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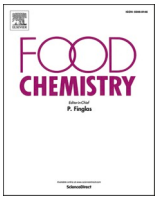

Targeted metabolic profiling of the revived ancient 'Corbella' olive cultivar during early maturation

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ABSTRACT

'Corbella' is an ancient olive cultivar whose cultivation has recently been revived and hence little is known about its composition. This is the first work studying the metabolic profile of 'Corbella' olives during early maturation. Olives with a ripening index (RI) < 1 yielded considerably less oil content (<40%) but had more concentration of phenolic compounds (148.41–219.70 mg/kg), carotenoids (9.61–14.94 mg/kg) and squalene (521.41–624.40 mg/kg). Contrarily, the levels of α-tocopherol were higher at the RI of 1.08 and 1.96 (64.57 and 57.75 mg/kg, respectively). The most abundant phenolic compound was oleuropein aglycone (>50% of the phenolic composition), suggesting a high hydrolytic activity of β-glucosidase in the fruit. The antioxidant capacity was barely affected, while oleic/linoleic ratio reached its highest at RI of 1.96. Therefore, olives with an RI below 2 could be good candidates to produce high-quality olive oils with good level of stability.

1. Introduction

Recently, ancient olive cultivars such as 'Corbella' have been revived and brought back into cultivation. 'Corbella' olive trees are originally from the Cardener Valley in the Bages and Solsonès districts but are now also grown in other areas of Catalonia (Spain). The olives have a medium size, half-moon shape, they are asymmetric and become totally black at the last stage of maturation. The stone is long and big with some rugosity. This cultivar produces a unique extra virgin olive oil (EVOO) with a pleasant sweet and fruity taste (Ninot et al., 2019), but when harvested at the reddish to black ripening stage the resulting oil is unstable and easily degraded.

The olive oil composition is mainly composed of triglycerides (97–99%) and minor compounds (1–3%), which are the principal responsible for its biological properties and sensory attributes. The most abundant fatty acids (FA) are oleic (55–85%), palmitic (7–20%), linoleic (2.5–21%), stearic (0.5–5%), palmitoleic (0.3–3.5%), and α-linolenic ($\leq$1%) (International Olive Council, 2022). The minor compounds include hydrocarbons (like squalene), tocopherols (like vitamin E), pigments (chlorophylls and carotenoids), aliphatic and aromatic alcohols, sterols, triterpene acids (like maslinic acid), volatile compounds, wax, and phenolic compounds (Boskou et al., 2006).

One of the factors affecting the oil composition is the olive cultivar. Therefore, the study of the olive fruit composition can give information about the oil. The various chemical processes taking place throughout olive maturation cause variations in the composition of the fruit (Conde et al., 2008). A ripening index (RI) has been defined (Uceda & Frías, 1975), which divides olives into 8 categories according to their skin and flesh color, ranging from 0 (deep green skin) to 7 (black skin color and purple flesh all the way to the stone). The optimal RI for a high-quality EVOO depends on the olive cultivar (Fernández-Poyatos et al., 2021; Kafkaletou et al., 2021; López-Yerena et al., 2021; Yorulmaz et al., 2013). In a previous study, where 'Corbella' EVOOs were produced using olives with a wide range of ripeness, those with a low RI yielded oil with a higher total phenolic content (López-Yerena et al., 2021). Based on that finding, and to differentiate the present study from previous research, we here decided to restrict the RI to values below 2.

Many studies have been conducted on the evolution of chemical

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parameters of olive oil during fruit ripening (Fernández-Poyatos et al., 2021; Kafkaletou et al., 2021; López-Yerena et al., 2021; Yorulmaz et al., 2013). However, this paper is focused on the evolution in the olive fruit. Phenolic compounds are probably the most investigated bioactive constituents of olives and olive oils, because of their antioxidant properties and health benefits (Rahman et al., 2021). Literature about the olive fruit have shown great variability in the phenolic content of olives, which can decrease or increase as the fruits ripen, depending on the cultivar (Fernández-Poyatos et al., 2021; Yorulmaz et al., 2013). Other powerful antioxidant components are carotenoids, which contribute to the color of the oil and decrease during maturation (Yorulmaz et al., 2013), and α-tocopherol, also known as vitamin E, whose evolution during ripening seems to depend on the cultivar (Yorulmaz et al., 2013). The antioxidant squalene, found in olive oil in high quantities, is valuable for its detoxifying, immunomodulatory, skin protective, and above all, chemopreventive and anticancer activity (Kim & Karadeniz, 2012). It is an important intermediate in the production of sterols and a precursor in cholesterol biosynthesis (Martínez-Beamonte et al., 2020), and it has been found that its level decreases significantly with ripeness (Martínez-Beamonte et al., 2020). The fatty acid (FA) composition of olives also changes during maturation, and again, different cultivars show different trends (Hernández et al., 2009, 2021; Menz & Vriesekoop, 2010). The FA composition and antioxidant levels play an important role in EVOO stability (Velasco & Dobarganes, 2002). The lower the content of unsaturated FAs and the higher the content of antioxidant compounds, especially phenolics and tocopherols, the more stable the oil.

As this cultivar has only recently been revived, just one previous study has information about the phenolic composition of 'Corbella' EVOOs (López-Yerena et al., 2021), but no information about the olive fruit composition can be found in the literature. Therefore, the aim of this work was to provide comprehensive data about the composition of this forgotten olive cultivar in the early stages of ripeness using a targeted metabolic approach and to envisage which RI could be more favorable for the production of a high quality EVOO with enhanced stability.

2. Material and methods

2.1. Reagents

n-Hexane, 0.5 N sodium methoxide, 14% boron trifluoride–methanol, Trolox, diphenyl-1-picryl-hydrazyl (DPPH), and Folin–Ciocalteu's reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA); acetic acid, formic acid, methanol, acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF), and tertbutylmethyleter (TBME) from Sigma-Aldrich (Madrid, Spain); and sodium chloride (NaCl) and sodium carbonate (Na_2CO_3) from Panreac Química SLU (Castellar del Vallès, Spain). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA).

Regarding the standards ($\geq$90% purity), oleocanthal was purchased from Merck (Darmstadt, Germany), and oleacein, oleuropein aglycone, and elenolic acid from Toronto Research Chemical Inc. (ON, Canada). Oleuropein, ligstroside, pinoresinol, gallic acid, vanillic acid, caffeic acid, verbascoside, rutin, lutein, β-carotene, squalene, and (α)-tocopherol were acquired from Sigma-Aldrich; apigenin, ferulic acid and *p*-coumaric from Fluka, and hydroxytyrosol from Extrasynthese (Genay, France). Methyl tridecanoate (C13:0), used as a standard for the analysis of FAs, was acquired from Sigma-Aldrich.

2.2. Olive samples

The olive orchard is in Valls de Torroella (Barcelona, Spain) which is sited at latitude 41°52′12.9″N and longitude 1°44′35.9″E with 400 m altitude and 87 km from Barcelona. It consists of 450 olive trees auto rooted of an average age of 20 years old. The plantation frame is 6 × 7 m. The soil has green manure and a loamy texture. Alga, potassium, nitrogen, and phosphor are used as fertilizers 3 or 4 times a year, and copper as pest treatment. Drip irrigation is supplied at 18,000 L/h. The climatic data of the year of harvesting (2021) can be found in the Supplementary material (Table S1). Information about the monthly average temperature, accumulated precipitation, relative humidity, and solar irradiance is given. The olives for this study were collected over five weeks, from September 20 to October 19, 2021 (Table S2), with an RI ranging from 0 to 2. Two high yielding 'Corbella' olive trees of the orchard were selected. Every week, two replicate samples of 2 kg of olives were harvested and immediately sent to the IRTA-Mas Bové Center to be processed. The physical characterization of olives was carried out within 24 h of harvesting. Olives were stored at − 80 °C until chemical analysis, prior to which they were ground to a powder using an IKA® A11 basic mill (IKA®-Werke GmbH & Co., Staufen, Germany) with liquid nitrogen and stored at − 80 °C. The analyses were performed from February 2022 to June 2022.

2.3. Physical characterization of the olives

Following the methodology described in Uceda & Frías (1975), the olive RI was determined according to the color of the olive skin and mesocarp, and by calculating the weighted average number of fruits in each category (from 0 to 7) from a sample of 50 olives. The weight (g) of the fruit, the mesocarp and the stone were determined with a laboratory balance, and the mesocarp/stone ratio was calculated by dividing the weight of the mesocarp and the stone. The oil content (% dry matter) was assessed in powdered fruits using the Soxhlet methodology (International Organization for Standardization, 2009) and a VELP device model SER158 (VELP Scientifica Srl – HQ, Usmate, Italy), with hexane as the solvent.

2.4. Extraction, identification, and quantification of olive phenolic compounds

2.4.1. Extraction of the phenolic fraction

Phenolic compounds underwent a liquid–liquid extraction, as described in López-Yerena et al. (2021), with minor modifications. 1 g of a powdered olive sample was weighed in a 10 mL tube and 2 mL of methanol:water (8:2) was added. After stirring for 3 min, the samples were ultrasonicated in an ice bath for 10 min. Then, 1 mL of hexane was added followed by 3 min of stirring. After centrifuging the samples at 2760 rcf for 20 min at 4 °C, the methanol phase with the polyphenols was separated in a new 10 mL tube, and washed with 1 mL of hexane. The tube with the hexane phase was treated again with 2 mL of methanol:water (8:2). Both tubes were stirred for 1 min and centrifuged as before. Finally, all methanol phases were collected in a new tube after filtration at 0.22 μm and evaporated under reduced pressure (miVac DNA concentrator, Genevac™, Ipswich, UK). The phenolic extracts were reconstituted with 800 μL of ACN, filtered again at 0.22 μm into amber vials, and stored at − 80 °C until analysis.

2.4.2. Identification of phenolic compounds

An exhaustive characterization of phenolic compounds was performed by liquid chromatography coupled to high-resolution mass spectrometry (LC-LTQ-Orbitrap-MS). An Accela chromatograph (Thermo Scientific, Hemel Hempstead, UK) equipped with a quaternary pump, and a thermostated auto-sampler (established at 4 °C) was employed. For chromatographic separation, a BEH C18 column (50 mm × 2.1 mm) i.d., 1.7 μm (Milford, MA, United States), maintained at 30 °C, pumped at a flow-rate of 400 μL/min and with an injection volume of 5 μL, was used. The mobile phase consisted of an A phase of water (0.1% formic acid) and a B phase of acetonitrile (0.1% formic acid). The gradient conditions applied were: 0–5% B (0–2 min); 18% B (2–15 min); 100% B (15–26 min), followed by a decrease of B to 5% (maintained for one min). Finally, the starting condition was re-established and

maintained for 2 min to re-equilibrate the column (total run time: 30 min).

For the MS analysis, the LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK), equipped with an electrospray ionization source, was operated in negative mode. The parameters were as follows: source voltage, 3 kV; sheath gas, 50 a.u. (arbitrary units); auxiliary gas, 20 a.u.; and sweep gas, 2 a.u. During the analysis, the capillary temperature was 375 °C. A preliminary analysis of 5 μL of olive fruit extract was carried out in Fourier transform mass spectrometry (FTMS) mode at a resolving power of 30,000 at *m/z* 900, and data-dependent MS/MS events were collected with a resolving power of 15.000 at *m/z* 900. The most intense ions detected in the FTMS spectrum were selected for the data-dependent scan. Parent ions were fragmented by high-energy collisional dissociation with normalized collision energy of 35 a.u.. Data processing and instrument control were performed with Xcalibur 3.0 software (Thermo Fisher Scientific Inc., Waltham, MA, USA). Phenolic compounds were identified using a commercial standard or, if no reference standard was available, identification was based on the chemical composition and MS/MS fragmentation pattern, carried out as before.

2.4.3. Quantification of phenolic compounds

Phenolic compound quantification was carried out by liquid chromatography coupled to mass spectrometry in tandem mode (LC-MS/MS). An Acquity TM UPLC (Waters; Milford, MA, USA) coupled to an API 3000 triple-quadrupole mass spectrometer (PE Sciex, Concord, ON, Canada) with a turbo ion spray source was used, employing an Acquity UPLC® BEH C18 column (2.1 × 50 mm, i.d., 1.7 μm particle size) and Acquity UPLC® BEH C18 Pre-Column (2.1 × 5 mm, i.d., 1.7 μm particle size) (Waters Corporation®, Wexford, Ireland).

Three identification methods were used for the quantification: method (a) for the major secoiridoids (oleacein, oleocanthal, and ligstroside and oleuropein aglycones) (Lozano-Castellón et al., 2021), and for other phenolic compounds methods (b) (López-Yerena et al., 2021), and (c) (Moreno-González, Juan, & Planas, 2020; Rinaldi de Alvarenga et al., 2019). In method (a), the mobile phases were methanol (A) and water (B), both with 0.1% formic acid. An increasing linear gradient (v/v) of A was used (t (min), %A): (0, 5); (2, 5); (4, 100); (5, 100); (5.50, 5); (6.5, 5). In method (b), the mobile phases were ACN (A) and water with 0.05% acetic acid (B). An increasing linear gradient (v/v) of A was used (t (min), %A): (0, 2); (2, 5); (7.5, 40); (7.6, 100); (8.5, 100); (8.6, 5); (9, 2), (10, 2). In method (c), the mobile phases were methanol (A) and water (B), both with 0.1% formic acid. An increasing linear gradient (v/v) of A was used (t (min), %A): (0, 5); (2, 25); (2.2, 25); (2.4, 75); (2.6, 100); (4, 100); (4.1, 75); (4.2, 5); (5, 5). The three methods had a constant flow rate of 0.6 mL/min, an injection volume of 5 μL, and the temperature of the column was 50 °C.

Ionization in negative mode was performed using electrospray ionization, and all the compounds were monitored in multiple monitoring mode using the settings described in López-Yerena et al. (2021), Lozano-Castellón et al. (2021), Moreno-González, Juan, & Planas (2020), and Rinaldi de Alvarenga et al. (2019) for methods a, b and c, respectively. The system was controlled by Analyst version 1.4.2 supplied by ABSciex, and the chromatograms were integrated using the same software.

The quantification was done with a calibration curve using the following standards: apigenin, hydroxytyrosol, vanillic acid, *p*-coumaric acid, pinoresinol, oleuropein, ligstroside, oleocanthal, oleacein, oleuropein aglycone, elenolic acid, ferulic acid, verbascoside, 4-hydroxybenzoic acid, caffeic acid, and rutin. Compounds without a corresponding commercial standard were quantified using standards of phenolic compounds with a similar chemical structure.

2.5. Extraction and determination of the total phenolic content (TPC) and antioxidant capacity (DPPH free radical scavenging assay) of the olives

For the extraction, 0.5 g of olive fruit powder was dissolved in 1 mL of hexane in a 10 mL centrifuge tube and shaken for 3 min. Then, 2 mL of methanol:H_2O (8:2) was added, the samples were shaken again for 3 min, and the two phases were separated by centrifugation at 2760 rcf and 4 °C for 20 min. The methanolic fraction was collected in another centrifuge tube and underwent a second cleaning with 1 mL of hexane, whereas the hexane fraction was again treated with 2 mL of methanol:H_2O (8:2) to recover the remaining phenolic compounds. All tubes were shaken for 1 min and centrifuged at 2760 rcf and 4 °C for 20 min. The methanolic phases were recovered together and stored at − 20 °C until the TPC and DPPH analysis.

The TPC was determined based on the Folin–Ciocalteu procedure (Singleton et al., 1999) with minor modifications, the results being expressed as μg of gallic acid equivalents (GAE) per mg of fruit. In a 96-well microplate, 30 μL of extract was added to 150 μL of Folin-Ciocalteu's reagent (1:10, v/v) and 120 μL of 7.5% (w/v) Na_2CO_3 solution in each well. The microplate was incubated at 45 °C for 15 min and then placed at room temperature in the dark for 30 min. The absorbance was read at 765 nm. Gallic acid was used as the standard to plot the calibration curve (linearity range = 5–80 ppm, $R^2 > 0.973$).

The DPPH radical scavenging activity of extracts was evaluated based on the reduction of the DPPH• radical by antioxidants, according to Pinto et al. (2021), with minor modifications. In a 96-well microplate, 30 μL of extract and 270 μL of an ethanolic solution containing DPPH (6×10^5 M) were added to each well. The microplate was incubated at room temperature for 40 min and absorbance was measured at 525 nm. Trolox was used as the standard to prepare a calibration curve (linearity range: 5–100 μg/mL, $R^2 > 0.927$), and results were expressed as μg of Trolox equivalents (TE) per mg of fruit.

2.6. Extraction and determination of olive fatty acid composition

For FA extraction and determination, the method for FA methyl esters (FAME) described in Olmo-Cunillera et al. (2022) was followed, weighting 100 mg of a powdered olive sample. Fast GC analyses were performed on a Shimadzu GC-2010 Gas Chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Shimadzu AOC-20i Autoinjector. Separation of FAME was carried out on a capillary column (40 cm × 0.18 mm i.d. × 0.1 μm film thickness) coated with an RTX-2330 stationary phase of 10% cyanopropyl phenyl − 90% biscyanopropyl polysiloxane from Restek (Bellefonte, USA). The operating conditions of the GC and the equation used for calculating the FA concentration and percentage are detailed in Olmo-Cunillera et al. (2022).

2.7. Extraction and determination of olive carotenoids, α-tocopherol, and squalene

The extraction of carotenoids (lutein and *β*-carotene), *α*-tocopherol (vitamin E), and squalene was done as in Martakos et al. (2021), with minor modifications. 0.5 g of powdered olive sample was weighed in a 10 mL tube and 2 mL of DMF was added. After stirring for 4 min, followed by ultrasonication with ice for 10 min, the samples were centrifuged at 4312 rcf for 20 min at 4 °C. The liquid was transferred into a volumetric flask, and a second extraction of the solid fraction was carried out with 2 mL of hexane. The samples were stirred for 4 min and centrifuged as before. The hexane was collected in the same tube as the DMF and evaporated at 80 °C using a rotary evaporator system with dried ice refrigeration. Finally, the samples were reconstituted with 800 μL of TBME and filtered at 0.22 μm before storing in amber vials at − 20 °C until analysis.

The compounds were determined by LC, using an Acquity TM UPLC coupled to a photodiode detector (PDA) (Waters Corporation®; Milford,

MA, USA). The column was an YMCTM C30 (250 × 4.6 mm, i.d., 5 μm particle size) (Waters Corporation®, Milford, MA, USA). The mobile phases were TBME:methanol (8:2 v/v) (A) and methanol (B). An increasing linear gradient (v/v) of A was used (t (min), %A) as follows: (0, 10); (10, 25); (20, 50); (25, 70); (35, 90); (43, 94); (45, 19); (55, 10). The method had a constant flow rate of 0.6 mL/min, and an injection volume of 10 μL. The absorbance was measured at 450 nm for carotenoids (lutein and β-carotene) and at 210 nm for α-tocopherol and squalene.

For the quantification of each compound, a calibration curve of the corresponding commercial standard was employed (lutein, β-carotene, α-tocopherol and squalene).

2.8. Statistical and multivariate analyses

For every RI there were two batches of olive samples. All the analyses were done in triplicate for every olive sample batch. Results are expressed on a fresh weight basis, except for the oil content, which is expressed on a dry weight basis. Statgraphics Centurion 18 software, version 18.1.13 (Statgraphics Technologies, Inc., The Plains, Virginia, USA), and RStudio, version 2022.02.3 Build 492 (Posit® PBC, Boston, MA, USA), were used to perform the statistical analysis. First, the normality of data and the homogeneity of variance were tested by Saphiro-Wilk test and Levene's test, respectively. An analysis of variance of one factor (one-way ANOVA) with a Tukey test was applied to evaluate the effect of the RI on the olive samples when the assumptions of normality and homogeneity of variance were met ($p \geq 0.05$). If any of these assumptions was not met ($p < 0.05$), a nonparametric statistical test was applied (Kruskal-Wallis with Bonferroni test).

Additionally, multivariate analysis was performed with all the data collected in the present study, using the software SIMCA 13.0.3.0 (Umetrics, Umeå, Sweden). Phenolic compounds were grouped by classes (secoiridoids, phenolic acids, phenolic alcohols, and flavonoids), and only the most meaningful compounds were included in the figures individually. First, an unsupervised approach, specifically a principal component analysis (PCA), showed that the samples could be separated by their RI. Then, supervised analysis, specifically the orthogonal projections to latent structures–discriminant analysis (OPLS-DA) model, was conducted in order to find the discriminating variables that separated the olive samples according to their RI. The olive samples were distributed on the X-axis according to the RI (0, 0.36, 0.66, 1.08 and 1.96). The quality and reliability of the model were assessed by the following parameters. R^2Y (explained variation) was 0.940, which referred to the goodness of fit (how well the data of the training set can be mathematically reproduced) and Q^2 (predicted variation) was 0.767, which referred to the predictive power of the model. Additionally, to assess the reliability of the OPLS-DA model, a cross-validated ANOVA (CV-ANOVA) was performed, and a p-value of < 0.01 was obtained, indicating that it was a significant model. The permutation test (200 permutations) was carried out to exclude overfitting. Hotelling's T2 and DModX were performed to identify strong and moderate outliers, and none were found.

Furthermore, variable importance in the projection (VIP) values of $>$ 1 were accepted as the most influential for the model and compared with their coefficient values. Coefficient values of > 0 and < 0 express how strongly variables are positively and negatively correlated with the X classes (RI), respectively, as long as their confidence interval does not include zero.

3. Results and discussion

3.1. Physical characterization of 'Corbella' olives during early maturation

The harvested olives were classified into 5 groups according to their RI: 0.00, 0.36, 0.66, 1.08 and 1.96. The oil content ranged between 31 and 46.2% dry matter (Table S2), and increased with the RI, as in other studies (Menz & Vriesekoop, 2010). The weight (g) of the fruit, the mesocarp, and the stone, as well as the mesocarp/stone ratio is shown in Table S2. There was a clear increasing tendency in the mesocarp/stone ratio as the olives ripened (Fig. 1B), as it has been reported for other cultivars (Emmanouilidou et al., 2020; Ivancic et al., 2022). This agrees with the fact that the mesocarp develops and gains weight during the maturation process due to cell division, expansion, and accumulation of storage components, such as oil (Conde et al., 2008; Hammami et al., 2011). The oil accumulation reaches its maximum when the fruit is completely developed, a little bit before its skin color changes from green to yellowish green (RI = 1) (Del Río & Caballero, 2008), which coincides with our results (Fig. 1A). Morelló et al. (2004) also observed a slight rise of the oil content between olive samples with an RI = 0 and an RI = 1 in 'Arbequina', 'Farga', and 'Morrut' cultivars, and it remained practically unchanged at higher RI. The same pattern has also been observed in other cultivars (Emmanouilidou et al., 2020).

The results indicated that harvesting 'Corbella' olives with an RI below 1 might entail a considerable loss of oil yield, especially below 0.50 (Fig. 1A), whereas a maximum oil yield could be achieved with an RI between 1 and 2. The yield is expected to plateau at a higher RI, as an average oil content of about 43.7% ± 1.8 (dry basis) is described for this cultivar (Tous & Romero, 2004).

3.2. Phenolic composition and content in 'Corbella' olives during early maturation

A total of 55 phenolic compounds were identified by LTQ-Orbitrap-MS (Table S3). Half of the compounds were secoiridoids, reflecting the large size and complexity of this phenolic group in *Olea europaea*. A type of terpenoid derived from iridoids, secoiridoids are abundant in the *Oleacea* family and other plants (Obied et al., 2008), but oleoside secoiridoids are restricted to the *Oleacea*. This group of compounds, which include oleuropein, ligstroside, elenolic acid and all their derivatives, possess the oleoside nucleus, a combination of elenolic acid and a glucosidic residue (Soler-Rivas et al., 2000).

An exhaustive search of the literature revealed that some of the phenolic compounds identified in 'Corbella' olives have been extensively reported in other cultivars (Fayek et al., 2021; Kanakis et al., 2013; Martakos et al., 2021; Obied et al., 2008; Olmo-García et al., 2018). In contrast, other 'Corbella' phenolic compounds have been scarcely reported, such as oleoside methylester, also known as elenolic acid glucoside, which is the precursor of oleuropein and is formed by the action of esterases (Gutierrez-Rosales et al., 2010), and nuzhenide and salidroside, both specific to olive seeds (Obied et al., 2008).

Finally, the following phenolic compounds identified in 'Corbella' olives were also recently determined in a study of 'Picual', 'Manzanillo', 'Koroneiki' and 'Coratina' cultivars (Fayek et al., 2021): oleuropein-sinapinic acid, hydroxy-methyl-dihydrooleuropein aglycone, dihydrooleuropein aglycone, hydroligstroside aglycone, acyclodihydroelenolic acid, acyclodihydroelenolic acid hexoside derivative, dihydrooleoside dimethylester, oleoside-*O*-(hydroxycinnamoyl), and oleoside-*O*-(hydroxydimethyloctenoyl) (secoiridoids); dihydroxyphenyl glycerol methyl ether (simple phenol); and apigenin rutinoside and trihydroxy-dimethoxyflavan (flavonoids). Not all the phenolic compounds identified were present in each of the four studied cultivars; for example, oleuropein-sinapinic acid and trihydroxy-dimethoxyflavan were only found in 'Picual', and hydroligstroside aglycone in 'Manzanillo'. Other cultivar-dependent phenolics are demethyloleuropein and verbascoside (Obied et al., 2008), only the latter being detected in 'Corbella' olives.

LC-MS/MS quantification of 23 of the 55 identified phenolic compounds revealed that the olive phenolic content was affected by the RI (Table S4), as expected. The total amount of phenolic compounds decreased as the RI increased (depletion of 77.67%) (Fig. 2A). This trend was also observed for secoiridoids, the predominant phenolic group (depletion of 78.65%) (Fig. 2A), as well as phenolic alcohols and acids

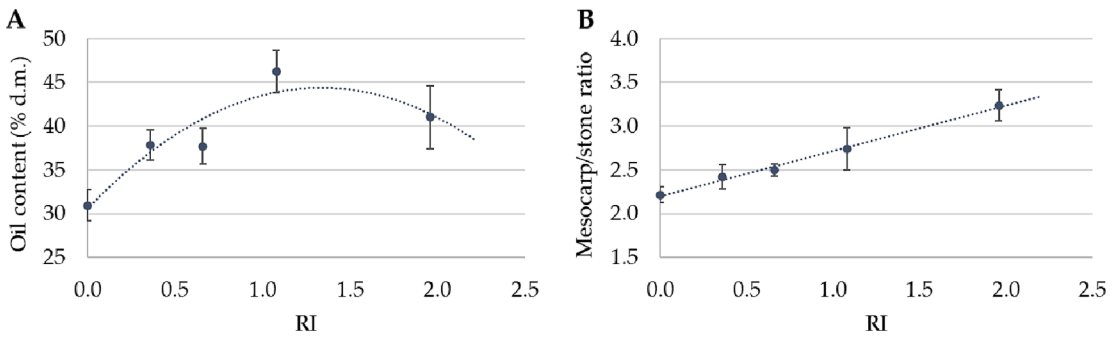

Fig. 1. Relationship between the oil content (% dry matter) and the RI (A), and the mesocarp/stone ratio and the RI (B).

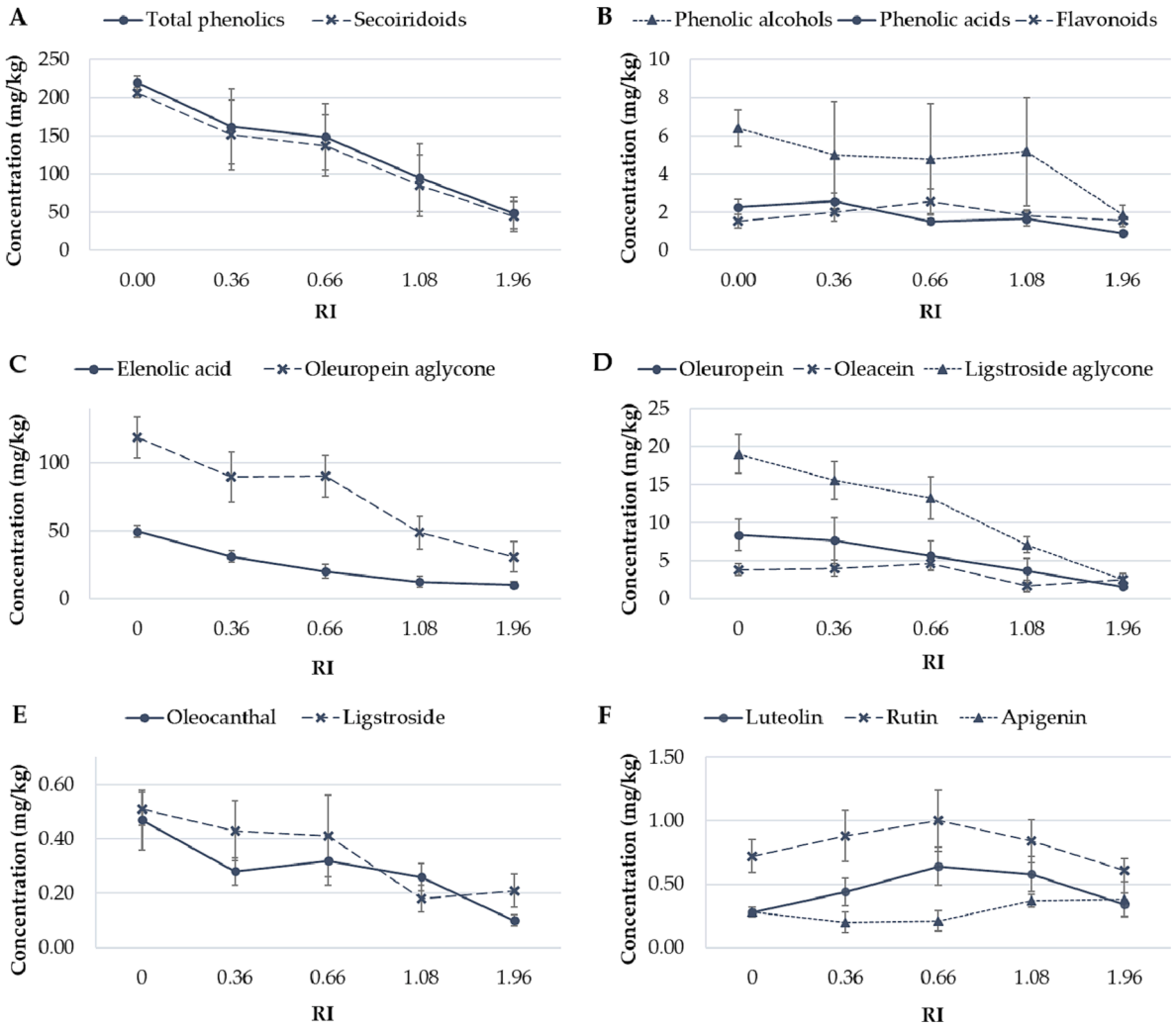

Fig. 2. Evolution of the concentrations (mg/kg) with the ripening index (RI) of the total amount of phenolics and secoiridoids (A); phenolic alcohols, phenolic acids and flavonoids (B); elenolic acid and oleuropein aglycone (C); oleuropein, oleacein and ligstroside aglycone (D); oleocanthal and ligstroside (E); and luteolin, rutin and apigenin (F).

(Fig. 2B). In contrast, the flavonoid concentration increased until an RI of 0.66, decreasing thereafter (Fig. 2B). These results are in accordance with López-Yerena et al. (2021), who analyzed 'Corbella' EVOOs produced with olives at different stages of maturation and found that the phenolic concentration was negatively affected by a higher RI.

The reduction in phenolic content during maturation shows different patterns, depending on the cultivar (Fernández-Poyatos et al., 2021; Gutierrez-Rosales et al., 2010; López-Yerena et al., 2021), sometimes starting at the very beginning of the process, as occurs in 'Corbella' olives. Gutierrez-Rosales et al. (2010) reported that active phenol synthesis takes place mainly in young fruits. Thus, once the initial massive synthesis is complete, the biosynthetic capacity declines, and the phenolic content drops due to a lack of precursors and the activity of endogenous enzymes.

The major group of phenolic compounds in olives and olive oil generally are the secoiridoids, among which oleuropein, oleacein, and oleocanthal are the most important for oil quality and health benefits. The rate of secoiridoid biosynthesis depends not only on the cultivar but

also on climatic and environmental factors (Gutierrez-Rosales et al., 2012). Although oleuropein is usually the predominant phenolic compound in olive fruit (Yorulmaz et al., 2013), its concentration depends on its anabolic and catabolic biosynthetic pathways and the cultivar (Ranalli et al., 2009), so other phenols can occur in higher amounts (Fernández-Poyatos et al., 2021; Gutierrez-Rosales et al., 2010; Kanakis et al., 2013; Martakos et al., 2021). This is the case with comselogoside in 'Cornezuelo' or oleuropein aglycone and oleacein in 'Hojiblanca' and 'Arbequina' cultivars. In 'Corbella' olives, the major phenolic compound detected was oleuropein aglycone, a hydrolytic product of oleuropein (Domínguez-López et al., 2021; Gutierrez-Rosales et al., 2010) (Table S4). The predominance of this derivative could be attributed to a high activity of the hydrolytic enzyme β-glucosidase, which transforms oleuropein to its aglycone (Gutierrez-Rosales et al., 2010; Obied et al., 2008), and to a low activity of the methylesterase that converts oleuropein aglycone to oleacein (Volk et al., 2019). Second in abundance was elenolic acid, a nonphenolic compound that constitutes the iridoid part of the secoiridoids, thought to be formed in the olive fruit from oleoside-11-methyl ester, the precursor of oleuropein, also by the intervention of β-glucosidase (Gutierrez-Rosales et al., 2010). Another indicator of high β-glucosidase activity is the lower concentration of ligstroside compared to its aglycone form (Gutierrez-Rosales et al., 2010), whereas the low concentration of oleocanthal suggests low methylesterase activity (Volk et al., 2019). As ligstroside is reported to be a precursor of oleuropein (Gutierrez-Rosales et al., 2010), its relatively low levels are in accordance with the higher concentration of the latter.

Monitoring the activity of β-glucosidase in 'Arbequina' and 'Hojiblanca' olives, Gutierrez-Rosales et al. (2010) found its peak coincided with the highest content of oleuropein aglycone, which occurred in late July for both cultivars. Throughout October and November, the activity was very low, as was the content of oleuropein aglycone, ligstroside aglycone and elenolic acid. If the same pattern occurred in 'Corbella' olives, it could explain the decrease of these β-glucosidase products. The enzymatic activity of olives harvested with the same RI can differ greatly between cultivars (Ramírez et al., 2014); for example, a high activity of polyphenol oxidase, β-glucosidase, and esterase was found in 'Gordal' but not in 'Hojiblanca'. Therefore, considering that oleuropein aglycone and elenolic acid were the most abundant phenolic compounds in 'Corbella' olives, a high hydrolytic activity may be assumed, which could partially explain why 'Corbella' EVOO is less stable than other oils.

The content of oleuropein, oleuropein aglycone and ligstroside aglycone decreased as the RI increased (depletion of 66.03%, 74.06% and 86.44%, respectively) (Table S4, Fig. 2C and 2D), as reported in other studies (Cardoso et al., 2006; Martakos et al., 2021; Yorulmaz et al., 2013). As mentioned, this could be associated with enzymatic generation but also with transformation into other derivatives (Gutierrez-Rosales et al., 2010; Obied et al., 2008); for example, a study found that oleuropein can form oligomers (Cardoso et al., 2006). In 'Corbella' olives, the oleuropein concentration did not differ significantly at RIs between 0 and 0.66, suggesting an equilibrium between its catabolism and anabolism. The depletion of oleuropein aglycone and ligstroside aglycone could be also related to their mobilization in other anabolic routes toward other biosynthetic intermediates (Gutierrez-Rosales et al., 2010).

Elenolic acid, a secoiridoid degradation product (Domínguez-López et al., 2021), decreased significantly from an RI of 0 to 0.66 and then remained constant (depletion of 79.47%) (Fig. 2C). This depletion could be attributed to a low hydrolytic degradation of secoiridoids, which remained constant during this period of maturation, and only started to decline significantly at an RI > 1, when the elenolic acid concentration was stable, indicating an increase in hydrolytic activity.

Hydroxytyrosol, the main phenolic alcohol quantified, did not differ significantly between samples. As this compound is both a precursor and hydrolytic product of the secoiridoid pathway (Domínguez-López et al., 2021), this constancy could be explained by its simultaneous use to form secoiridoids and generation by hydrolysis. In reports in the literature, the hydroxytyrosol content in olives mostly increases with maturation (Kafkaletou et al., 2021) due to secoiridoid hydrolysis, although in other cultivars it decreases (Damak et al., 2008). It is noteworthy that most of this research has been performed with olives at a wider range of ripeness than in the present study, where the RI was restricted to below 2. Within such a limited maturation phase and harvesting time, differences are less likely to be significant.

A higher content of oleacein than oleocanthal was found in 'Corbella' olives, in accordance with studies of other cultivars (Kanakis et al., 2013; Martakos et al., 2021). Oleacein was constant until an RI of 1.08, when it started to decrease, whereas oleocanthal levels were already diminishing at an RI of 0.36, remaining unchanged until 1.08 and decreasing again (Fig. 2D and 2E). The enzymatic activity that forms these two secoiridoids mainly occurs during oil production, when cell breakage favors interaction between enzymes and their substrates (Domínguez-López et al., 2021).

Verbascoside levels remained unchanged from an RI of 0 to 1.08 and decreased from 1.08 to 1.96, a trend observed elsewhere (Kafkaletou et al., 2021). The main flavonoid was rutin, as reported in other olive cultivars (Yorulmaz et al., 2013). All the flavonoids in 'Corbella' olives decreased during maturation after an initial increase, except for apigenin which remained constant in all the RI studied ($p < 0.05$) even though its trend was to increase (Fig. 2F). A similar pattern has been reported for other cultivars (Fernández-Poyatos et al., 2021), as well as in 'Corbella' EVOO. This early increase may be attributed to the activity of PAL, a crucial enzyme in the formation of flavonoids (Ortega-García & Peragón, 2009). A study conducted with 'Koroneiki' cultivar described only a decreasing trend (Kafkaletou et al., 2021). However, the initial RI was 0.9 which is higher than ours (0). Interestingly, the initial decreasing point observed in our study (from RI 0.66 to 1.08) does not disagree with those results.

The contribution of the phenolic compounds to human health is well known (Rahman et al., 2021), and food rich in these strong antioxidants is highly appreciated. Therefore, one of the fields of interest of olive oil research is the obtention of EVOO with high content of phenolics. The European Food Safety Authority (EFSA) authorized a health claim for olive oil containing at least 250 mg/kg of hydroxytyrosol or derivatives (oleuropein complex and tyrosol, i.e., the secoiridoid group and its derivatives) (European Commission, 2012). The claim states that "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress" with a daily intake of 20 g of olive oil. Additionally, oleocanthal and oleacein are two secoiridoids with promising health properties (Lozano-Castellón et al., 2019). Therefore, from a health point of view, 'Corbella' olives with an RI below 1 are the best to obtain EVOOs with high content of phenolics, especially, secoiridoids.

3.3. TPC and antioxidant capacity of 'Corbella' olives during early maturation

When analyzing the TPC by the HPLC-MS/MS method, it was found to decrease with the RI, whereas no significant differences were found between maturation stages when using Folin Ciocalteu analysis (Table S5). An explanation for this discrepancy is that HPLC-MS/MS is used to quantify specific phenolic compounds, whereas the reductive Folin Ciocalteu reaction estimates the content of a wide range of phenolics and is also affected by non-phenolic compounds (Ainsworth & Gillespie, 2007). Variable results have been reported for the evolution of TPC during olive ripening (Fernandez-Orozco et al., 2011), with significant differences observed between stages in studies of a longer maturation phase than here.

The antioxidant capacity of the olives did not change except at the highest RI (1.96), when it was significantly lower (Table S5), despite no significant decrease in the TPC at this ripening stage. Both parameters are closely correlated in olives (Fernandez-Orozco et al., 2011), but individual phenolics vary in their antioxidant properties. *o*-Diphenols

(hydroxytyrosol, oleuropein and their derivatives) are reported to have higher antioxidant activity (Velasco & Dobarganes, 2002), an in the 'Corbella' olives lower content of these phenolics are found at an RI of 1.96, possibly contributing to the decrease in antioxidant capacity. In this study the DPPH assay was applied in the olive fruit. Nevertheless, DPPH assay is very commonly applied to study the antioxidant capacity of olive oil. A positive correlation has been found between the radical scavenging activity measured as DPPH and the TPC in olive oil of different geographical origins, while a negative correlation has been described with maturation (Giuffre, 2018; Hmida et al., 2022). Hmida et al. (2022) also found a positive correlation between the antioxidant activity and the *o*-diphenol and flavonoid content during ripening. Controversially, although the TPC decreased with maturation in the 'Chondrolia Chalkidikis' Greek cultivar, the antioxidant capacity was not correlated, suggesting the implication of content and synergism of individual phenols and/or other constituents (such as tocopherols and pigments) (Psathas et al., 2022).

Considering these results, it could be stated that during the maturation process the TPC and antioxidant capacity of the 'Corbella' cultivar decrease, hence harvesting 'Corbella' olives with an RI below 2 could be the optimum maturation stage to have more TPC and antioxidant capacity.

3.4. Fatty acid profile of 'Corbella' olives during early maturation

The FA profile of the olive samples was the same regardless of the RI. Oleic acid (C18:1n-9) was the main FA (72.72 to 79.20%), followed by palmitic (C16:0) (10.46 – 12.61%), linoleic (C18:2n-6) (6.25 – 10.81%), stearic (C18:0) (1.77 – 1.91%), α-linolenic (C18:3n-3) (0.63 – 0.73%), 9-palmitoleic (C16:1n-7) (0.45 – 0.65%), arachidic (C20:0) (0.27 – 0.31%), gondoic (C20:1n-9) (0.22 – 0.23%), and behenic (C22:0) (0.09 – 0.11%) acids. The rest of the FAs had a composition percentage of $<$ 0.10%. If these percentages were maintained in 'Corbella' EVOO, the FA composition would be similar to the EVOO produced from the Catalan cultivars 'Arbequina', 'Argudell', 'Empletre', 'Morrut' and 'Sevillenca', whereas it would have a lower content of oleic acid and a higher content of linoleic acid than 'Farga' EVOO (Tous & Romero, 2004). The percentage of palmitic acid in 'Corbella' olives is lower than in 'Gordal Sevillana' cultivar, whereas the percentage of oleic and α-linolenic acids is higher (Menz & Vriesekoop, 2010).

The ripening stages studied apparently did not significantly affect the content of most individual FAs (Table S6), including the main FA, oleic acid, as well as stearic, arachidic, gondoic, behenic and most of the minor acids. In contrast, myristic (C14:0), pentadecanoic (C15:0), pentadecenoic (C15:1), and 7-palmitoleic (C16:1n-9) acids increased significantly with the RI. The concentration of palmitic, 9-palmitoleic, heptadecenoic (C17:1), linoleic and α-linolenic acids was constant from an RI of 0 to 0.66, increasing from 0.66 to 1.08, and then decreasing from 1.08 to 1.96. The same pattern was observed for saturated FAs and PUFA. Menz and Vriesekoop (2010) monitored the entire maturation process in the 'Gordal Sevillana' cultivar, and observed a decrease in palmitic and oleic acid, and an increase in α-linolenic acid. A study on the cultivars 'Klon-14′ and 'Abou Kanani' reported an increase of oleic, linoleic and α-linolenic acid during ripening (Hernández et al., 2021). In 'Arbequina' olives, the content of linoleic acid initially increased and then decreased, and α-linolenic acid decreased, while in 'Picual' both FAs increased (Hernández et al., 2009). Differences in gene expression as well as enzyme activity may explain the differences among cultivars (Hernández et al., 2009; Hernández et al., 2021). As the RI range in the present study was limited to 0–1.96, covering only the beginning of the maturation process, it cannot be concluded that the evolution of FAs in 'Corbella' olives is different from or similar to the trends reported elsewhere.

The monounsaturated FA/polyunsaturated FA (MUFA/PUFA) and oleic/linoleic ratios give information about the oxidative stability and rancidity of oils (Hernández et al., 2021): the higher the values, the more stable and less rancid they are. Since oleic acid is the main MUFA in olive and linoleic acid is the main PUFA, these two ratios are correlated. Although the values here refer to the olive fruit, they can provide insight into the ratios of the resulting oils. The ratio values did not differ between the first two RIs (0 and 0.36), increased at 0.66, and began to decrease at 1.08. Accordingly, the most stable oils would be those produced with olives with an RI of 1.96 (MUFA/PUFA $=$ 11.51 $\pm$ 0.78, oleic/linoleic $=$ 12.73 $\pm$ 0.97) (Table S6).

Hernández et al. (2021) studied the FA composition of oils from 89 cultivars selected from the Worldwide Olive Germplasm Bank of Cordoba, in which the oleic/linoleic ratio ranged from 1.74 ('Abou Kanani') to 22.68 ('Kalokerida'). In an additional group of 36 samples, the ratio was between 1.71 ('Abou Kanani') and 23.71 ('Picual'). If the oleic/linoleic ratio of 'Corbella' olives at an RI of 1.96 was maintained, the resulting EVOO would be ranked 18th in the group of 89 samples, and 8th among the 36 samples, indicating a higher stability than EVOOs from most of the other cultivars. Although 'Corbella' EVOOs need to be produced from olives at this RI to verify whether the ratio can be maintained, it seems that 'Corbella' might be a cultivar with a high oleic/linoleic ratio.

The content of MUFA and phenolic compounds in olive oil has been associated to a lower risk of cardiovascular disease and all-cause mortality (Xia et al., 2022). 'Corbella' olives at an early stage of maturation showed to have between 75–80% of MUFA, which is a high percentage. In addition, the high oleic/linoleic ratio could contribute to the health properties. It is worth mentioning that the omega-3 EPA was also detected, even though in a low percentage (0.01%). Considering these results, both the 'Corbella' olive and the EVOO obtained from olives during early maturation can have a very healthy FA profile.

3.5. Carotenoids, α-tocopherol, and squalene content of 'Corbella' olives during early maturation

The RI had a significant effect on the carotenoid, α-tocopherol, and squalene content of the olives (Table S5). The concentration of lutein and β-carotene increased at the start of maturation (RI from 0 to 0.36), and then began to decrease. Carotenoids are related to the chlorophylls of photosynthetic tissues, and both are usually catabolized simultaneously during ripening, whereas their rates of degradation can vary. In most olive cultivars, carotenoids degrade gradually during maturation, although there are exceptions, such as 'Arbequina', in which the carotenoid concentration initially increases (Roca & Mínguez-Mosquera, 2001). It therefore seems that the 'Corbella' cultivar might follow the same carotenoid pattern as 'Arbequina' during the maturation process.

Squalene levels remained constant from an RI of 0 to 0.36 and then decreased. A review compiling 98 values of squalene content in olives found the level decreased significantly with ripeness (Martínez-Beamonte et al., 2020), but this reduction begins at different points of the maturation process according to the cultivar. In the present study, although we cannot predict the evolution of squalene in subsequent stages of maturity, the results point to a similar decreasing trend. As squalene is an intermediate in the biosynthesis of phytosterols and terpenes in plants (Martínez-Beamonte et al., 2020), its decrease could be linked to these metabolic pathways.

In contrast, the α-tocopherol content increased slightly with the RI until 1.08, and then levelled off. Muzzalupo, Stefanizzi, Perri, and Chiappetta (2011) found a similar increase in α-tocopherol with ripening in several cultivars, whereas a decrease was reported in the 'Koroneiki' cultivar (Georgiadou et al., 2016). Again, this variable behavior could be due to different genotypes and gene regulation patterns (Georgiadou et al., 2016).

Carotenoids, squalene and α-tocopherol also contribute to the health properties of olive oil (Cooperstone & Schwartz, 2016; Eroglu et al., 2023; Kim & Karadeniz, 2012; Rizvi et al., 2014). Furthermore, carotenoids might have potential gut-related health-beneficial effects. Therefore, from a health point of view, it would be interesting to use

'Corbella' olives at an early stage of maturation for EVOO production.

3.6. Multivariate analysis by OPLS-DA

The OPLS-DA model had a predictive variability (R^2X) of 0.489 and an orthogonal variability (R^2X) of 0.374, which indicated that 48.9% of the sample variation correlated with the RI and 37.4% with other variables. The model, which had four predictive components and five orthogonal components, accounted for 86.3% of the X-variation (R^2X) and 94.0 of the Y-variation (R^2Y).

The score scatter plot (Fig. 3) shows that the two olive samples with the highest RI (1.08 and 1.96) are clearly separated in two clusters, whereas the other three samples (RI of 0, 0.36 and 0.66), although also separated, are clustered more closely together, especially 0 and 0.36. This indicates that the olive samples with an RI from 0 to 0.66 have similar characteristics, while those with an RI of 1.08 and 1.96 differ from the others to a greater degree.

The loading plot (Fig. 4) shows the characteristics of the samples according to the analyzed variables, as well as their correlations. The variables located in the upper middle-right were characteristic of the samples with the lowest RI (0, 0.36 and 0.66), those in the bottom middle with an RI of 1.08, and in the upper left with an RI of 1.96. These results were verified by the VIP and coefficient values.

Considering the variables that most influenced the OPLS-DA model (VIP > 1) and their coefficients, *p*-coumaric acid, hydroxytyrosol acetate, oleuropein aglycone, secoiridoids, total phenolics, oleacein, ligstroside aglycone and hydroxytyrosol were positively correlated with olives with an RI of 0; *β*-carotene, lutein, and squalene with an RI of 0.36; luteolin and flavonoids with an RI of 0.66; C16:1n-7, C18:2n-6, C16:0, C17:1 and C15:1 with an RI of 1.08; and oleic/linoleic and C16:1n-9 with an RI of 1.96. In contrast, oleic/linoleic, *β*-carotene, lutein, and squalene, were negatively correlated with olives with an RI of 1.08, and C16:1n-7, C16:0 C18:2n-6, luteolin, *p*-coumaric acid, ferulic-*O*-hexoside acid, oleuropein aglycone, hydroxytyrosol, and secoiridoids with an RI of 1.96. Interestingly, although the TPC had a VIP value below 1, its coefficient was negatively correlated with the RI of 1.96.

These results agree with the statistical findings of the previous sections. The highest amounts of phenolic compounds, especially secoiridoids, were found in 'Corbella' olives with an RI of 0, and the lowest in those with an RI of 1.96. Even though the TPC did not differ significantly between the samples (Table S5), the OPLS-DA model revealed a negative correlation with the RI of 1.96, which supports the decreasing tendency of the phenolic content during olive maturation. The highest levels of carotenoids (*β*-carotene and lutein) and squalene were found at an RI of 0.36, and luteolin and flavonoids at 0.66. 'Corbella' olives with an RI of 1.08 stood out for having the highest levels of linoleic acid, leading to the lowest oleic/linoleic ratio. Finally, the olives with an RI of 1.96 had the lowest content of C18:2n-6, and so the highest ratio of oleic/linoleic. Neither *α*-tocopherol nor DPPH influenced the OPLS-DA model (VIP < 1).

4. Conclusions

This study provides insight into the metabolic profile of 'Corbella' olives harvested at early stages of maturation, and it is the first metabolic study performed on this revived ancient cultivar. The resulting information sheds light on the low stability of the EVOOs produced with this olive cultivar, and how this may be addressed to improve oil quality.

Olives with an RI < 1 had a considerably lower oil yield, especially below 0.5. Within the short maturation period studied (RI of 0 to 1.96), the total amount of quantified phenolic compounds was depleted by 77.67%, which represents a considerable loss. The most abundant phenolic compound was oleuropein aglycone, followed by the secoiridoid degradation product elenolic acid, indicating a high hydrolytic activity, especially of *β*-glucosidase, which could explain, at least partially, the low stability of 'Corbella' EVOO. A further investigation of the enzymatic activity of 'Corbella' olives would be useful to verify this hypothesis. However, both the TPC and the antioxidant capacity was maintained throughout the ripening period studied, the latter decreasing only at an RI of 1.96. After an initial increase, the carotenoid level decreased, as did squalene, whereas *α*-tocopherol increased. Finally, most individual FAs remained constant throughout. Interestingly, the highest oleic/linoleic ratio was found in olives with an RI of 1.96, which accordingly would produce a more stable EVOO with a lower tendency to rancidity, a hypothesis that requires testing. In fact, 'Corbella' might be an olive cultivar with a high oleic/linoleic ratio.

Considering these results, together with the information obtained from the OPLS-DA model, which was not influenced by the variables of *α*-tocopherol or DPPH (VIP < 1), it could be concluded that 'Corbella' olives harvested with an RI from 0 to 0.66 will have the highest content of phenolic compounds, carotenoids, and squalene, and thus a good antioxidant capacity. On the other hand, olives harvested with an RI of around 2 will have lower levels of these compounds while retaining a

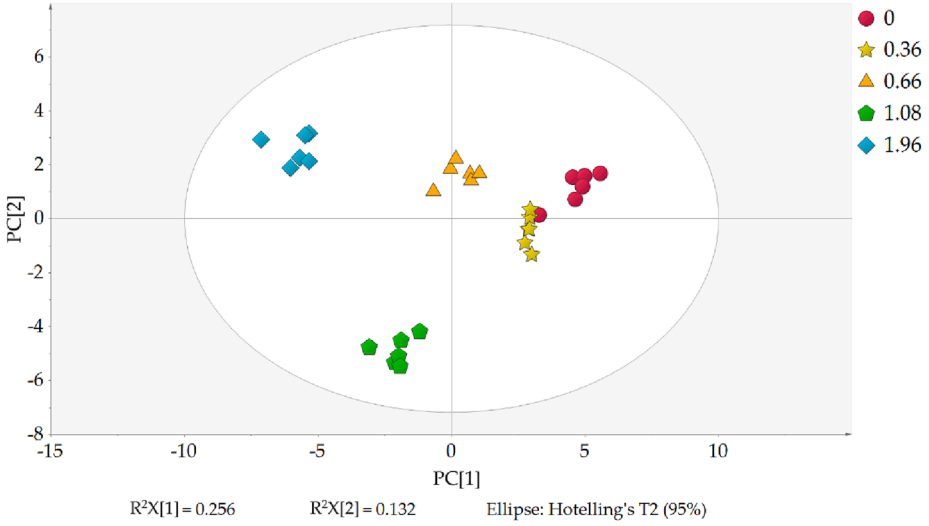

Fig. 3. Score scatter plot of the OPLS-DA. 'Corbella' olive samples are colored according to the RI (0, 0.36, 0.66, 1.08 and 1.96). $R^2X[1]$ and $R^2X[2]$ are the values with variation in the two predictive components based on the RI. Their sum is $R^2X = 0.389$, which refers to the variation correlated with the RI. All samples were inside the Ellipse Hotelling's T2, indicating there were no strong outliers.

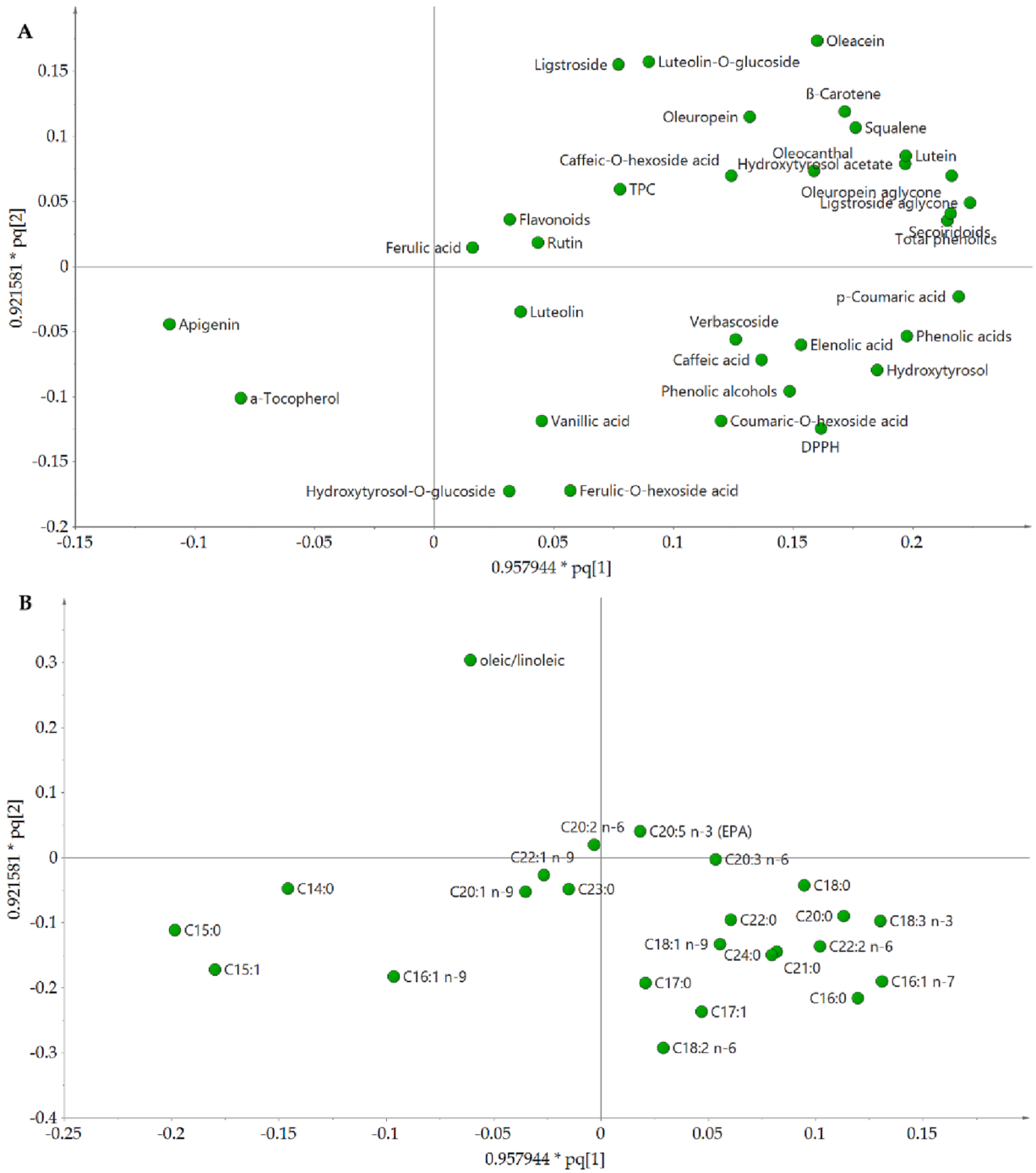

Fig. 4. Loading scatter plots of the OPLS-DA showing the distribution and correlation of the different variables analyzed in the 'Corbella' olive samples. (A) Distribution and correlation of the phenolic compounds, carotenoids (β-carotene and lutein), α-tocopherol, squalene, total phenolic content (TPC) and antioxidant capacity (DPPH). (B) Distribution and correlation of the fatty acids and the oleic/linoleic ratio.

good antioxidant capacity, which could be due to high oleic/linoleic ratio, among other factors. Therefore, a priori, olives within an RI range of 0 to 2 should produce EVOOs with similar levels of stability. Whether the different concentrations of metabolites, especially phenolic compounds, could influence the oil stability will be tested in a future study. Therefore, the optimum RI at which 'Corbella' olives should be harvested will depend on whether the aim is to produce an EVOO rich in phenolic content. From a health point of view, 'Corbella' olives harvested at an early stage of maturation seem to be great candidates to obtain high-quality EVOOs.

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CRediT authorship contribution statement

Alexandra Olmo-Cunillera: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Maria Pérez:** Writing – review & editing, Supervision. **Anallely López-Yerena:**

Investigation, Writing – review & editing. **Mohamed M. Abuhabib:** Investigation. **Antònia Ninot:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing. **Agustí Romero-Aroca:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing. **Anna Vallverdú-Queralt:** Methodology, Writing – review & editing. **Rosa Maria Lamuela-Raventós:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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