



Bioactive compounds in Spanish extra virgin olive oils: Migration and stability according to the culinary technique used

Rosario Pérez Córdoba^a, José Javier Quesada-Granados^{a,b,*}, Jessica del Pilar Ramírez-Anaya^c, Jaime Peña-Díaz^d, Rosa Blanca-Herrera^a, Cristina Samaniego-Sánchez^{a,b}

^a Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Campus Cartuja s/n, C.P. 10871 Granada, Spain

^b Instituto de Nutrición y Tecnología de los Alimentos "José Mataix Verdú", Centro de Investigación Biomédica, Avenida del Conocimiento s/n, Parque tecnológico de Ciencias de la Salud, Universidad de Granada, 18100-Armilla, Granada, Spain

^c Department of Computational Sciences and Technological Innovation, Centro Universitario del Sur (UdeG), Av. Enrique Arreola Silva 883, Ciudad Guzmán C.P. 49000, Jalisco, Mexico

^d Member of María José Faus Dader Pharmaceutical Care Académic Center, University of Granada, Campus Cartuja s/n, C.P. 10871 Granada, Spain

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ABSTRACT

Extra virgin olive oil (EVOO) is a basic food of the Mediterranean diet and an important source of bioactive compounds, especially phenolic substances. The culinary techniques to which the oil is subjected before consumption cause the migration of these compounds, hence the importance of studying their stability before and after culinary treatment. We determined the behaviour of the phenols present in EVOO and its total antioxidant capacity before and after the use of various culinary techniques such as deep frying, boiling (in a water/oil mixture (W/O) and sauteing, observing that the study parameters varied according to the variety of oil and the culinary technique used. Significant statistical differences were observed between the different varieties of EVOO according to the culinary technique used. But this was not the case with respect to polyphenol content, for which no statistically significant differences were observed among the different varieties of EVOO according to the culinary techniques employed ($p > 0.05$), except with the Arbequina variety ($p < 0.05$). With respect to the individual polyphenols – tyrosol, p-vainillin, vanillic acid, gallic acid, *trans*-caffeic acid, ferulic acid and luteolin – our analysis shows that although there were differences in content between raw EVOO and EVOO treated with each of the culinary techniques, these differences were not statistically significant ($p > 0.05$). There were significant losses of oleocanthal with the W/O boiling technique, but content increases were observed following sauteing and deep frying with respect to raw EVOO. Total antioxidant capacity presented a similar pattern in all samples, with increases after sauteing and decreases after W/O boiling and deep frying. ABTS was the most suitable technique for determining antioxidant capacity in EVOO. In short, the behaviour of the bioactive compounds in EVOO depends on the temperature and the cooking medium used.

1. Introduction

The Mediterranean diet is characterized by the high consumption of extra virgin olive oil (EVOO), fruit, cereals, vegetables and grains; moderate consumption of fish and poultry; low consumption of dairy products, red and processed meats and dessert sweets; and wine in moderation consumed with meals (Colomer & Menéndez, 2006; Scotece et al., 2015). Olive oil is the main fat in the Mediterranean diet, due to its organoleptic characteristics, nutritional properties and cultural influence (García-Vico et al., 2017; Polari et al., 2018). The consumption of

olive oil is related to health benefits such as protection against cancer (Giulitti et al., 2021; Tzekaki et al., 2021; Almanza-Aguilera et al., 2023; Donat-Vargas et al., 2023), type II diabetes (Njike, Ayettey, Treu, Doughty & Katz, 2021; Calabrese, Valentini & Calabrese, 2021) and cardiovascular disease (Katsiki, Pérez-Martínez & Lopez-Miranda, 2021). These properties are attributed to the saponifiable fraction of its composition, which constitutes 98–99% of the oil and contains triglycerides rich in monounsaturated fatty acids (Reboredo-Rodríguez et al., 2018; Nikou et al., 2019). Olive oil is also composed of a polar fraction (or total phenolic fraction - TPF), commonly termed the

* Corresponding author at: Dept. of Nutrition and Food Science, School of Pharmacy, University of Granada, Campus Cartuja S/N, 18071 Granada, Spain.

E-mail addresses: charopc96@gmail.com (R.P. Córdoba), quesadag@ugr.es (J.J. Quesada-Granados), jessicar@cusur.udg.mx (J.P. Ramírez-Anaya), direccion@lenisan.es (J. Peña-Díaz), rblanca@ugr.es (R. Blanca-Herrera), csama@ugr.es (C. Samaniego-Sánchez).

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polyphenols or biophenols, which represents its antioxidant component. The chemical classes that characterise olive oil polyphenols are very diverse, and include phenylalcohols, phenolic acids, secoiridoids, flavonoids and lignans (Nikou et al., 2019; Kanakis et al., 2013). The main phenolic compounds found in EVOO are tyrosol, hydroxytyrosol, oleuropein and oleocanthal (Bendini et al., 2007). The presence and availability of these phenols is an important parameter determining the quality and nutritional value of EVOO. In addition, these phenols largely determine the oil's shelf life and organoleptic characteristics (Reboredo-Rodríguez et al., 2018; Romani et al., 2019; Servili et al., 2014). One of the most important is oleocanthal, which is a natural polyphenolic anti-inflammatory agent found only in EVOO. This phenol has numerous beneficial health effects. Of special importance is its intense anti-inflammatory action, which is comparable to that of ibuprofen, thanks to its ability to inhibit COX-1 and COX-2 cyclooxygenases but not 15-lipoxygenase (Beauchamp et al., 2005; Parkinson & Keast, 2014; Montoya et al., 2021). Beauchamp et al. (2005) coined the term oleocanthal from "oleo" (Oil), "canth" from acanthos (itch) and "Al" from aldehyde, reflecting the fact that this substance provokes the sensation of itchy throat caused by many EVOOs.

The bioactive capacity of the phenols present in EVOO is of great interest due to their proven benefits to health and their antioxidant power, which enables them to capture free radicals, not only preventing oxidative stress, but also delaying and/or preventing the development of some chronic non-communicable diseases associated with this condition (Brand-Williams, Cuvelier & Berset, 1995; Bertelli et al., 2020; Shahidi & Zhong, 2015). EVOO is commonly used in preparing dishes that form part of the Mediterranean diet. However, the culinary techniques used to increase the food's palatability may cause a partial loss of the phenols present in the oil (Cattivelli et al., 2023; Lozano-Castellón et al., 2022).

In view of these considerations, the aim of the present study is to study the migration and stability of the bioactive compounds present in different varieties of olive oil, before and after subjecting them to different culinary treatments. In addition, we examine how these treatments affect the oil's antioxidant capacity, with particular attention to the content of bioactive compounds.

2. Material and methods

2.1. Standards and reagents

All chemicals used were analytical reagent grade. The reagents used to measure the individual phenolic compounds – gallic acid, vanillic acid, syringic acid, caffeic acid, pinoresinol, p-coumaric, o-coumaric and ferulic acids, chlorogenic acid, tyrosol, quercetin, apigenin, luteolin, p-hydroxybenzoic and p-hydroxyphenylacetic – were obtained from Sigma-Aldrich SL (Madrid, Spain). Tyrosol and o-vanillin standards were supplied by Fluka Chemicals, oleuropein by Extrasynthèse, and rutin by HWI Analytik GmbH. Hydroxytyrosol standard was synthesised at the organic chemistry laboratory in the School of Pharmacy at Granada University (Spain). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

The following reagents were used to measure antioxidant capacity: DPPH (2,2-diphenyl-1-picryl hydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid) and Trolox standard ((\pm) -6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (all supplied by Sigma-Aldrich SL, Madrid, Spain); and potassium peroxodisulphate (K₂S₂O₈), sodium acetate 3-hydrate, glacial acetic acid, ferric chloride 6-hydrate (supplied by Panreac Quimica SL, Barcelona, Spain). The 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) used for the ferric iron (FRAP) method was obtained from Fluka Chemicals (Buchs, Switzerland). Folin-Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Hesse, Germany) and the anhydrous sodium carbonate (Na₂CO₃) used to determine the TPC was obtained from Carlo-Erba (Rodano, Milan, Italy). Methanol and ethanol were provided by Sigma-Aldrich (Milan, Italy). All NMR solvents and reagents used to measure oleocanthal and

derivates were analytical reagent grade and pure standards obtained from "Sociedad Andaluza del Oleocanthal (SAO)" and served both for the identification and quantification of the molecules under study.

2.2. EVOO samples and cooking processes

The EVOOs analysed in 2021, were acquired in one-litre bottles from commercial establishments in Granada (Spain). Two bottles per type of sample to be analyzed were purchased. The samples were of different varieties of EVOO, Picual (P), Hojiblanca (H), Cornicabra (C) and Arbequina (A), all of them with guarantees of quality and variety (according to its labelling for being EVOOs with a Protected Designation of Origin according to Spanish Legislation), and each one of them produced during 2021 harvest. These varieties were chosen because they are the most commonly consumed in Spain. Prior to analysis, all oils were stored in the dark and with no headspace. To standardise the experimental conditions, all EVOO samples subjected to cooking processes were treated under identical conditions. Two replicates were treated and each sample was analysed three times.

In this case, and contrary to other studies (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir & De La Serrana, 2015), only possible changes in the phenolic composition of the extra virgin olive oil are being studied based on the culinary technique employed, and therefore no vegetable matrix has been used.

The domestic cooking techniques used to study the migration and stability of bioactive compounds were Deep frying and Sauteing (using EVOO), and Boiling (in a water/oil mixture, W/O). Table 1 details the times, temperatures and conditions involved in each cooking technique, as slightly modified in our laboratory (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir & De La Serrana, 2015) from earlier work in this area (Bello, 1998; Mc Gee, 2004). The temperature of the heat transfer medium was 180 °C for deep frying, 80–100 °C for sauteing and 100 °C for boiling. In the latter process, the water:oil proportion was 9:1. In all cases, the processing times were maintained for 10 min. The heat transfer medium was recovered after 5 min. In addition, the W/O medium was separated in a decantation device. Each sample was stored in cool, dark conditions until the bioactive compounds were extracted. Each cooking technique was performed and analysed three times.

2.3. Methodology for EVOO quality indicator parameters

The chemical parameters (free fatty acids, peroxide and UV K₂₇₀ and K₂₃₂ light absorption) were determined, in triplicate, following the official analytical methods described in European Commission Regulation No. 2568/1991 and Regulation (EU) No. 1604/2019 (Commission Regulation (EEC), 1991; Commission Implementing Regulation (EU), 2019). The quality values for all EVOOs are summarised in Table 2. Acidity is expressed as a percentage of oleic acid. All samples presented low acidity values, in accordance with the regulation for EVOO acidity, $\leq 0.8\%$ (Commission Regulation (EEC), 1991; Commission Implementing Regulation (EU), 2019).

As shown in Table 2, the initial peroxide indices, too, were always within the limit stipulated in EU Regulation No. 1604/2019, max. 20 Meq O₂/kg (Commission Implementing Regulation (EU), 2019). A

Table 1
Summary of EVOO cooking treatment.

Culinary technique	Deep frying	Saute	Boiling in water/oil
Heat transfer medium	EVOO	EVOO	Water and EVOO
Amount of cooking medium (mL)	600	60	600:66
Proportion	–	–	9:1
Time (min)	10	10	10
Temperature (°C)	180	80–100	100
Analysis fraction	Oil	Oil	Oil

Table 2
Quality parameters of extra virgin olive oil.

SAMPLE	Acidity (%)	Peroxide (Meq O ₂ /Kg)	UV light absorption (K ₂₇₀)	UV light absorption (K ₂₃₂)
Picual (P)	0.153 ± 0,02	2.378 ± 0,33	0.138 ± 0	1.906 ± 0.1
Hojiblanca (H)	0.148 ± 0,01	2.962 ± 0,38	0.121 ± 0,01	1.907 ± 0,05
Cornicabra (C)	0.204 ± 0,04	3.039 ± 0,64	0.12 ± 0,02	1.778 ± 0,44
Arbequina (A)	0.197 ± 0,02	4.218 ± 0,51	0.137 ± 0	1.92 ± 0,41

Data are expressed as mean ± standar deviation.

spectrophotometric assay was conducted to determine the presence of conjugated trienes (K₂₇₀) that may have formed during autoxidation of the oil, following a positional shift of double bonds. The final parameter analysed was K₂₃₂. When the EVOO was in a medium state of oxidation, it presented maximum absorption at 232 nm. The K₂₇₀ and K₂₃₂ coefficients were within the permitted legal limits for EVOO, max. 2.50 and 0.22, respectively (Commission Regulation (EEC), 1991; Commission Implementing Regulation (EU), 2019).

In view of the characteristics observed, the olive oils used in our analysis can all be considered EVOO.

2.4. Extraction of phenolic compounds

The extracts were obtained following an adaptation of the method described by Montedoro et al. (1992), and later modified in our laboratory (Samaniego-Sánchez et al., 2007; El Haouhay, Samaniego-Sánchez, Asehraou, Villalón-Mir & López-García De La Serrana, 2015; Rueda, Cantarero, Seiquer, Cabrera-Vique & Olalla, 2017; Ramirez-Anaya, et al., 2019). The extracts were obtained by combining 10 g of oil with 10 mL of 80% methanol (1:1 w/v) and shaking for 60 min. The mixture was then centrifuged at 6800 G for 15 min. After recovery of the polar fraction, the procedure was repeated. Volume was adjusted to 25 mL with 80% methanol. The extracts were stored at - 40 °C for no longer than two months.

2.5. Phenol contents

2.5.1. Total phenol content

The Total Phenol Content (TPC) of the water or methanolic oil extract used was determined in triplicate using the Folin-Ciocalteu colorimetric method (El Haouhay, Samaniego-Sánchez, Asehraou, Villalón-Mir & López-García De La Serrana, 2015) modified in our laboratory (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir & De La Serrana, 2015; Samaniego-Sánchez, Stagno, Quesada-Granados, Blanca-Herrera & Brandolini, 2014; Ramirez-Anaya et al., 2019). The absorbance was measured at 700 nm using a Boeco S-22 ul-traviolet-visible (UV-VIS) spectrophotometer (Hamburg, Germany). The TPC was calculated by a calibration curve of gallic acid (0.5–7.5 µg/mL) (R² = 0.999) and expressed as µg gallic acid equivalent (GAE)/g.

2.5.2. Individual phenolic compounds: Chromatographic and mass spectrometer operating conditions, identification and quantification

The use of HPLC/UPLC methodology for the identification and quantification of polyphenolic species has been widely reported in the scientific literature since the early 1990s (Monedero, Olalla, Quesada, Lopez & Lopez Martinez, 1998; Gruz, Novák & Strnad, 2008; Suárez, Macià, Romero & Motilva, 2008; Guo et al., 2020). For our study, the UPLC technique was chosen due to the experience that the research team had in the use and interpretation of results for polyphenolic compounds with this chromatographic technique. So, the polyphenols were

identified using a UPLC-QTOF-MS (Rueda et al., 2017), as modified by Esteban-Muñoz et al. (Esteban-Muñoz, Sánchez-Hernández, Samaniego-Sánchez, Giménez-Martínez & Olalla-Herrera, 2021). The electrospray ionisation (ESI)-MS experiments were performed on a liquid chromatography system with a SYNAPT G2 HDMS QTOF hybrid mass spectrometer (Waters, Milford, CT, USA). The UPLC separation was performed using an Acquity UPLC™ HSS T3 2.1 × 100 mm, 1.8 µm C18 column (Waters, Milford, CT, USA). The chromatography programme was set with a binary gradient consisting of (A) water with 0.5% acetic acid and (B) acetonitrile, as follows: 0.0–15.0 min, 5% (B); 15.0–15.1 min, from 5 to 95% (B); and 15.1–18.0 min, from 95% to 5% (B). In this last part, the column was reconditioned. Ten microlitres of sample were injected, at a flow rate of 0.4 mL/min. The time-of-flight (TOF) conditions (with measurement ranging from 50 to 1200 Da and the following mass spectrometer settings: nebuliser gas: 2 bar; drying temperature: 180 °C; capillary: 3100 V; drying gas: 6 L/min) consisted of a full MS and data-dependent scanning, performed in negative mode with electrospray ionisation (ESI). The fragment spectra for each mass were acquired using collision energy set to 40 eV with the MS/MS mode.

Phenolic compounds were identified using the exact mass of the isotopes, considering negative masses obtained from previously recorded research and then comparing the ion fragments detected with prior database records (ChemSpider, FooDB and Pub-Chem) using MassLynx V4 software (Waters, Milford, CT, USA) for instrument control, data acquisition and data analysis. Individual phenolic compounds were quantified by obtaining a series of solutions, with a concentration of 0.1–40 mg/L of standards with different retention times (Table S1; Fig. 1). For each phenolic compound selected, a five-point calibration curve was created, with R² ≥ 0.93 to ensure the linearity of the method. The standards were analysed under the same working conditions as the samples.

The results obtained were validated by reference to the analytical parameters of selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ) and precision, in accordance with the Association of Official Analytical Chemists guidelines for the validation of analytical methods (AOAC International, 2012).

2.6. Antioxidant capacity

The antioxidant capacity (AC) of the samples was measured by three different methods, in a BMG Labtech FLUOstar Omega plate reader (Offenburg, Germany) using the Omega control program and MARS data analysis software (BMG LABTECH, Ortenberg, Germany). The DPPH scavenging assay was used, as proposed by Brand-Williams et al. (Brand-Williams, Cuvelier & Berset, 1995). This method derives the ferric-reducing ability of FRAP, applying the procedure described by Benzie and Strain (Benzie & Strain, 1996) and the antioxidant equivalent capacity as radical scavenging activity, based on the reduction of the radical cation ABTS assay, following the procedure of Re et al. (1999). These assays were modified in our laboratory and these methods have been described in detail by our research group (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir & De La Serrana, 2015; Samaniego-Sánchez et al., 2007; Samaniego-Sánchez, Stagno, Quesada-Granados, Blanca-Herrera & Brandolini, 2014; Ramirez-Anaya et al., 2019; Samaniego-Sánchez, Oliveras-López, Quesada-Granados, Villalón-Mir & López-García de la Serrana, 2012). In all of the methods applied, the dilution of methanolic extracts that gave a linear response was determined. The absorbance signal was translated into antioxidant activity using Trolox as the external standard. Different calibration curve ranges were used depending on the method employed. The results of the assays are expressed as micromoles of Trolox equivalent per gram (µmol TE/g). All experiments were conducted in triplicate and the values are expressed as average ± standard deviation.

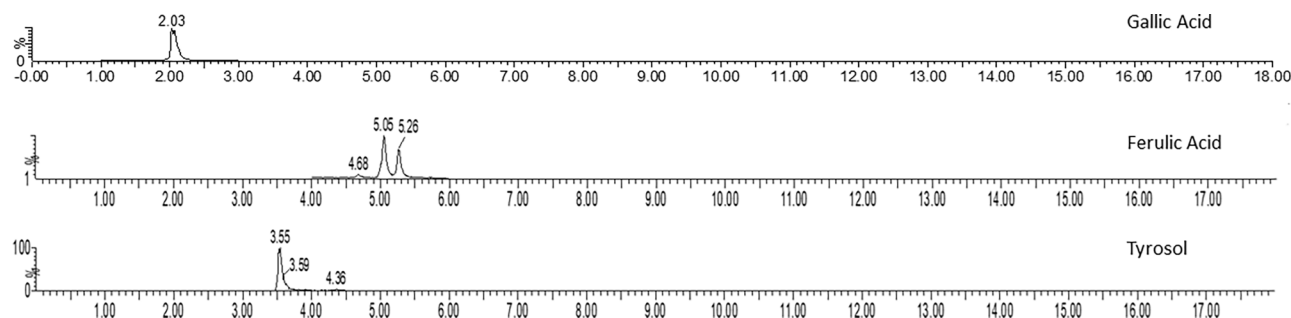


Fig. 1. Example chromatogram of standard phenolic compound.

2.7. Nuclear magnetic resonance analysis. Olive oil extraction and sample preparation

Nuclear magnetic resonance (NMR) is one of the most important food analysis techniques available (Mannina, Sobolev & Viel, 2012), and specifically for bioactive secoiridoids compounds (Karkoula, Skantzari, Melliou & Magiatis, 2012; Karkoula, Skantzari, Melliou & Magiatis, 2014; Consonni & Cagliani, 2019; Starec, Calabretti, Berti & Forzato, 2021). The strength of ^1H NMR analysis lies in its speed and ability to simultaneously detect several chemical species (sugars, lipids, amino acids and organic acids), which enables them to be identified and quantified after straightforward sample preparation (Jacobsen, 2007; Karkoula, Skantzari, Melliou & Magiatis, 2012; Karkoula, Skantzari, Melliou & Magiatis, 2014; Claridge, 2016).

For NMR analysis, the samples must first be extracted and prepared. In the present case, the extracts were obtained using an adaptation of the method described by Karkoula et al. (2012), and modified in our laboratory. Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (20 mL). The mixture was homogenised using a vortex mixer for 120 s and then centrifuged at 4000 rpm for 12 min. Part of the cyclohexane phase (15 mL) was collected and evaporated under reduced pressure using a rotary evaporator (Buchi, Flawil, Switzerland). Finally, the residue of the above procedure was dissolved in CDCl_3 (750 μL) and an accurately measured volume of the solution (550 μL) was transferred to a 5 mm NMR tube. ^1H NMR spectra were recorded at 500 MHz using an Agilent DDR-1 NMR spectrometer. Fig. 2 shows an example of one of the ^1H NMR spectra obtained in our study. Identification and

quantification were performed by Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multibond Correlation (HMBC), as described by Karkoula et al., 2014 and Karkoula et al., 2012. For the preparation of the solutions used for identification and quantification, standards supplied by the SAO dissolved in cyclohexane were used. Identification was done by comparing the spectra obtained for the pure patterns. For quantification, increasing solutions of the pure standards were prepared in cyclohexane, and a calibration curve was obtained through linear regression, which served to calculate the concentrations of the molecules in the oils subjected to different culinary techniques. The results obtained for selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ) and precision, were in accordance with AOAC (AOAC International, 2012).

The verification of recoveries after the extraction processes was carried out by adding known solutions of pure standards to an extra virgin olive oil of known concentration in the studied molecules, and subsequently comparing it with the same extra virgin olive oil without the addition of pure standards. Likewise, the results obtained were in accordance with AOAC.

2.8. Statistical analysis

Relative standard deviations were calculated for each group of data to determine their distributive characteristics and enable a correct choice of statistical test (i.e., ANOVA, Bartlett, Kruskal-Wallis or Student's *t*-test). Differences were considered statistically significant at $p \leq 0.05$. To obtain a global vision of the characteristics of the samples,

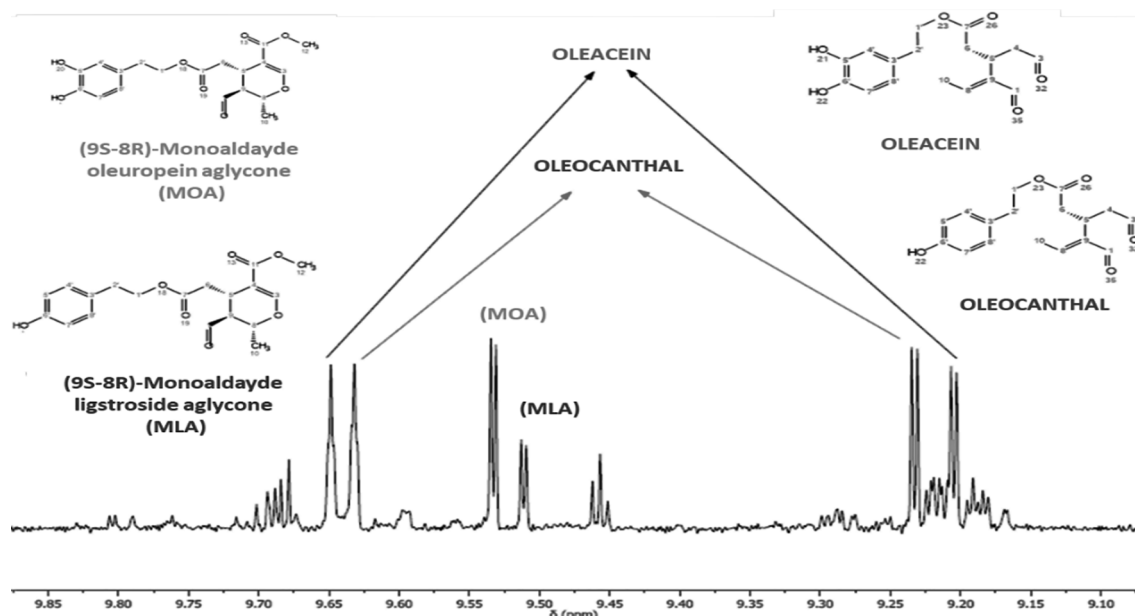


Fig. 2. Oleocanthal (R = H), Oleacein (R = OH) and derivatives observed using NMR analysis. Profile in the 9.1–9.8 ppm region in the ^1H NMR spectrum.

different analyses were performed using multivariate techniques such as cluster analysis and Pearson's test. The statistical package Statgraphics® Centurion XVI (v16. StatPoint Technologies, Inc.) was used to interpret the data obtained.

3. Results and discussion

3.1. Phenol contents

3.1.1. Total phenol content

Fig. 3 shows the variations recorded in total phenol content (TPC) of EVOO after application of the different culinary techniques.

No statistically significant differences ($p > 0.05$) were observed between the different varieties (Picual, Arbequina, Hojiblanca and Cornicabra) before any culinary technique was applied. Subsequently, however, significant differences were recorded between the varieties according to the culinary technique used. Raw (unused) EVOO presents TPC concentrations ranging from 93.13 to 48.00 $\mu\text{g ac.gallic/g}$ ($p \leq 0.001$). By order of concentration, the ranking is Picual > Cornicabra > Arbequina > Hojiblanca. Following deep frying, the EVOO varieties presented total phenol concentrations ranging from 48.61 to 33.92 $\mu\text{g ac.gallic/g}$ ($p \leq 0.01$), in the order Picual > Cornicabra > Hojiblanca > Arbequina. After sauteing, the concentrations ranged from 129.92 to 61.79 $\mu\text{g ac.gallic/g}$ ($p \leq 0.001$), in the order Hojiblanca > Picual > Cornicabra > Arbequina. Finally, after W/O boiling, the concentrations ranged from 21.08 to 8.98 $\mu\text{g ac.gallic/g}$ ($p \leq 0.001$) in the order Arbequina > Cornicabra > Hojiblanca > Picual.

The culinary techniques applied clearly influence TPC ($p \leq 0.001$), in all the varieties examined. For example, after sauteing, all EVOO varieties presented an increased TPC compared to the levels recorded in raw oil. The Hojiblanca variety presented the sharpest increase, to 129.92 $\mu\text{g/g gallic acid}$ ($p \leq 0.001$). The other varieties reflected the same trend ($p \leq 0.001$), but the increase was not so marked. By contrast, deep frying and W/O boiling both led to a decrease in TPC, in all varieties. This outcome was especially apparent in the Picual variety (with TPC falling from 93.13 to 8.98 $\mu\text{g/g gallic acid}$ for the W/O boiling treatment) (Fig. 3).

The thermal oxidation, polymerisation and hydrolysis reactions which occur during deep frying at high temperatures, in an oxygen environment, are the main causes of the losses of polyphenols from EVOO. So, Canaj (2021) found that heating at 220 °C for 6 h has a

significant impact on decreasing the amount of antioxidant and polyphenolic compounds in olive oil, especially in high-quality samples such as extra-virgin olive oil, with changes even in the color and flavor of the olive oil after the heating process. These losses can also result from the formation of covalent bonds between oxidised phenols and proteins or amino acids, and/or from the polymerisation of oxidised phenols (Friedman, 2006). A similar significant decrease in phenolic content was observed during frying at 180 °C for 90 min in extra-virgin olive oils, with significant changes in the polyphenolic profile (Criado-Navarro, López-Bascón, Ledesma-Escobar & Priego-Capote, 2021). Fundamentally, therefore, the losses of phenols from EVOO are attributed to the increased temperatures produced during food processing. This hypothesis is supported by previous observations according to which the heating of EVOO progressively decreases the concentration of polyphenols, which may disappear entirely with prolonged treatment at very high temperatures (313 °C). In the sauteing treatment, since temperatures are not so high, this loss does not occur (Cerretani, Bendini, Rodríguez-Estrada, Vittadini & Chiavaro, 2009).

The loss of phenols during boiling may be due to their water-soluble nature; thus, the EVOO loses phenols to the water in which it is boiled (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir & De La Serrana, 2015; Ramírez-Anaya et al., 2019).

3.1.2. Individual phenolic compounds: Chromatographic and mass spectrometer operating conditions, identification and quantification.

Table 3 lists the individual polyphenols found in the different EVOO samples according to the techniques described in Section 2, classified by variety and culinary technique and independently of the extraction and determination method used. Oleuropein is omitted from the table because, despite being investigated, it was not detected in any of the samples. Specifically, the reduction to the total loss of oleuropein is a consequence of the high temperatures used for the different culinary techniques, which range from 80 °C for sautéing to 180 °C for deep frying (Attya, Benabdelkamel, Perri, Russo & Sindona, 2010). Although it is not the situation described in our study, the presence of food during the use of the culinary techniques studied here, can also lead to a considerable degradation of oleuropein as a consequence of the small amounts of water released on the surface of the food (Silva, Pinto, Carrola & Paiva-Martins, 2010).

In general, Table 3 shows there were no statistically significant differences in polyphenol content among the EVOO varieties considered (p

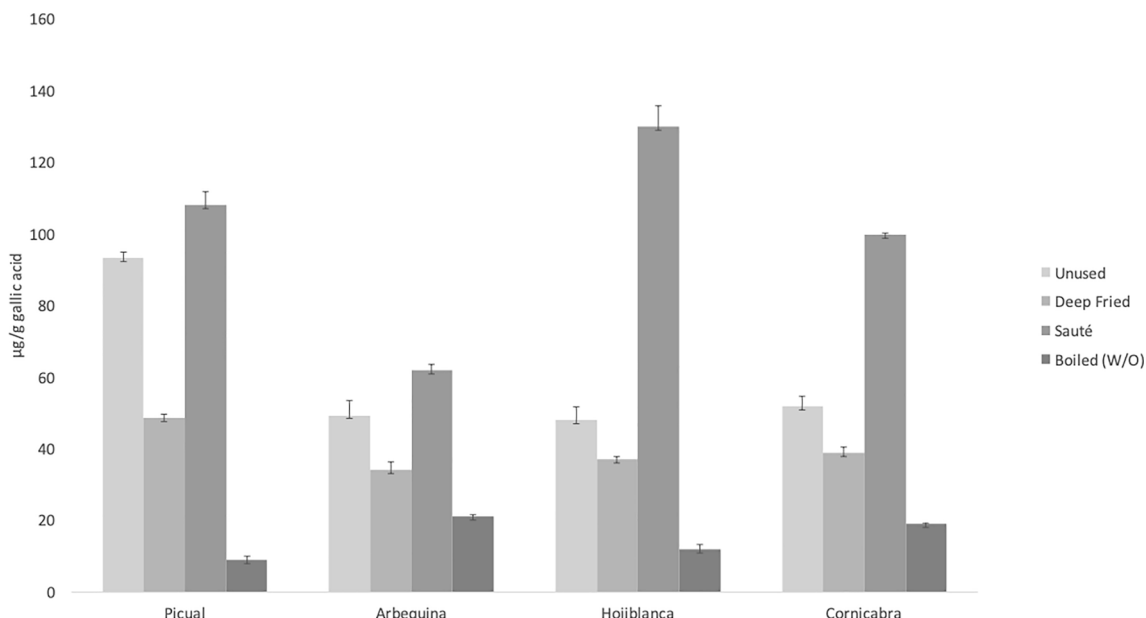


Fig. 3. Changes in the total phenol content ($\mu\text{g/g gallic acid}$) of EVOO varieties according to the culinary technique used.

Table 3Percentage of Individual phenolic compounds (ppm) in EVOO according to culinary technique. Different superscripts, $p \leq 0.05$. MOA, Monoaldehyde oleuropein aglycone. MLA, Monoaldehyde ligstroside aglycone.

EVOO variety	Culinary technique	Tyrosol ⁽⁺⁾	p-Vainillin ⁽⁺⁾	Hydroxytyrosol ⁽⁺⁾	p-Coumaric acid ⁽⁺⁾	Vanillic acid ⁽⁺⁾	Gallic acid ⁽⁺⁾	trans Caffeic acid ⁽⁺⁾	Ferulic acid ⁽⁺⁾	Apigenin ⁽⁺⁾	Luteolin ⁽⁺⁾	Oleacein ^(*)	Oleocanthal ^(*)	MOA ^(*)	MLA ^(*)
Picual⁽⁺⁾	Unused ^(a)	5.75 ± 0.73	2.25 ± 0.50	0.50 ± 0.10	0.25 ± 0.05	0.25 ± 0.06	0	0	0	0.25 ± 0.05	2.00 ± 0.27	519.24 ± 2.95	763.15 ± 2.87	227.67 ± 2.70	150.46 ± 1.93
	DeepFried ^(a)	4.00 ± 0.46	2.00 ± 0.86	0.25 ± 0.09	1.00 ± 0.15	2.00 ± 0.22	0	0	0	0.25 ± 0.09	1.50 ± 0.28	328.48 ± 2.48	801.42 ± 1.54	0	0
	Sauté ^(a)	7.25 ± 1.12	4.75 ± 1.02	0.50 ± 0.08	1.50 ± 0.12	4.75 ± 0.50	0.25 ± 0.07	0	0	0.50 ± 0.07	2.00 ± 0.28	415.75 ± 2.50	780.18 ± 3.60	188.47 ± 2.67	124.32 ± 1.49
	Boiled (W/O) ^(a)	7.75 ± 1.77	3.75 ± 1.06	0	1.75 ± 0.32	2.75 ± 0.77	0.25 ± 0.05	0	1.00 ± 0.05	0.50 ± 0.02	1.25 ± 0.11	0	0	0	0
Hojiblanca⁽⁺⁾	Unused ^(b)	6.00 ± 1.22	3.00 ± 0.48	0.25 ± 0.07	0.25 ± 0.09	0.25 ± 0.05	0	0	0	0.25 ± 0.06	2.00 ± 0.34	437.97 ± 3.75	643.88 ± 4.74	191.56 ± 2.68	126.58 ± 1.44
	DeepFried ^(b)	8.75 ± 1.88	5.25 ± 0.57	0.25 ± 0.02	2.75 ± 0.22	14.00 ± 0.85	0.50 ± 0.09	0	1.75 ± 0.27	1.50 ± 0.12	2.25 ± 0.30	401.27 ± 3.91	704.36 ± 3.31	0	0
	Sauté ^(b)	7.25 ± 1.13	2.00 ± 0.37	0.50 ± 0.05	6.50 ± 0.37	9.75 ± 0.52	0.75 ± 0.07	0	0.25 ± 0.03	2.00 ± 0.54	3.00 ± 0.49	656.96 ± 2.22	965.82 ± 4.23	287.34 ± 2.74	189.87 ± 1.37
	Boiled (W/O) ^(b)	197.50 ± 1.49	4.00 ± 0.61	0	4.00 ± 0.76	4.00 ± 0.27	0.50 ± 0.06	0.25 ± 0.02	0	1.25 ± 0.08	1.75 ± 0.21	0	0	0	0
Cornicabra⁽⁺⁾	Unused ^(c)	5.25 ± 0.23	0.75 ± 0.08	0.50 ± 0.03	0.25 ± 0.06	0	0	0	0	0.25 ± 0.03	1.25 ± 0.15	273.73 ± 1.63	402.43 ± 3.63	119.73 ± 1.66	79.11 ± 1.13
	DeepFried ^(c)	50.25 ± 0.84	4.75 ± 0.98	0.25 ± 0.02	6.50 ± 0.42	7.75 ± 0.83	0	0.50 ± 0.05	0	1.50 ± 0.09	1.25 ± 0.20	248.25 ± 1.20	505.71 ± 2.16	0	0
	Sauté ^(c)	13.75 ± 0.50	16.25 ± 1.79	0.50 ± 0.09	7.75 ± 0.38	7.25 ± 0.98	0.50 ± 0.04	0.50 ± 0.05	0	3.50 ± 0.26	2.25 ± 0.76	492.72 ± 2.59	724.37 ± 4.66	215.51 ± 1.50	142.41 ± 1.27
	Boiled (W/O) ^(c)	11.25 ± 0.75	16.25 ± 0.72	0	6.25 ± 0.21	12.00 ± 1.53	1.00 ± 0.03	0	0	1.75 ± 0.06	2.25 ± 0.18	0	0	0	0
Arbequina⁽⁺⁾	Unused ^(d)	4.50 ± 0.30	1.00 ± 0.09	0.50 ± 0.08	0.25 ± 0.04	0.50 ± 0.07	0	0	0	0.25 ± 0.06	2.75 ± 0.22	602.22 ± 2.55	885.34 ± 3.16	263.4 ± 1.38	174.05 ± 1.32
	DeepFried ^(e)	7.00 ± 0.71	3.75 ± 0.32	0.25 ± 0.03	3.00 ± 0.17	6.25 ± 1.10	0	0	0.50 ± 0.06	2.00 ± 0.19	1.25 ± 0.20	273.73 ± 1.33	1602.71 ± 4.33	0	0
	Sauté ^(f)	8.50 ± 0.89	7.25 ± 0.80	0.25 ± 0.03	2.00 ± 0.67	3.00 ± 0.37	0	0	0	1.75 ± 0.23	4.75 ± 0.32	1040.19 ± 5.11	1529.22 ± 4.51	454.96 ± 2.14	300.63 ± 2.96
	Boiled (W/O) ^(g)	3.75 ± 0.29	1.75 ± 0.45	0	0.25 ± 0.02	0	0	0	0	0.50 ± 0.05	1.50 ± 0.15	0	0	0	0

Data are expressed as mean ± standard deviation. Different letters superscripts, $p \leq 0.05$. MOA, Monoaldehyde oleuropein aglycone. MLA, Monoaldehyde ligstroside aglycone. Superscript (+), polyphenolic species determined using UPLC-QTOF-MS. Superscript (*), polyphenolic species determined using NMR.

> 0.05) or according to the different culinary techniques applied ($p > 0.05$), except between those used with Arbequina ($p \leq 0.05$). Neither were there any statistically significant differences in polyphenol contents for the same culinary technique between the different varieties of EVOO ($p > 0.05$).

For some individual polyphenols – tyrosol, p-vainillin, vanillic acid, gallic acid, trans caffeic acid, ferulic acid and luteolin – although there were differences in content between raw EVOO and the oils treated with culinary techniques, they were not statistically significant ($p > 0.05$).

For hydroxytyrosol, important and statistically significant losses ($p \leq 0.05$) were observed with deep frying and, especially, with W/O boiling. When the sauteing technique was applied, TPC remained unchanged from the original level. Studies have shown that the loss of phenols such as hydroxytyrosol and its derivatives is linear with respect to the number of frying operations performed. In general, the loss of polyphenols after high-temperature treatment is also related to the antioxidant capacity of the compound and to its chemical structure (Gómez-Alonso, Fregapane, Salvador & Gordon, 2003).

Similarly, for p-coumaric acid and apigenin, significant increases ($p \leq 0.05$) in TPC were observed with all the culinary techniques studied compared with those measured in raw EVOO. For oleacein, significant losses were observed after W/O boiling, compared to the other culinary techniques ($p \leq 0.05$). Oleacein contents were homogeneous in raw EVOO and after deep frying, but increased after sauteing. Likewise, oleocanthal presented a significant decrease in TPC after W/O boiling, but increased after sauteing and deep frying (Criado-Navarro, López-Bascón, Ledesma-Escobar & Priego-Capote, 2021), with respect to the levels in raw EVOO (Fig. 4). These variations in the TPC of oleocanthal were statistically significant in every case ($p \leq 0.05$). For MOA and MLA, the greatest losses were observed with W/O boiling and deep frying; with sauteing, on the other hand, there was a statistically significant increase ($p \leq 0.01$). It has been reported that the increased temperature during the application of culinary techniques can produce a supramolecular breakdown of the glycosidic bridges between the phenolic fractions and the sugars (Gómez-Alonso, Fregapane, Salvador & Gordon, 2003), which may explain the increase in the concentration of phenols observed in some samples of EVOO after sauteing. Similarly, culinary processing can alter the chemical structure of polyphenols, converting insoluble phenols into more soluble forms in aqueous media, which are ultimately lost in the cooking water, as in the W/O boiling technique (Bunea et al., 2008; Silva, Pinto, Carrola & Paiva-Martins, 2010; Santos, Cruz, Cunha & Casal, 2013).

Much has been written about the behavior of polyphenolic species

when subjected to culinary treatments involving temperature increase (Danesi & Bordoni, 2008). The increase in phenol concentrations in extra virgin olive oil subjected to thermal treatments is unclear and unpredictable. Authors such as Bunea et al., (2008) have already studied this phenomenon and attributed it to reactions between the glycone and phenolic fractions of the oil, as well as their conversion into more soluble forms (Cohen, Sakihama & Yamasaki, 2001).

3.2. Antioxidant capacity

Of the three analytical techniques (Table 4), ABTS produces the highest values, because it measures both liposoluble and hydrosoluble compounds. In contrast, FRAP presents very low values because this method only measures the reducing capacity based on the ferric ion.

With the DPPH assay, the values obtained for the methanolic extracts of raw EVOO ranged from $0.14 \pm 0.01 \mu\text{mol/g}$ (for Arbequina) to $0.46 \mu\text{mol/g} \pm 0.04$ (for Picual). Significant differences were only observed for these two varieties. After subjecting the food to sauteing, the Antioxidant Capacity (AC) values increased in all varieties, exceeding those of raw oil. This increase was greatest in Hojiblanca, rising from $0.21 \mu\text{mol/g} \pm 0.01$ to $0.77 \mu\text{mol/g} \pm 0.02$. In contrast, the deep-frying technique produced a decrease in AC in all varieties, although the differences between them were not statistically significant. This decrease in DPPH activity coincides with what has been found in other studies, in which in addition to a decrease in DPPH antioxidant capacity, it was observed how it affected all physicochemical properties (Giuffrè, Zappia & Capocasale, 2017). The lowest value measured was $0.04 \mu\text{mol/g}$, for the Cornicabra variety. W/O boiling provoked a sharp decrease in AC, especially in Hojiblanca, which fell to $0.01 \mu\text{mol/g}$. However, only Arbequina presented significant differences from the other varieties in this respect, with a value of $0.09 \mu\text{mol/g}$.

The highest AC value obtained by ABTS corresponded to the methanolic extracts from raw oil, with the Picual variety ($1.16 \mu\text{mol/g} \pm 0.11$) followed by Arbequina, Cornicabra and Hojiblanca. Only Picual presented significant differences with respect to the other varieties. With this analytical method, not all varieties followed the same pattern after the culinary treatment. In contrast to the results obtained by DPPH, sauteing led to a decrease in AC with Picual and Arbequina, while the level remained unchanged with Cornicabra; however, AC levels increased in Hojiblanca, with significant differences from all the others. After deep frying, the AC decreased in all varieties except Hojiblanca, where there was a small increase. The values obtained with this technique followed the same pattern in all varieties, with AC decreasing in

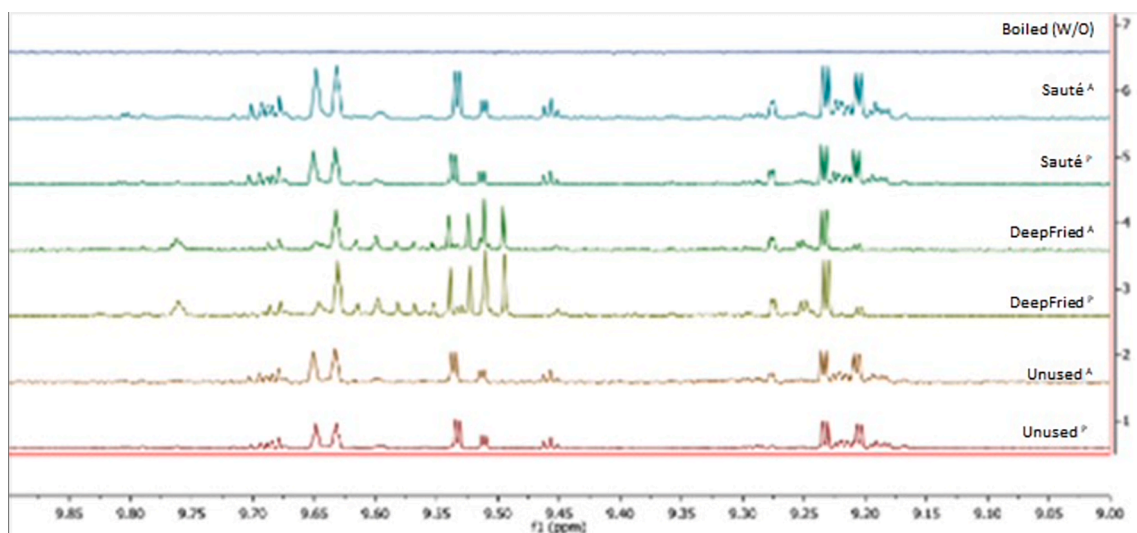


Fig. 4. Example profile in the 9.1–9.8 ppm region in the ^1H NMR spectrum in Spanish extra virgin olive oils according to the culinary technique used: Arbequina variety (A), Picual variety (P).

Table 4
Antioxidant capacity of extra virgin olive oil according to four analytical techniques.

EVOO VARIETY	TREATMENT	DPPH($\mu\text{mol TE/g}$)	ABTS($\mu\text{mol TE/g}$)	FRAP($\mu\text{mol TE/g}$)
Picual	Unused	0.45 \pm 0.04 ^a _e	1.11 \pm 0.11 ^a _e	0.9 \pm 0.03 ^a _e
	DeepFried	0.13 \pm 0.03 ^a _f	0.48 \pm 0.03 ^a _f	0.22 \pm 0.01 ^a _f
	Saute	0.58 \pm 0.05 ^a _e	0.55 \pm 0.05 ^a _f	0.84 \pm 0.03 ^a _e
	Boiled (W/O)	0.05 \pm 0 ^a _g	0.20 \pm 0.04 ^a _g	0.11 \pm 0.03 ^a _g
Hojiblanca	Unused	0.21 \pm 0.01 ^b _e	0.45 \pm 0.07 ^b _e	0.39 \pm 0.05 ^b _e
	DeepFried	0.09 \pm 0.02 ^a _f	0.52 \pm 0.01 ^a _e	0.15 \pm 0.03 ^b _f
	Saute	0.77 \pm 0.02 ^b _g	0.69 \pm 0.05 ^b _f	1 \pm 0.05 ^b _g
	Boiled (W/O)	0.01 \pm 0.05 ^a _h	0.14 \pm 0.04 ^b _g	0.08 \pm 0.01 ^b _f
Cornicabra	Unused	0.22 \pm 0.02 ^b _e	0.53 \pm 0.04 ^b _e	0.37 \pm 0.01 ^b _e
	DeepFried	0.04 \pm 0.01 ^a _f	0.33 \pm 0.05 ^b _f	0.13 \pm 0 ^b _f
	Saute	0.57 \pm 0.02 ^a _g	0.5 \pm 0.01 ^c _e	0.74 \pm 0.03 ^c _g
	Boiled (W/O)	0.06 \pm 0.01 ^a _f	0.26 \pm 0.01 ^c _f	0.12 \pm 0.01 ^a _f
Arbequina	Unused	0.14 \pm 0.01 ^c _e	0.45 \pm 0.13 ^b _e	0.11 \pm 0 ^c _e
	DeepFried	0.06 \pm 0.03 ^a _f	0.34 \pm 0.02 ^b _f	0.15 \pm 0 ^b _f
	Saute	0.36 \pm 0.01 ^c _g	0.42 \pm 0 ^d _e	0.52 \pm 0.01 ^d _g
	Boiled (W/O)	0.09 \pm 0 ^b _f	0.26 \pm 0 ^c _g	0.16 \pm 0.02 ^c _f

Data are expressed as mean \pm standard deviation. Different superscript by treatment and antioxidant capacity technique (a, b, c, d), $p \leq 0.05$. Different superscript for variety and culinary treatment (e, f, g, h), $p \leq 0.05$.

all varieties, with significant statistical differences for Picual and Hojiblanca with respect to Cornicabra. A notable result was the AC value of 0.14 $\mu\text{mol/g}$ for Hojiblanca, the variety that underwent the sharpest decrease.

The AC values quantified by the FRAP method ranged from the 0.08 $\mu\text{mol/g}$ for the Hojiblanca oil subjected to W/O boiling to the 0.84 $\mu\text{mol/g}$ of the sautéed Picual EVOO. In the samples of raw oil, significant statistical differences were observed between Hojiblanca and Cornicabra compared to Picual and Arbequina. The FRAP technique revealed the same pattern of AC variation as obtained by DPPH after sautéing, with increases in all varieties except Picual, although the latter variation (in which AC levels fell) was not statistically significant. The highest value observed with the frying technique was for the Hojiblanca variety, where AC rose to 1 $\mu\text{mol/g}$, while the lowest value was recorded for Arbequina, at 0.52 $\mu\text{mol/g}$. After deep frying, only Picual presented significant differences from the other oils. AC decreased in all varieties except Arbequina, in which there was a small increase. The same pattern was observed after each of the culinary techniques, with respect to the values for raw oil, with an increased AC in Arbequina to 0.16 $\mu\text{mol/g}$, and lower values in the other varieties.

In general, cooking has a destructive effect on TAC (Giuffrè, Caracciolo, Zappia, Capocasale & Poiana, 2018), but in some cases this parameter increases significantly with respect to raw food (Ji, Wu, Gao, Yang & Guo, 2011). The severity of damage to antioxidant substances caused by culinary treatments such as frying, steaming or boiling depends on whether lipophilic or hydrophilic compounds are evaluated. The increase in temperature during the application of culinary techniques, in the case of sautéing, can produce a supramolecular rupture of the glycosidic bridges between the phenolic fractions and the sugars, which may explain the increase in the concentration of phenols observed

in some oil samples (Bunea et al., 2008). The sautéing treatment can alter the chemical structure of antioxidant compounds, which makes possible the conversion of insoluble phenols into more soluble forms, and hence the greater affinity for the different antioxidant methods (Cohen, Sakihama & Yamasaki, 2001). Studies have shown that repeated use of the same oil for frying can decrease AC due to the loss of important antioxidant compounds such as hydroxytyrosol and its derivatives (Gómez-Alonso, Fregapane, Salvador & Gordon, 2003).

3.3. Global analysis

The health-giving characteristics of EVOO are derived from bioactive compounds in its composition that promote its antioxidant potential. When an EVOO is subjected to a culinary process, the oil's composition and therefore its antioxidant potential may be altered. Raw EVOO, by definition, presents the most suitable composition in this respect. In selecting an appropriate culinary technique for EVOO, preference should be given to preserving the oil's health-giving characteristics as far as possible, taking into account the differences between different varieties according to the technique applied.

The analysis of individual data groups for EVOO varieties shows how they are affected by different culinary techniques, but does not provide sufficient information about the influence of these techniques on the global antioxidant characteristics of EVOO. For this purpose, we must perform a joint analysis of all the data obtained, using a multivariate technique such as cluster analysis or Pearson's test.

3.3.1. Pearson's test

The Antioxidant Capacity (AC) of EVOO largely arises from the bioactive compounds of a polyphenolic nature present in its unsaponifiable fraction. Given the great variety of these polyphenols and the diverse means by which AC can be quantified, it is necessary to determine which polyphenolic bioactive compounds exert most influence on AC in each of the analytical methods available.

Accordingly, Pearson's correlation test (Table 5) was applied to reveal the extent to which the polyphenols included in our analysis influenced the results obtained by the DPPH, FRAP, ABTS and TPC techniques.

Table 5 shows the results obtained by Pearson's test, highlighting the correlations presenting statistical significance. As can be seen, only four of the polyphenolic components – hydroxytyrosol, oleacein, MOA and MLA – presented statistically significant correlations with the techniques used to measure AC. These results are consistent with the fact that these four polyphenols are known to act as antioxidants in EVOO, making them the most likely to participate in AC (Reboredo-Rodríguez et al., 2018; Bendini et al., 2007). However, two other polyphenolic species, tyrosol and oleocanthal, which are also known to be bioactive

Table 5
Statistically significant correlations between polyphenols and total antioxidant capacity, according to Pearson's test. (*) $p \leq 0.05$.

	DPPH	FRAP	ABTS	TPC
Tyrosol			(*)	
p-Vainillin				
Hydroxytyrosol	(*)	(*)	(*)	(*)
p-Coumaric acid				
Vanillic acid				
Galic acid				
trans caffeic acid				
Ferulic acid				
Apigenin				
Luteolin				
Oleacein	(*)	(*)	(*)	(*)
Oleocanthal			(*)	
Monoaldehyde oleuropein aglycone (MOA)	(*)	(*)	(*)	(*)
Monoaldehyde ligstroside aglycone (MLA)	(*)	(*)	(*)	(*)

(*) $p \leq 0.05$.

antioxidant components in EVOO, only appear to contribute to AC when measured by ABTS. These findings are in line with those reflected in Table 4 and with the observations made in Section 3.2 regarding ABTS, in that this technique reveals a greater number of polyphenolic species contributing to AC than any of the other methods considered.

3.3.2. Cluster analysis

Fig. 5 shows the dendrogram obtained from the cluster analysis of the following data: total polyphenolic content (TPC), individual polyphenol content according to UPLC and NMR, and antioxidant capacity (AC).

This dendrogram clearly shows that the culinary techniques applied influence the antioxidant characteristics of the EVOOs used. Thus, the raw EVOOs are all grouped in the lowest levels (1–4). However, they are not grouped together or at level 1. Those with greatest similarity are on the left of the dendrogram while the least similar ones are on the right. The lowest level of grouping corresponded to Picual, in its raw state (PU) or after sauteing (PS). The latter is the least aggressive culinary technique, and the one that best maintains the original composition and antioxidant characteristics of this EVOO. The next level of grouping (level 2) is occupied by the raw state of the Hojiblanca and Cornicabra varieties, reflecting the similarity of these two oils. Interestingly, level 3 contains the same oils in the level 2 cluster (i.e., HU and CU), together with Picual, when subjected to deep frying. This grouping shows that the application of this culinary technique to Picual does not significantly impair its characteristics, which remain similar to those observed in Hojiblanca and Cornicabra in their raw states. When subjected to deep frying, however, the EVOO are grouped at much higher levels (8, 10 and 11, for ADF, CDF and HDF, respectively). The Picual variety of EVOO would be the most suitable with this culinary technique, although even in this case there is a significant linear loss of phenols such as hydroxytyrosol and its derivatives, according to the number of frying operations performed. If carried to excess, this technique would produce a total loss of these compounds.

The loss of polyphenols is not only due to high-temperature culinary treatment but is also determined by the compound's chemical structure and antioxidant capacity (Gómez-Alonso, Fregapane, Salvador & Gordon, 2003). In our cluster analysis, level 4 contains the last of the raw-state EVOO (Arbequina), closely associated with the EVOO found in level 3 (PDF). From these findings, we conclude that the compositional and antioxidant characteristics of Picual after deep frying are

intermediate between those of Hojiblanca, Cornicabra and Arbequina in their raw states. Accordingly, the use of Picual, despite its known limitations (Gómez-Alonso, Fregapane, Salvador & Gordon, 2003), remains the best option for deep frying, as it maintains similar characteristics to the raw oil despite the considerable thermal impact (180 °C) experienced.

At level 5, two varieties (Picual and Arbequina) represent the first appearance of EVOOs subjected to W/O boiling. This level is well above those of the raw EVOOs, although the thermal impact (100 °C) of this culinary technique is not, a priori, excessively high. The other EVOOs subjected to this technique (Hojiblanca and Cornicabra) are found at levels 12 and 15, respectively, well above those of the oils in their raw states, reflecting the unsuitability of these varieties for this purpose. Finally, the level 8 classification of Arbequina, after deep frying, is the last of the groupings presenting characteristics similar to those of the raw oils. Level 9 contains Hojiblanca after sauteing (HS), and is located at a significant distance from the preceding groups, as can be seen in Fig. 5. Above this level, there appear Hojiblanca and Cornicabra (whether deep fried, sautéed or boiled) and Arbequina (sautéed). From the results shown in the dendrogram, we conclude that the varieties grouped up to level 8, i.e. Picual and Arbequina, best resist the culinary treatments described, while Hojiblanca and Cornicabra are the most sensitive and are significantly affected by the use of these treatments.

4. Conclusions

According to our analysis, the total polyphenol content in raw EVOO was highest for the Picual variety, followed by Cornicabra, Arbequina and Hojiblanca. Frying and boiling decreased this value in all cases, but sauteing increased it. All four varieties, in their raw state, presented similar profiles for individual bioactive compounds. The total antioxidant capacity (AC) also followed a similar pattern in all the samples, being increased by sauteing and decreased by boiling and frying. The higher the content of bioactive compounds, the higher the AC. The most suitable technique for measuring this parameter was ABTS. The composition of bioactive compounds, after cooking, was most similar to that of the original raw EVOO when the Picual variety was subjected to sauteing or frying. Picual and Arbequina best resisted the different culinary treatments, while Hojiblanca and Cornicabra were the most sensitive and the most strongly affected by these treatments. The properties and reactions of the bioactive compounds in EVOO – whether

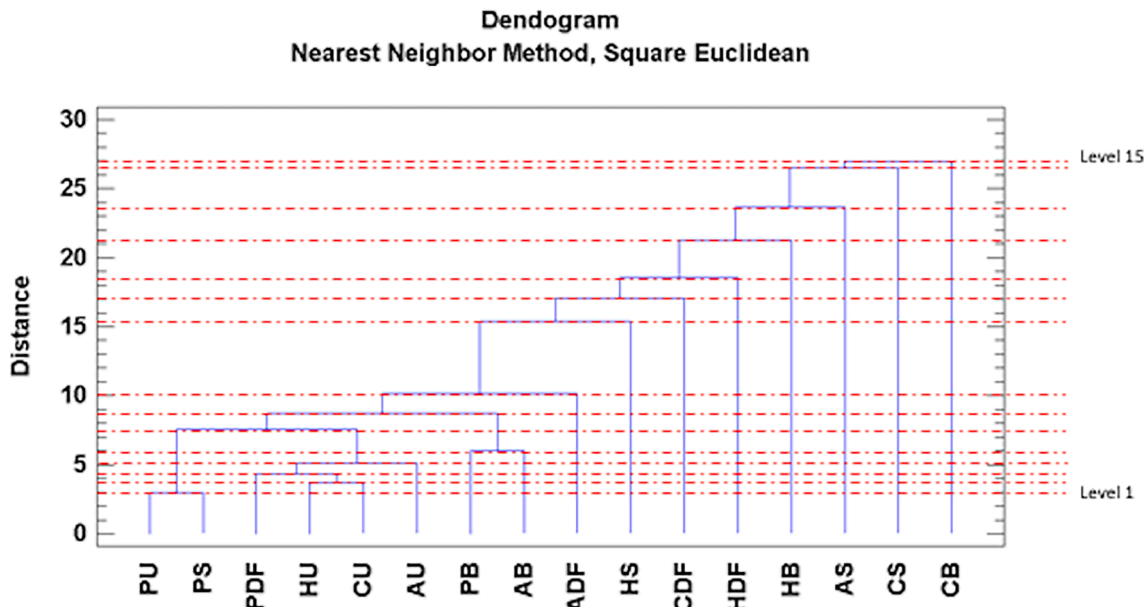


Fig. 5. Dendrogram derived from the cluster analysis of the EVOO samples analyzed.

raw or subjected to culinary treatment – are important due to their proven benefits to human health.

CRedit authorship contribution statement

Rosario Pérez Córdoba: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **José Javier Quesada-Granados:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition. **Jessica del Pilar Ramírez-Anaya:** Resources, Writing – review & editing. **Jaime Peña-Díaz:** Software, Resources. **Rosa Blanca-Herrera:** Resources, Funding acquisition. **Cristina Samaniego-Sánchez:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, 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 authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

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