Dugo, Mondello, Cogliandro, d'Alcontres, & Cotroneo, 1994),* (Weber et al., 2006), (Dugo et al., 1996), (Tatum & Berry, 1972), (Li, Lo, Wang, & Ho, 2010), (Mondello, Dugo, & d'Alcontres, 1993), (Gaydou, Berahia, Wallet, & Bianchini, 1991), (Bianchini & Gaydou, 1981), (Dugo et al., 2011b), (Donato, Bonaccorsi, Russo, & Dugo, 2014) (Russo et al., 2015)	Orange Bergamot Mandarin	358.1013 343.0732 312.0978	$C_{19}H_{18}O_7$ $C_{18}H_{15}O_7$ $C_{18}H_{16}O_5$	11.04 6.64 6.32

10	355.1534	354.1456	8.63	$C_{21}H_{22}O_5$	3.17	Aurantiumal	(Dugo & McHale, 2002)	Grapefruit	163.0414	$C_9H_7O_3$	11.54
11	287.0919	286.0841	8.85	$C_{16}H_{14}O_5$	0.08	Oxypeucedanin	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (McHale & Sheridan, 1988), (Marin et al., 2000), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Dugo et al., 1998), (Glandian et al., 1978), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al., 1997) (Russo et al., 2015)	Lemon Lime	203.0351 147.0448	$C_{11}H_7O_4$ $C_9H_7O_2$	3.28 1.33
12	403.1385	402.1307	8.92	$C_{21}H_{22}O_8$	1.91	Nobiletin isomer 1	(Dugo et al., 1999a), (Dugo et al.,	Orange Mandarin Grapefruit	373.0907 355.0771	$C_{19}H_{17}O_8$ $C_{19}H_{15}O_7$	4.40 13.17

							2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Dugo et al., 1996), (Feger et al., 2006), (Tatum & Berry, 1979), (Weber et al., 2006), (Dugo et al., 1994), (Dugo et al., 1996) (Tatum & Berry, 1972), (Li et al., 2010), (Mondello et al., 1993), (Gaydou et al., 1991), (Bianchini & Gaydou, 1981), (Dugo et al., 2011b), (Verzera, Mondello, Trozzi, & Dugo, 1997) (Russo et al., 2015)				
13	403.1378	402.1300	9.36	$C_{20}H_{22}O_8$	3.65	Nobiletin isomer 2	(Dugo et al., 1999a), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989),	Orange Mandarin Bigarade Grapefruit	373.0894 355.0772	$C_{19}H_{17}O_8$ $C_{19}H_{15}O_7$	7.89 12.89

							(Bonaccorsi et al., 2000), (Dugo et al., 1996), (Dugo et al., 2011a), (Feger et al., 2006), (Tatum & Berry, 1979), *(Mencherini et al., 2013)*, (Weber et al., 2006), (Dugo et al., 1994), (Dugo et al., 1996), (Tatum & Berry, 1972), (Li et al., 2010), (Mondello et al., 1993), (Gaydou et al., 1991), (Bianchini & Gaydou, 1981), (Dugo et al., 2011b) (Russo et al., 2015)				
14	343.1167	342.1089	9.40	$C_{19}H_{18}O_6$	4.2	Tetra-O-methylscutellarein	(Dugo et al., 1999a), (Russo et al., 2012), (Bonaccorsi et al., 2000), (Weber et al., 2006), (Dugo et al., 1994),	Orange Mandarin	313.0670 282.0853	$C_{17}H_{13}O_6$ $C_{17}H_{14}O_4$	13.45 13.85

							(Dugo et al., 1996), (Tatum & Berry, 1972), (Li et al., 2010), (Mondello et al., 1993), (Gaydou et al., 1991), (Bianchini & Gaydou, 1981), (Russo et al., 2015)				
15	359.1121	358.1043	9.57	$C_{19}H_{18}O_7$	2.66	Hydroxytetramethoxyflavone isomer 1	(Russo et al., 2012), (Li et al., 2010), (Dugo et al., 2011b)	Bergamot Orange Mandarin	344.0842 298.0835	$C_{18}H_{16}O_7$ $C_{17}H_{14}O_5$	15.70 2.09
16	433.1489	432.1411	9.58	$C_{22}H_{24}O_9$	2.16	Heptametoxyflavone	(Dugo et al., 1999a), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Dugo et al., 1996), (Feger et al., 2006), (Weber et al., 2006), (Dugo et al., 1994), (Dugo et al., 1996), (Tatum & Berry, 1972), (Li et al., 2010),	Orange Mandarin Grapefruit	403.0963	$C_{20}H_9O_9$	16.39

							(Mondello et al., 1993), (Gaydou et al., 1991), (Bianchini & Gaydou, 1981), (Dugo et al., 2011b) (Russo et al., 2015)				
17	387.1802	386.1724	9.69	$C_{22}H_{26}O_6$	1.39	unknown 1		Dist. lime			
18	271.0962	270.0884	9.83	$C_{16}H_{14}O_4$	2.99	Imperatorin	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 2009), (Russo et al., 2012), (Casabianca, 2012), (Bonaccorsi et al., 2000), (McHale & Sheridan, 1988), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Nigg et al., 1993) (Russo et al., 2015)	Expr. lime lemon	203.0342	$C_{11}H_7O_4$	1.15
19	359.1123	358.1045	9.86	$C_{19}H_{18}O_7$	2.1	Hydroxytetramethoxyflavone isomer 2	(Russo et al., 2012), (Li et al., 2010), (Dugo et al.,	Bergamot Orange Mandarin Grapefruit	344.0849 315.0858	$C_{18}H_{16}O_7$ $C_{17}H_{15}O_6$	3.40 3.37

							2011b; Russo et al., 2015)				
20	373.1276	372.1198	9.90	$C_{20}H_{20}O_7$	2.96	Sinensetin isomer 2	(Dugo et al., 1999a), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Tatum & Berry, 1979), (Dugo et al., 1994), (Weber et al., 2006), (Dugo et al., 1996), (Tatum & Berry, 1972), (Li et al., 2010), (Mondello et al., 1993), (Gaydou et al., 1991), (Bianchini & Gaydou, 1981), (Dugo et al., 2011b) (Russo et al., 2015)	Mandarin Orange Bigarade Grapefruit	343.0749	$C_{18}H_{15}O_7$	20.04
21	389.1227	388.1149	10.02	$C_{20}H_{20}O_8$	2.36	Hydroxypentamethoxyflavone	(Weber et al., 2006), (Tatum & Berry, 1972), (Li et al., 2010), (Dugo et al., 2011b) (Russo et al.,	Mandarin Orange Bigarade Bergamot Grapefruit	359.1052 342.0840	$C_{19}H_{19}O_7$ $C_{18}H_{14}O_7$	21.93 29.37

							2015)				
22	315.1590	314.1511	10.03	$C_{19}H_{22}O_4$	2.26	Epoxyaurapten	(Dugo et al., 1999a), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (McHale & Sheridan, 1989), (Feger et al., 2006), (Tatum & Berry, 1979) (Russo et al., 2015)	Grapefruit	297.1488 163.0396 153.1279 135.1175	$C_{19}H_{21}O_3$ $C_9H_7O_3$ $C_{10}H_{17}O$ $C_{10}H_{15}$	0.91 0.50 0.26 0.92
23	301.1072	300.0994	10.06	$C_{17}H_{16}O_5$	1.24	Phellopterin	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (Bonaccorsi et al., 2000), (McHale & Sheridan, 1988), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997),	Lemon Expr. Lime	233.045 218.0168	$C_{12}H_9O_5$ $C_{11}H_6O_5$	0.007 21.66

							(Fisher & Trama, 1979), (Feger et al., 2006), (Stanley & Vanier, 1967) (Russo et al., 2015)				
24	403.1381	402.1303	10.10	$C_{21}H_{22}O_8$	2.9	Nobiletin isomer 3	(Dugo et al., 1999a), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Dugo et al., 1996), (Dugo et al., 2011a), (Feger et al., 2006), (Tatum & Berry, 1979), *(Mencherini et al., 2013)*, (Weber et al., 2006), (Dugo et al., 1994), (Dugo et al., 1996), (Tatum & Berry, 1972), (Li et al., 2010), (Mondello et al., 1993), (Gaydou et al., 1991),	Mandarin Orange Grapefruit Bigarade	373.0908 355.0762	$C_{19}H_{17}O_8$ $C_{19}H_{15}O_7$	4.13 15.71

							(Bianchini & Gaydou, 1981), (Dugo et al., 2011b) (Russo et al., 2015)				
25	245.1174	244.1096	10.19	$C_{15}H_{16}O_3$	1.41	Unknown 2		Bigarade Grapefruit	189.0546 131.0492	$C_{11}H_9O_3$ C_9H_7O	3.01 3.73
26	329.1020	328.0942	10.19	$C_{18}H_{16}O_6$	1.49	Hydroxytrimethoxyflavone		Bergamot	268.0751	$C_{16}H_{12}O_4$	5.75
27	301.1074	300.0996	10.22	$C_{17}H_{16}O_5$	0.58	Phellopterin isomer	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (Feger et al., 2006), (Stanley & Vanier, 1967) (Russo et al., 2015)	Expr. lime	233.0452	$C_{12}H_9O_5$.	0.86
28	355.1539	354.1461	10.39	$C_{21}H_{22}O_5$	1.76	Epoxybergamottin	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (McHale & Sheridan, 1989),	Grapefruit Bigarade	203.0346 153.1268	$C_{11}H_7O_4$ $C_{10}H_7O$	0.82 7.45

							(Bonaccorsi et al., 2000), (Dugo et al., 1996), (Dugo et al., 2011a), (Feger et al., 2006) (Russo et al., 2015)				
29	261.1120	260.1042	10.60	$C_{15}H_{16}O_4$	2.53	Meranzin isomer 3	(Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Dugo et al., 1996), (Dugo et al., 2011a), (Chouchi et al., 1996), (Feger et al., 2006), (Tatum & Berry, 1979), *(Mencherini et al., 2013)*, *(Boelens & Sindreu, 1986), (Boelens & Jimenez, 1989)* (Russo et al., 2015)	Bigarade Grapefruit	243.0999	$C_{15}H_{15}O_9$	9.13
30	355.1542	354.1464	10.97	$C_{21}H_{22}O_5$	0.91	Cnidicin isomer	(Dugo et al., 2009), (Russo et al.,	Lemon Lime expr. Grapefruit	219.0325 173.0279	$C_{11}H_7O_5$ $C_{10}H_5O_3$	14.39 23.29

							2012), (Casabianca, 2012), (Ziegler & Spiteller, 1992) (Russo et al., 2015)				
31	339.1592	338.1514	11.04	$C_{21}H_{22}O_4$	1.21	8-Geranyloxypsoralen	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (McHale & Sheridan, 1988), (Marin et al., 2000), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Dugo et al., 1998), (Fisher & Trama, 1979), (Glandian et al., 1978), (Feger et al., 2006),	Lemon Expr.lime	203.0338 147.0447	$C_{11}H_7O_4$ $C_9H_7O_2$	3.12 0.65

							(Bonaccorsi et al., 2011), (Dugo et al., 1997), (Nigg et al., 1993) (Russo et al., 2015)				
32	299.1640	298.1562	11.18	$C_{19}H_{23}O_3$	2.4	Aurapten	(Dugo et al., 1999a), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (McHale & Sheridan, 1989), (Fisher & Trama, 1979), (Feger et al., 2006), (Tatum & Berry, 1979) (Russo et al., 2015)	Grapefruit	163.0396	$C_9H_7O_3$	0.5
33	369.1699	368.1621	11.26	$C_{22}H_{24}O_5$	0.74	5-Geranyloxy-8-methoxypsoralen	(Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al., 1997), (Stanley & Vanier, 1967) (Russo et al., 2015)	Expr.lime	233.0464 173.0233	$C_{12}H_9O_5$ $C_{10}H_5O_3$	6.01 3.25

34	339.1593	338.1515	11.35	$C_{21}H_{22}O_4$	0.91	Bergamottin	*(Frérot & Decorzant, 2004)*, (Dugo et al., 1999a), (Dugo et al., 1999), (Costa et al., 2010), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Gionfriddo et al., 1997), (Casabianca, 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Calabro & Curro, 1976), (Mondello et al., 1993), (Dellacassa et al., 1997), (Calvarano et al., 1979), (Cieri, 1969), (McHale & Sheridan, 1988), (Marin et al., 2000), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Dugo et al., 1998),	Bergamote Lemon Lime Grapefruit	203.0359 147.0450	$C_{11}H_7O_4$ $C_9H_7O_2$	7.22 2.69

							(Madsen & Latz, 1970), (Fisher & Trama, 1979), (Glandian et al., 1978), (Feger et al., 2006), (Tatum & Berry, 1979), (Bonaccorsi et al., 2011), (Dugo et al., 1997), (Stanley & Vanier, 1967) (Russo et al., 2015)				
35	261.1124	260.1046	11.36	$C_{15}H_{16}O_4$	0.99	Meranzin isomer 4	(Dugo et al., 1999a), (Dugo et al., 1999b), (Dugo et al., 2009), (Russo et al., 2012), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), *(Mencherini et al., 2013)* (Russo et al., 2015)	Grapefruit	189.0542 131.0476	$C_{11}H_9O_3$ C_9H_7O	5.13 15.95
36	323.1051	322.0973	11.41			Unknown 3		Dist. Lime	251.0506	$C_{19}H_7O$	3.62
37	329.1746	328.1668	11.41	$C_{20}H_{24}O_4$	2.01	5-geranyloxy-7-methoxycoumarin	(Dugo et al., 1999a), (Dugo et al., 1999), (Costa et al.,	Lemon Lime Bergamot	251.0481 193.0506	$C_{19}H_7O$ $C_{10}H_9O_4$	6.33 2.67

							2010), (Dugo et al., 2009), (Dugrand et al., 2013), (Russo et al., 2012), (Gionfriddo et al., 1997), (McHale & Sheridan, 1989), (Bonaccorsi et al., 2000), (Calabro & Curro, 1976), (Mondello et al., 1993), (Dellacassa et al., 1997), (Cieri, 1969), (McHale & Sheridan, 1988), (Marin et al., 2000), (Ziegler & Spiteller, 1992), (Dellacassa et al., 1997), (Dugo et al., 1998), (Madsen & Latz, 1970), (Fisher & Trama, 1979), (Glandian et al., 1978), (Feger et al., 2006), (Bonaccorsi et al., 2011), (Dugo et al.,					

							1997), (Stanley & Vanier, 1967) (Russo et al., 2015)				
38	279.1596	278.1518	12.51	$C_{16}H_{22}O_4$	0.03	Unknown 4		Dist. lime	163.0396	$C_9H_7O_3$	0.5

Figure SM1 a) Chromatographic patterns of the extracted mass of bergamottin at different dilutions of a bergamot sample b) plot of regression line associated to the EDA approach

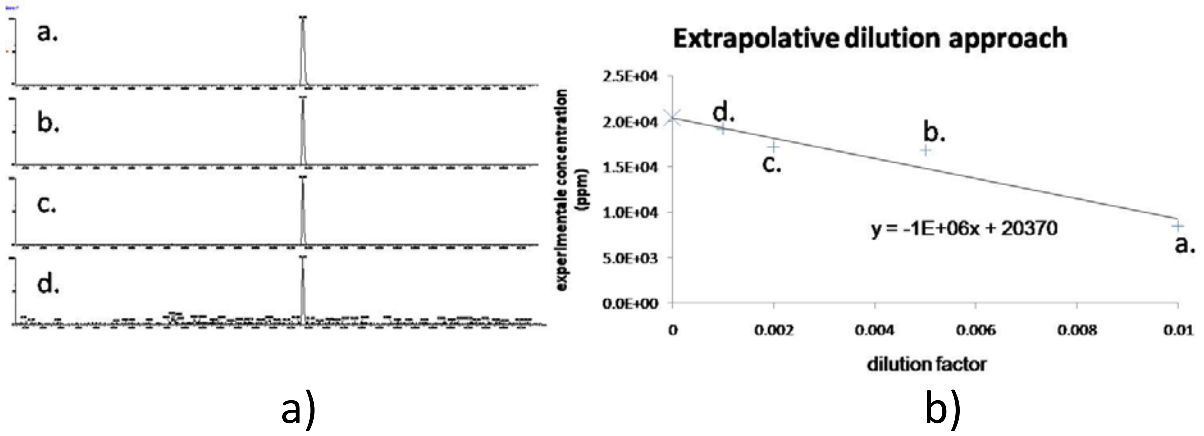


Figure SM1

Figure SM2 PCA scores plot of the investigated citrus EOs, taking the 18 Fcs and Cs as variables. R2[X](1)=0.22; R2[X](2)=0.15, Ellipse Hotelling's T2 (95%)

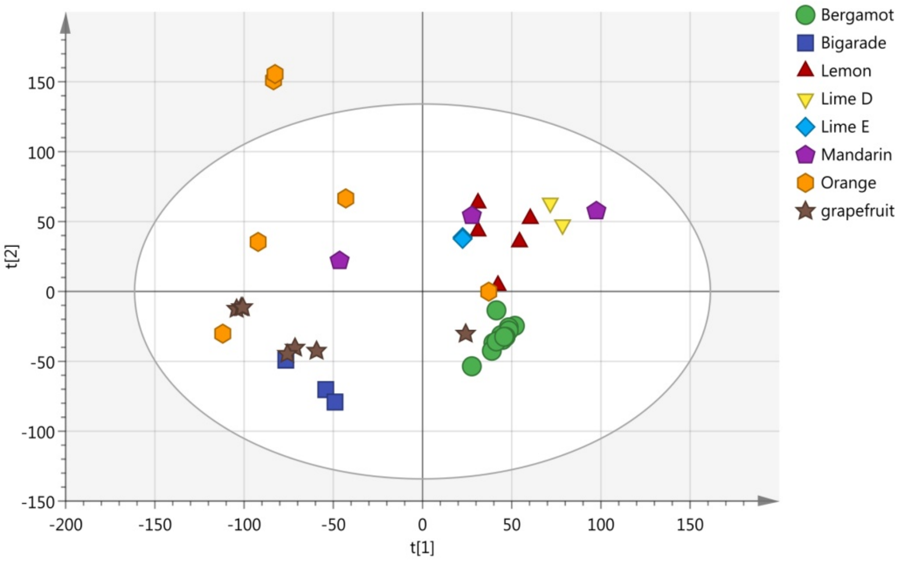
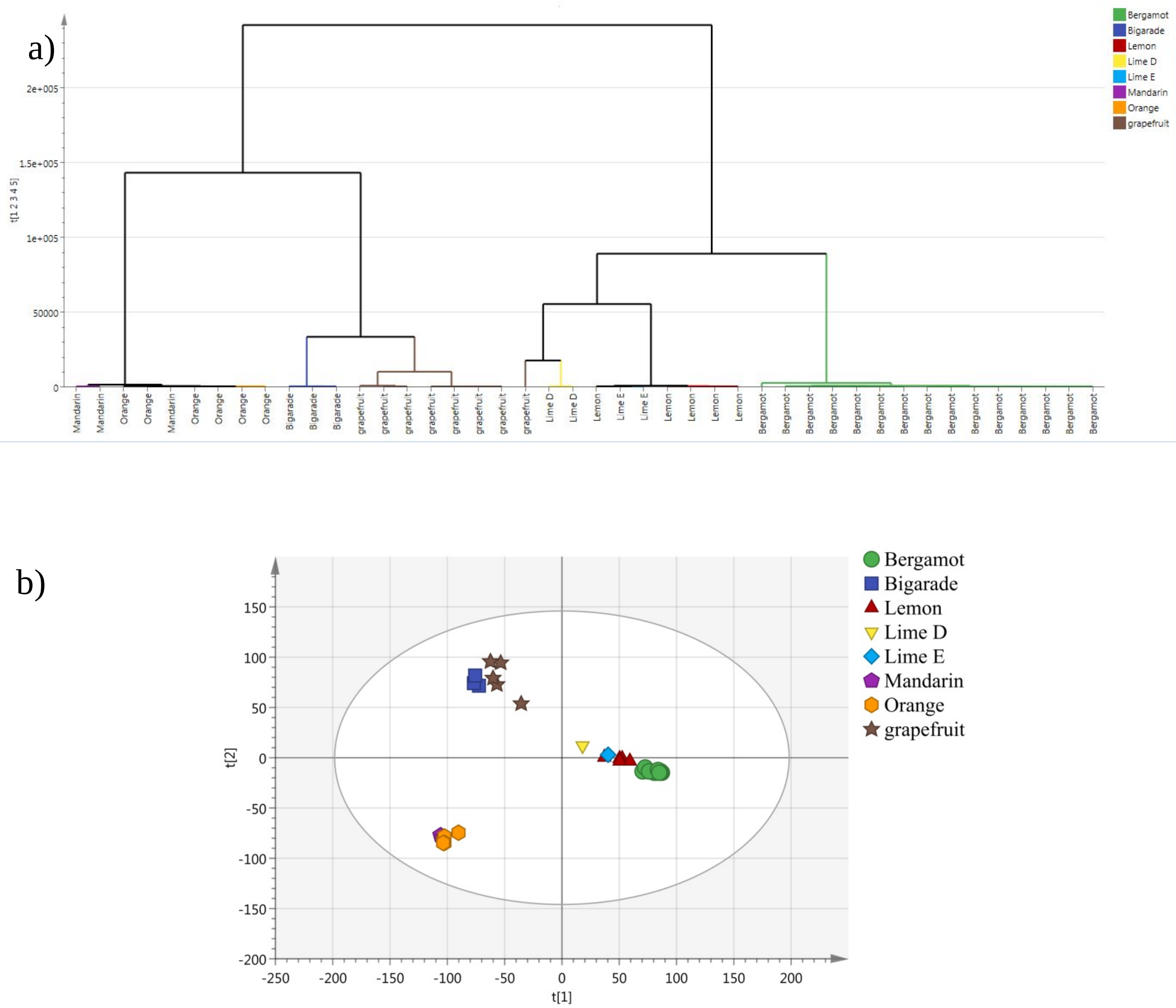


Figure SM3 a) HCA of the investigated citrus EOs, taking the 140 OHC compounds b) PCA scores plot with the 850 EMRTs resulting from the investigated citrus EOs.R2[X](1)= 0.34, R2[X](2) = 0.18, Ellipse Hotelling's T2 (95%)



Supporting Information References

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