

## **Characterisation of nutraceutical compounds from different parts of particular species of *Citrus sinensis* ‘Ovale Calabrese’ by UHPLC-UV-ESI-HRMS**

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### **Abstract**

Consumers are aware of diet causing health problems and therefore there is an increased demand for natural ingredients that are expected to be safe and health-promoting. Many of these compounds belong to the class of flavonoids and can be divided into these five groups: flavanones, flavones, flavonols, flavanols, isoflavones and anthocyanidins. Extracts from citrus fruits are usually used as functional ingredients for several products. The aim of this paper was to develop an UHPLC-UV-ESI-HRMS method to define the metabolite profile of different parts of citrus fruit, of a particular cultivar called ‘Ovale Calabrese’, and in its main by-products. The high resolution mass spectrometry analysis allowed the identification of 27 compounds belonging to the classes of flavonoids and terpenoids. The high contents of phytochemical compounds, reveal the potential use of the ‘Ovale Calabrese’ as a rich source of nutraceutical compounds.

**Keywords:** *Citrus sinensis*, flavonoids, terpenoids, Orbitrap, UHPLC-HRMS/MS

### **1. Introduction**

The genus *Citrus* and its varieties is one of the crops widely cultivated all over the world. According to the agricultural census (section cultivation and farming) conducted in 2010 by the Italian National Institute of Statistics (I.Stat) there is approximately 170.000 hectares which are dedicated to citrus cultivation with annual fruits production about 400.000 tons (Italian National Institute of Statistics 2010). Italy presents one of the highest production of citrus among European countries. Citrus benefits on human health are well demonstrated (Piccinelli et al. 2008; Mencherini et al. 2013; Gonçalves et al. 2017; Metro et al. 2017). A large number of studies have been carried out with the aim of identifying the bioactive components present in different parts of citrus fruits over the past decades, (Cicero et al. 2015; Salvo et al. 2016) in an attempt to gain a deeper understanding of the correlation between diet, health benefits and reduce risk of diseases (Liu 2003; Gonçalves et al., 2017). In this context, several studies have been associated to the consumption of food rich in bioactive compounds which have antihyperlipidemic effects (Santini and Novellino 2017) decrease the risk of different types of cancer, cardiovascular diseases (Yao et al. 2004) and also reduce the incidence of many disease as cardiovascular disease (Kris-Etherton et al. 2002). Citrus fruits are one of the richest dietary sources of flavonoids and for these reason can become a perfect candidate for

the formulation of food supplement and nutraceutical products (Santini et al. 2017). Citrus have shown antioxidant, antiedematogenic, anti-inflammatory and anti-ageing activity (Benavente-Garcia and Castillo 2008; Mencherini et al. 2013). Due to the benefits of phenolic compounds on health, the identification and/or structural determination as well as a number of analytical methods for the quantification and their identification were reported (Ignat et al. 2011; Gervasi et al. 2016; Montero et al. 2016; Pagano et al. 2016; Celano et al. 2017; Mansour et al. 2017; de Souza Figueiredo et al. 2017). The most effective analytical equipment as HPLC-UV, GC-MS and CE used for identification of flavonoids in citrus fruits and their by-products were reported (Cancalon and Bryan 1993; Justesen et al. 1998; Kanaze et al. 2003; Füzfaı and Molnár-Perl 2007; Londoño-Londoño et al. 2010). However, previous studies have primarily focused on the main components, such as naringin, eriocitrin and neohesperidin (Kanes et al. 1993; Pichaiyongvongdee and Haruenkit 2009). The flavonoids profile varies with the species and cultivars and can therefore used to distinguish between the different varieties (Nogata et al. 2006; Zhang et al. 2011; Corsaro et al. 2016). The aim of this research was develop an analytical method for the simultaneous determination and characterisation of flavonoids and terpenoids in citrus fruit using high-performance liquid chromatography coupled to UV detection and electrospray ionisation high resolution mass spectrometry (HPLC-DAD-ESI-HRMS/MS). The developed method was applied to evaluate the qualitative and quantitative composition of bioactive compounds in the different part of fruit such as peel, pulp, juice and pastazzo. The main advantages of method are the fully characterisation of the exhaustive extract in different parts of citrus fruit in one chromatographic run.

## **2. Experimental**

See Supplementary materials for samples and extraction, chemical and standards, chemical characterisation by UHPLC-PDA-ESI-HRMS and quantitative analysis.

## **3. Results and discussion**

### **3.1. UHPLC-PDA-ESI-HRMS analysis**

UHPLC-ESI-HRMS analyses were performed in negative and positive ionisation mode to obtain complementary information useful to characterise the peel, pulp, juice and pastazzo of 'Ovale Calabrese' orange. The HPLC conditions were optimised to maximised the chromatographic resolution and increase the MS signal. Figure S1 shows representative chromatograms of peel, pulp, juice and pastazzo extracts under optimal conditions. Metabolite assignments were carried out by comparing UV-vis, HRMS and MS/MS spectra with reference standards when available, or with chemo-taxonomic data reported in the literature and databases. The UHPLC-DAD-HRMSn analysis has allowed to identify 27 compounds that can be grouped into two major classes of secondary metabolites: limonoids and flavonoids (Table S1).

### **3.2. Limonoids**

The limonoids were detected in the extracts both in glycosidic and aglyconic form. Limonoid aglycones are water-insoluble and responsible for a bitter taste of the citrus fruits, while limonoid glucosides are water-soluble and tasteless (Russo et al. 2016). The limonoids were detected by UV at 210 nm, because they do not have chromophore groups into the chemical structure, on the other hand, they provided the highest ionisation of the ESI- in the HRMS profile. Six limonoid glucosides (4, 6, 9, 13–15) were detected and the fragmentation pathway observed in their MS2 spectra confirmed the

structure of the identified limonoid glucosides (Chidambara Murthy et al. 2011). Compounds 4, and 15 in (–)-MS2 spectra showed the characteristic losses of 44 and 206 Da, corresponding to [M-H-CO2]<sup>–</sup> and [M-H-CO2-hexose]<sup>–</sup>, respectively. Instead, in the (–)-MS2 spectra the compounds 6, 9, 13 and 14 showed the characteristic loss of 60 Da [M-H-CH3COOH]<sup>–</sup>. Furthermore, the compounds 14 showed as base peak the ions at m/z 607.2812 corresponding to [M-H-CH3COOH-CO2]<sup>–</sup>. The compound 21 was the only limonoid aglycones detected and it was identified by reference standard.

### 3.3. Flavonoids

Flavonoids are another important group of phenolic compounds, largely distributed in citrus fruit. The flavonoids may contribute to fruit and juice quality in many ways, influencing the appearance, the taste and the nutritional value of the fruits (Gattuso et al. 2007).

In the analysed extracts, ten flavonoids (2, 3, 7, 8, 10, 11, 12, 16, 17 and 18) were detected. All detected flavonoids, except naringenin (17) hesperetin (18), and methoxyflavone compounds were characterised as glycosylated derivatives. Two flavone C-glycosides, vicenin II (2) and diosmetin 6,8-di-C-glucoside (3), were identified. In their (–) MS2 spectra produced the characteristic fragmentation pathway, showing successive or simultaneous losses of glycosylic group (30, 60, 90 and 120 Da) (Cuyckens and Claeys 2004; Piccinelli et al. 2008). Three glycosylated derivatives of naringenin (7, 8 and 16), two of hesperetin (10 and 12) and one of diosmetin (11) were identified. These compounds generate in negative MS2 spectra the diagnostic ions at m/z 271.0506 (ppm 1.9), 301.0723 (ppm 0.9) and 299.0562 (ppm 0.7) generated by the elimination of one or more glycosidic units and corresponding to naringenin, hesperetin and diosmetin aglycon, respectively. The polymethoxyflavones, one subclass of flavonoids in which all or almost hydroxyls are capped by methylation, are widely distributed in citrus fruits particularly in *C. sinensis* (Xing et al. 2017). These compounds were detected at wavelength range of 283–325 nm and were eluted in a specific zone of the chromatogram (16–20 min) (Figure S1). Polymethoxyflavones showed better MS signal and characteristic fragmentation in the positive ionisation mode (ESI<sup>+</sup>). In the analysed extracts one tetramethoxyflavone (25); three pentamethoxyflavones (19, 22 and 27), three hexamethoxyflavones (20, 23 and 24) and one heptamethoxyflavones (26) were identified. In the MS2 spectra all detected polymethoxysaflavones showed mainly consecutive and/or simultaneous losses of methyl radicals (CH<sub>3</sub>·), generating, usually as the base peaks, the characteristic product ions [M + H – n × 15]<sup>+</sup> (Zhang et al. 2012). Other fragments such as neutral loss of H<sub>2</sub>O (18 Da), CH<sub>4</sub> + CH<sub>3</sub>· (31 Da), CO<sub>2</sub> (44 Da), H<sub>2</sub>O + CO (44 Da), and CH<sub>4</sub> + 2CH<sub>3</sub>· (61 Da) from [M + H – n × 15]<sup>+</sup> were detected (Zhang et al. 2012).

### 3.4. Other compounds

Two phenolic acids (1 and 5) in citrus extracts were detected, belonging to hydroxycinnamic acid class. As is well known, phenolic acids and their derivatives, ionised better in the negative mode (Ammar et al. 2015) Compound 1 showed a molecular ion at 355.1040 m/z generating the molecular formula C<sub>16</sub>H<sub>20</sub>O<sub>9</sub>, according to the literature (Gargouri et al. 2017). This compound was assigned as ferulic acid hexoside. Compound 5 with molecular ion at 561.1254 m/z and molecular formula C<sub>26</sub>H<sub>26</sub>O<sub>14</sub> was identified as diferuloyl-glucaric acid ester (Risch et al. 1988).

### 3.5. Secondary metabolites distribution in peel, pulp, juice and pastazzo

The distribution of identified metabolites in peel, pulp, juice and pastazzo is reported in Table S1. As shown in UHPLC-UV profiles of peel, pulp, juice and pastazzo (Figure S1) and according to the literature, hesperidin (10) and narirutin (7) were the most abundant components of the samples analysed (Tripoli et al. 2007). Peel showed the most distinctive chemical profile (Figure S1). It

showed hesperidin as the only main flavonoid glycoside, with traces of vicenin II (2) and diosmetin-6,8-di-C-glucoside (3). These last compounds are present at low levels in all the extracts analysed. Furthermore, peel profile is characterised by the presence of polymethoxyflavons in significant quantities. This class of compounds is absent in pulp and juice extracts; therefore, they could be considered as qualitative markers of peel. In fact, as reported in the literature, the polymethoxyflavons were exclusively detected in the peel and they can be considered as a quality parameter of the juice (Scordino et al. 2011). The presence of polymethoxyflavons in the latter indicates a bad quality of juice, due to excessive squeezing or/and contaminated with the peel constituents (Scordino et al. 2011).

Pulp and juice showed qualitatively comparable profiles. Belonging to flavonoids class besides hesperidin and narirutin, the didymin (16) was also detected in appreciable quantities. Furthermore, in pulp and juice extracts the limonoid glycosides were contained in greater quantities than pastazzo and peel. The pastazzo showed the most complex and complete profile among all analysed extracts, since it consists of a mix of seed, pulp and peel. In the pastazzo profile, hesperidin was the most abundant component and the presence of the polymethoxyflavones were observed as in the peel extract.

### **3.6. Quantitative analysis of peel, pulp, juice and pastazzo**

Quantification of hesperidin, limonin and limonin glucoside in various parts of 'Ovale Calabrese' orange (peel, pulp, juice and pastazzo) were carried out by HPLC-UV analysis, using the external standard method calibration. The concentrations of each compound in the extracts were calculated from the peak areas by interpolation to standard calibration curves, including hesperidin and limonin in the concentration range of 0.1–10 µg/mL (5 levels). Table 1 reports the quantitative analysis expressed as mg/100 g DM (relative standard deviation) of all the samples analysed.

According to the literature the UHPLC profiling revealed that the hesperidin was the dominant phenolic compound of peel, pulp, juice and pastazzo (Gattuso et al. 2007; Soji-Omoniwa et al. 2014; Molina-Calle et al. 2015). Hesperidin is one of the most abundant secondary metabolites contained in citrus fruits and is the major flavonoid in sweet orange and lemon fruits. In young immature oranges, hesperidin can be found up to 14% of the fresh weight of the fruit (Garg et al. 2001). In the analysed samples the highest content of hesperidin was detected in the pastazzo followed by the peel, pulp and juice. This result is justified by the fact that the pastazzo is composed mainly by residue of peel and pulp. The quantitative results show clearly that the pastazzo can be considered a rich source of hesperidin. As can be seen in Table 1 limonin aglycones were detected in peel, and as consequence in pastazzo, while limonin glucoside was the most abundant compound detected in juice. These results are in agreement with those reported previously in literature for other citrus species, in which limonoid glucosides are reported as more abundant in juices because they are water-soluble, while water-insoluble limonoid aglycones are mostly present in peels (Manners 2007; Breksa et al. 2009).

**Table 1.** Quantitative analysis of main compounds in peel, pulp, juice and pastazzo of ‘Ovale Calabrese’, expressed as mg/100 g DM (RSD<sup>a</sup>).

Compound	Peel	Pulp	Juice	Pastazzo
Hesperidin	954.3 (5.4)	415.6 (4.2)	200,9 (2.5)	1976.7 (6.9)
Limonin	6.5 (6.3)	nq	nq	32.0 (4.5)
Limonin glucoside <sup>b</sup>	29.1 (4.6)	5.4 (7.3)	105.3 (5.6)	18.2 (5.1)

Note: nq: not quantifiable.

<sup>a</sup>RSD, relative standard deviation of three replications.

<sup>b</sup>Expressed as equivalents of limonin.

## 4. Conclusions

Nowadays the beneficial effects of secondary metabolites as flavonoids and limonoids on human health are widely demonstrated by several studies. Citrus fruits and its products remain one of the naturally dietary sources. The developed UHPLC-ESI-HRMS/MS, in positive and negative ionisation modes, enables the characterisation of 2 class of compounds: flavonoids and limonoids. The developed method used in this work allowed the detection and characterisation of 27 secondary metabolites in three parts juice, pulps, and peels and in the main industrial by-product of a particular specie of *C. sinensis* commonly called ‘Ovale Calabrese’ that grow in the south of Italy (Calabria region). Several flavonoids and limonoids derivatives were identified and quantified. Considering the high content of phenolic compounds in particular hesperidin, in all the analysed parts, and given the well-known pharmacological activity of these compounds, the orange ‘Ovale Calabrese’ can be considered as a rich source of bioactive substances for the formulation of beverage with high nutraceutical value. Moreover, the great content of hesperidin in the pastazzo could be a starting point for the revalorize this by-product as potential source of bioactive compounds for the developing of new functional ingredients for functional food and nutraceutical formulation.

To the best of our knowledge, this is the first study that reports the fully characterisation of all parts of this species of citrus and its by-product. This analytical methodology could be used as a rapid, effective technique to screen and identify compounds from citrus and citrus by-products.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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### Supplemental data

Supplemental data for this article can be accessed at <https://doi.org/10.1080/14786419.2018.1443102>.

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running the laboratory work and ALP, supervised the laboratory work. ALP, LC, RC, IP, RSD, SC, contributed to analysis of the data. All contributed to drafted the paper. ALP, LR and RMt designed the study and contributed to critical reading of the manuscript.

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## SUPPLEMENTARY MATERIAL

### **Characterization of nutraceutical compounds from different parts of particular species of *Citrus sinensis* “Ovale Calabrese” by UHPLC-UV-ESI-HRMS**

#### **2. Experimental**

##### **2.1 Samples and extraction**

The citrus genus used in this work belonging to a particular cultivar of *Citrus sinensis* called “Ovale Calabrese” a fruit that grow especially in the south region of Italy, mainly in Calabria and Sicily. Fruits were provided by Frujit Soc. Coop. Srl company and were collected from the orchards of Calabria (Italy) on march 2016. Before the extraction, all fruits were washed, dried and stored at +4°C for a maximum of three days. In order to obtain representative samples of the different components of the fruits: peel, pulp and juice from 20 fruits were peeled and manually squeezed to obtain the juice and the other parts; pulps were recovered from the juice after 5 minutes of centrifugation at 6000 rpm (SL 16 centrifuge, Thermo Fisher Scientific, Milan, Italy). Pure juice was frozen and stored at -20 °C, while the peel and pulp were immediately lyophilized (Alpha 14 LD, Christ) and stored at -20 °C in the dark until the analysis. Pastazzo, the main by-product of the citrus, composed of seeds (1-2%), pulps (30-35 %), exhausted peels (60-65%) was supplied with the fruit samples from company

Frujit Soc. Coop. Srl. Homogenized samples of pastazzo were lyophilized and the resulted powder was manually grinded and stored in dark at -20 °C until use.

All solid samples were lyophilized and homogenised before the extraction by the ultra sound assisted solid-liquid extraction (USA-SLE). Briefly, each sample was extracted in triplicate for 20 minutes five times at 25 °C with a solution of water:ethanol (70:30 v/v) with a matrix:solvent ratio of 1:10. The extracts were pooled, filtered (Whatman No. 1 filter) and freeze-dried (freeze dryer Alpha 1-2 LD, Christ, Germany). Organic solvent was removed under vacuum at 40 °C in a rotary evaporator (Rotavapor R-200, Buchi Italia s.r.l, Cornaredo, Italy).

The extraction yields determined gravimetrically and expressed as g/100 g dry matter ( $\pm$  standard deviation) of ultrasound assisted solid-liquid extraction (USA-SLE), were 36.1( $\pm$  1.5), 77.1 ( $\pm$  4.9) and 44.4 ( $\pm$  2.6) for peel, pulp and pastazzo respectively . Before the analysis all extracts were filtered with a 0.45  $\mu$ m filter Xtra RC (Macherey-Nagel GmbH & Co. Germany) in order to remove solid materials.

## 2.2 Chemicals and standards

MS-grade acetonitrile (MeCN) and water were supplied by Romil (Cambridge, UK). Ultrapure water (18 M $\Omega$ ) was prepared by a Milli-Q purification system (Millipore, Bedford, USA). Analytical-grade methanol (MeOH) and ethanol (EtOH), MS-grade formic acid (HCOOH), hesperetin ( $\geq$ 98% HPLC), hesperidin ( $\geq$ 98% HPLC) and naringenin ( $\geq$ 98% HPLC) were obtained from Sigma-Aldrich (Milan, Italy). The standard stock solutions were prepared from 1 mg of each compound dissolved in 1 mL MeOH to obtain a final concentration of 1 mg/mL and stored at 4°C for a maximum of three months. Diluted solutions used for calibration curve and standard mixtures were prepared in MeOH /H<sub>2</sub>O 2:8, v/v.

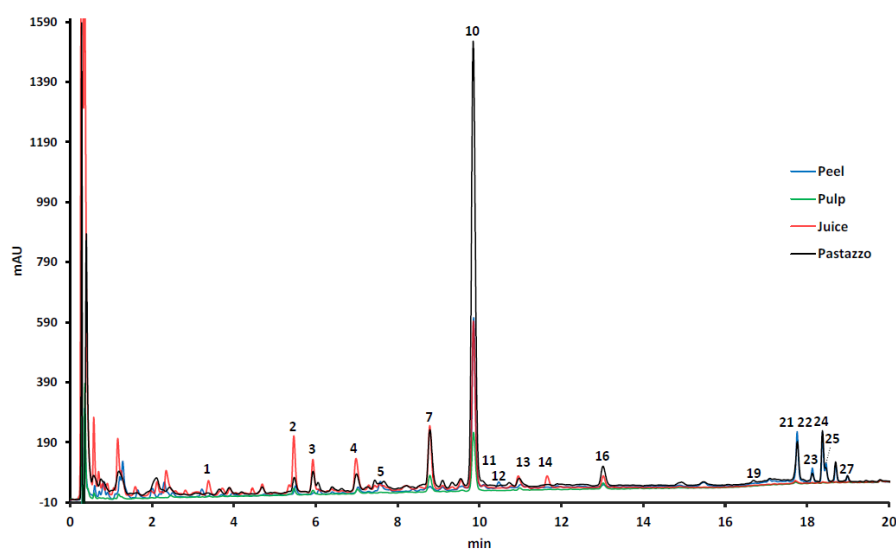
## 2.3 UHPLC-PDA-ESI-HRMS conditions and mobile phases.

Qualitative analysis were performed on a Platin Blue UHPLC system (KNAUER GmbH, Berlin, Germany) consisting of two Ultra High-Pressure Pumps, an autosampler, a column temperature manager and a diode array detector, coupled to a LTQ Orbitrap XL (Thermo Scientific, San Jose, CA) equipped with a electrospray ionization (ESI) probe. UHPLC separation was achieved with a Kinetex Biphenyl (50 x 2.1 mm I.D., 2.6  $\mu$ m) column protected by a Biphenyl Guard Cartridge (2.1 mm I.D.), both from Phenomenex (Torrance, CA, USA) held at 30 °C for all chromatographic run. The mobile phase consisted of water (A) and acetonitrile (B), both with 0.1% HCOOH and the following gradient was used: 0-15 min, 5-25% B; 15-20 min, 25-60% B; 20-21 min, 60-98% B. After each injection, the column was washed with 98% B for 4 min and re-equilibrated (5 min). A flow rate of 0.4 mL/min and an injection volume of 5  $\mu$ L (partial loop injection mode) were used.

In order to increase the method sensitivity detection by diode array was performed a three different wavelengths: 210, 283 and 325 nm and the UV spectra were recorded over a 200-600 nm range. The HRMS and HRMS/MS were performed with an ESI source in the negative and positive ion mode. High-purity nitrogen (N<sub>2</sub>) was used as both drying gas and nebulizing gas, and ultra-high pure helium (He) as the collision gas. The operating parameters were optimized as follows: source voltage 3.5 kV, capillary voltage -42 V, tube lens voltage -45.6 V, capillary temperature 280 °C, sheath and auxiliary gas flow (N<sub>2</sub>) 30 e 10 (arbitrary units), respectively. The MS profile was recorded in full scan mode (scan time = 1 micro scans and maximum inject time 500 ms) with resolution of 60000. For the HRMS/MS acquisitions, a data-dependent method, setting the normalized collision energy in the ion trap of 35%, was used. Detected compounds were characterized according to the corresponding spectral characteristics: UV and mass spectra, accurate mass, characteristic fragmentation, and retention time. Xcalibur software (version 2.2) was used for instrument control, data acquisition and data processing.

## 2.4 Quantitative analysis

Quantitative analysis was carried out by using the UHPLC equipment and chromatographic conditions used for qualitative analysis. The UV chromatograms were recorded at 283 and 210 nm for the quantification of hesperidin and limonin/limonin glucoside, respectively. The hesperidin and limonin were quantified using the calibration curves of the corresponding standards, while limonin - 10 190 390 590 790 990 1190 1390 1590 0 2 4 6 8 10 12 14 16 18 20 mAU min Peel Pulp Juice Pastazzo glucoside was quantified using the calibration curve of limonin and expressed as limonin equivalent. Standard calibration curves were obtained in a concentration range of 0.1 – 10 µg/mL with five concentration levels (0.1, 1.0, 2.5, 5.0 and 10.0 µg/mL) and triplicate injections for each level. UV peak areas of the external standard were plotted against the corresponding standard concentrations using weighed linear regression to generate standard curves. The R<sup>2</sup> values for the linear regression of external standards, for both calibration curves, was 0.999. All extracts were injected at a concentration of 0.25 mg/mL and 5 µL was injected for analysis. The amount of the compounds was expressed as grams of compound per 100g of DM (g/100g DM), as the mean of triplicate determinations.



**Figure S1** UHPLC-UV chromatograms of analysed samples of “Ovale Calabrese” orange recorded at 210nm

N.	T <sub>r</sub> (min)		(+) <sup>a</sup> HRMS			(-) <sup>a</sup> HRMS			Molecular formula	Compound	Distribution
	UV	MS	m/z	ppm	Product ions MS/MS	m/z	ppm	Product ions MS/MS			
1	3.4	3.5	357.1165	-4.2	/	355.1041	4.5	193.2711 [M-H-hexose] <sup>-</sup>	C <sub>16</sub> H <sub>20</sub> O <sub>9</sub>	Ferulic acid glucoside	Pe, Pu, J, Pa
2	5.5	5.5	595.1627	-5.1	/	593.1515	2.3	503.1201 [M-H-C <sub>3</sub> H <sub>6</sub> O <sub>3</sub> ] <sup>-</sup> ; 473.1074 [M-H-C <sub>4</sub> H <sub>8</sub> O <sub>4</sub> ] <sup>-</sup> ; 383.0773 [M-H-C <sub>7</sub> H <sub>14</sub> O <sub>7</sub> ] <sup>-</sup>	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	Vicenin II	Pe, Pu, J, Pa
3	6	5.7	625.1741	-3.6	/	623.1624	2.7	533.1317 [M-H-C <sub>3</sub> H <sub>6</sub> O <sub>3</sub> ] <sup>-</sup> ; 403.1194 [M-H-C <sub>4</sub> H <sub>8</sub> O <sub>4</sub> ] <sup>-</sup> ; 413.0868 [M-H-C <sub>7</sub> H <sub>14</sub> O <sub>7</sub> ] <sup>-</sup>	C <sub>28</sub> H <sub>32</sub> O <sub>16</sub>	Diosmetin 6,8-di-C-glucoside	Pe, J, Pa
4	7	6.7	673.2434 <sup>b</sup>	-4.9	/	649.2514	3.6	605.2553 [M-H-CO <sub>2</sub> ] <sup>-</sup> ; 443.2121 [M-H-CO <sub>2</sub> -hexose] <sup>-</sup>	C <sub>32</sub> H <sub>42</sub> O <sub>14</sub>	Limonin-glucoside	Pe, Pu, J, Pa
5	7.6	7.7	563.1366	-4.2	/	561.1254	3.0	367.0667 [M-H-Ferulic acid] <sup>-</sup>	C <sub>26</sub> H <sub>26</sub> O <sub>14</sub>	Diferuloyl-glucaric acid ester	Pe, Pu, J, Pa
6		8.1	693.2687 <sup>b</sup>	-6.1	/	669.2776	3.4	609.2519 [M-H-CH <sub>3</sub> COOH] <sup>-</sup>	C <sub>32</sub> H <sub>46</sub> O <sub>15</sub>	Deacetyl nomilinic acid glucoside	Pe, Pu, J, Pa
7	8.8	8.9	581.1838	-4.6	/	579.1722	2.4	417.1549 [M-H-hexose] <sup>-</sup> ; 271.0606 [M-H-hexose-deoxyhexose] <sup>-</sup>	C <sub>27</sub> H <sub>32</sub> O <sub>14</sub>	Narirutin	Pe, Pu, J, Pa
8		8.6	435.1267	-4.2	/	433.114	2.5	271.0588 [M-H-hexose] <sup>-</sup>	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	Naringenin-glucoside	Pe, Pu, J, Pa
9		9.2	675.2598 <sup>b</sup>	-3.8	/	651.2666	2.9	591.2512 [M-H-CH <sub>3</sub> COOH] <sup>-</sup>	C <sub>32</sub> H <sub>44</sub> O <sub>14</sub>	Deacetyl nomilin glucoside	Pe, Pu, J, Pa
10	9.9	10	611.1941	-4.9	/	609.1831	2.7	301.0723 [M-H-hexose-deoxyhexose] <sup>-</sup>	C <sub>28</sub> H <sub>34</sub> O <sub>15</sub>	Hesperidin <sup>a</sup>	Pe, Pu, J, Pa
11	10.5	10.5	609.1786	-4.5	/	607.1647	-1.7	299.0562 [M-H-hexose-deoxyhexose] <sup>-</sup>	C <sub>28</sub> H <sub>32</sub> O <sub>15</sub>	Neodiosmin	Pe, Pa
12		9.8	465.1374	-3.7	/	463.1247	2.7	301.0693 [M-H-hexose] <sup>-</sup>	C <sub>22</sub> H <sub>24</sub> O <sub>11</sub>	Hesperetin glucoside	J
13	11	10.7	717.2701 <sup>b</sup>	-3.9	/	693.2767	2.1	633.2124 [M-H-CH <sub>3</sub> COOH] <sup>-</sup>	C <sub>34</sub> H <sub>46</sub> O <sub>15</sub>	Nomilin glucoside	Pu, J, Pa
14	11.4	11.3	735.2807 <sup>b</sup>	-3.7	/	711.2881	3.1	651.2714 [M-H-CH <sub>3</sub> COOH] <sup>-</sup> ; 607.2812 [M-H-CH <sub>3</sub> COOH-CO <sub>2</sub> ] <sup>-</sup>	C <sub>34</sub> H <sub>48</sub> O <sub>16</sub>	Nomilinic acid glucoside	Pu, J, Pa
15		11.7	657.2493 <sup>b</sup>	-3.7	/	633.2556	2.3	589.2312 [M-H-CO <sub>2</sub> ] <sup>-</sup> ; 471.2124 [M-H-CO <sub>2</sub> -hexose] <sup>-</sup>	C <sub>32</sub> H <sub>42</sub> O <sub>13</sub>	Obacunone glucoside	Pu, J, Pa

16	13	13.1	595.1994	-4.6	/	593.1877	2.0	285.0769 [M-H-hexose-deoxyhexose] <sup>b</sup>	C <sub>28</sub> H <sub>34</sub> O <sub>14</sub>	Didymin	Pe, Pu, J, Pa
17		13.7			/	271.0613	10.5	/	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	Naringenin <sup>a</sup>	Pe, Pa
18		14.7			/	301.0719	10.5	/	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>	Hesperetin <sup>a</sup>	Pe, Pa
19	17	16.9	373.1269	-3.3	358.1033 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 343.0802 [M+H-2CH <sub>3</sub> ] <sup>+</sup> ; 329.1010 [M+H-CO-H <sub>2</sub> O] <sup>+</sup> ; 312.0985 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>20</sub> H <sub>20</sub> O <sub>7</sub>	Sinensetin isomer	Pe, Pa
20		17.2	403.1376	-2.8	388.1135 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 373.0905 [M+H-2CH <sub>3</sub> ] <sup>+</sup> ; 342.1093 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>21</sub> H <sub>22</sub> O <sub>8</sub>	Hexamethoxyflavone isomer	Pe, Pa
21	17.7	17.6	471.1995	-3.9	/	515.1924 <sup>c</sup>	12.5	/	C <sub>27</sub> H <sub>30</sub> O <sub>10</sub>	Limonin <sup>a</sup>	Pe, Pa
22	17.8	17.5	373.1269	-3.3	358.1033 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 343.0802 [M+H-2CH <sub>3</sub> ] <sup>+</sup> ; 329.1010 [M+H-CO-H <sub>2</sub> O] <sup>+</sup> ; 312.0985 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>20</sub> H <sub>20</sub> O <sub>7</sub>	Sinensetin	Pe, Pa
23	18.1	17.9	403.1371	-4.1	388.1135 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 373.0905 [M+H-2CH <sub>3</sub> ] <sup>+</sup> ; 342.1093 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>21</sub> H <sub>22</sub> O <sub>8</sub>	3,5,6,7,3',4'-hexamethoxyflavone	Pe, Pa
24	18.4	18.2	403.1372	-3.7	388.1135 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 373.0905 [M+H-2CH <sub>3</sub> ] <sup>+</sup> ; 342.1093 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>21</sub> H <sub>22</sub> O <sub>8</sub>	Nobiletin	Pe, Pa
25	18.5	18.3	343.1165	-3.3	328.0932 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 299.0910 [M+H-CO-H <sub>2</sub> O] <sup>+</sup> ; 282.0881 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>	5,6,7,4'-tetramethoxyflavone	Pe, Pa
26	18.7	18.5	433.1476	-4	418.0522 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 389.0254 [M+H-CO-H <sub>2</sub> O] <sup>+</sup> ; 372.0415 [M+H-CH <sub>4</sub> -2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>22</sub> H <sub>24</sub> O <sub>9</sub>	3,5,6,7,8,3',4'-heptamethoxyflavone	Pe, Pa
27	19	18.9	373.1266	-4.3	358.1034 [M+H-CH <sub>3</sub> ] <sup>+</sup> ; 343.0802 [M+H-2CH <sub>3</sub> ] <sup>+</sup>		/		C <sub>20</sub> H <sub>20</sub> O <sub>7</sub>	Tangeretin	Pe, Pa

Abbreviations: Pe: peel; Pu: pulp; J: juice; Pa: pastazzo <sup>a</sup> Compared with reference standards. <sup>b</sup> *m/z* values corresponding to [M+Na]<sup>+</sup> <sup>c</sup> *m/z* values corresponding to [M-H+HCOOH]<sup>-</sup>.

**Table S1.** UHPLC-HRMS/MS data of compounds detected in peel, pulp, juice and pastazzo of “Ovale Calabrese”.