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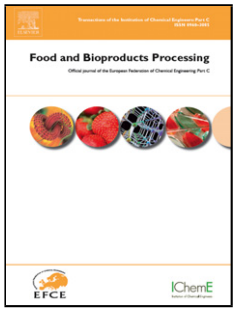

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Freeze dried extract from olive leaves: valorisation, extraction kinetics and extract characterization

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Graphical abstract

Bioactive compound	mg/gFDE
Oleuropein	118 ± 7
Luteolin-7-O-glucoside	13.8 ± 0.5
Catechin	6.2 ± 0.1
Verbascoside	5.74 ± 0.08
Luteolin	0.51 ± 0.08
Hydroxytyrosol	2.96 ± 0.07
Rutin	n.d.
Mannitol	173.7 ± 0.2
Sucrose	78 ± 1
Glucose	39 ± 4
Ch-a	1.3 ± 0.1
Ch-b	0.54 ± 0.05
Cx+c	0.49 ± 0.04

Highlights

- OL presented 25 % extractives, 10 % protein and 30 % structural carbohydrates (w/w)
- Bioactive compounds were extracted and kinetics were modelled
- 80 % ethanol yielded the highest oleuropein and other phenolic compounds yield
- A freeze dried extract was obtained, more than 11 % oleuropein and 17 % mannitol
- Bioactive compounds and antioxidant capacity were preserved for two months

Abstract

Valorization of olive leaves (OL) in a biorefinery context should include extraction of bioactive compounds, specially taking into account the high content of extractives of this by-product. Extraction of bioactive compounds from Spanish OL (cultivar "*Serrana de Espadán*") was studied by conventional and ultrasound assisted extraction (UAE). Faster extraction was observed by UAE, although similar final extraction yield was reached by both technologies. The best extraction solvent was an 80 % ethanol hydroalcoholic mixture at a ratio of 20 mL per gram of dried OL (DOL). At these conditions the highest content of oleuropein and luteolin-7-O-glucoside was determined as 31 ± 2 and 4.1 ± 0.2 mg/g_{DOL}. The power law and the Weibull models fitted the total phenolic compounds extraction kinetics quite well. The major soluble carbohydrate was mannitol, with a content of 4.48 ± 0.09 mg/g_{DOL} in the extract. The influence of OL source was also studied and it was concluded that the leaves collected as wastes from the factory presented the highest phenolic yield and antioxidant capacity.

The optimum extract was freeze dried resulting in a solid power with more than 11 % of oleuropein and 17 % of mannitol. Antioxidant activity of the freeze-dried extract was preserved for two months.

Keywords: olive leaves, phenolic compounds, oleuropein, mannitol, kinetic model, freeze-dried extract.

Introduction

The olive tree (*Olea europaea* L.) is one of the oldest known cultivated plants. It is usually native to Mediterranean countries and its cultivation has spread globally during the past two decades due to the healthiness attributed to the consumption of olive oil. More than 8 million of olive trees are cultivated worldwide; almost 98% of them are in the Mediterranean basin (Peralbo-Molina and

Luque deCastro, 2013). Spain is the country with the largest olive orchard acreage and with the highest number of olive trees (IOOC, 2012).

Olive leaves (OL) are one of the byproducts of olive farming and they can be generated during the pruning of olive trees and also in the separation process before olive processing (about 10 % of the weight of the olives) (Abaza et al., 2015). The production of OL from pruning is about 25 kg per olive tree plus 1 extra kg of leaves that can be collected at the oil mill (Molina-Alcaide and Yáñez-Ruiz, 2008). Lama Muñoz *et al.*, 2019 (Lama-Muñoz et al., 2019a) reported that the amount of OL that accumulate annually from the olive industry may exceed 1 million tons, being this residue an attractive candidate to be considered as raw material in a biorefinery context.

In many countries OL have traditionally been used as animal feed or simply burned them together with the branches from pruning (Guinda, 2006). However, OL present a chemical composition with important amounts of extractives, lignin, protein and carbohydrates (Rahmanian et al., 2015). A complete biomass valorization should include first the extractive fraction valorization. Extractives valorization is especially important for OL since they contain an important fraction of extractives reaching values up to 45 % with a high content of polyphenol compounds (Manzanares et al., 2017). Phenolic composition of the OL extracts can vary as a function of the extraction method applied and sample origin (Rahmanian et al., 2015). Most of the studies have reported oleuropein as the main phenolic compound in OL. Oleuropein has anti-inflammatory, antioxidant and antimicrobial properties. Other important phenolic compound in OL is luteolin-7-O-glucoside that is a potent drug against colon carcinogenesis (Lama-Muñoz et al., 2019a). Therefore, due to the increasing demand for the replacement of chemical additives by natural ones, OL extracts could be considered as an important, easily available and inexpensive raw material to be considered as natural antioxidant. Romero-García et al. (Romero-García et al., 2014) reviewed different applications of OL, within the context of a biorefinery based on olive biomass. These authors

reported health and medical food applications but also in supplemented foods or even nutraceuticals.

Other valuable compounds present in OL are polyols or sugar alcohols, mainly mannitol. Mannitol comprises a significant portion of the soluble carbohydrate pool (Oddo et al., 2002) and presents a sweetness potency equivalent to 70 % sucrose, but with low caloric values (2 kcal/g) (Guinda, 2006). Furthermore, mannitol has also antioxidant properties that make it useful for applications in the food and pharmaceutical industries (Ghoreishi and Shahrestani, 2009). Currently mannitol is obtained by catalytic hydrogenation from glucose-fructose mixtures; however, low mannitol yield is reached and high reaction temperature and pressure are used. Therefore, OL can be considered as a natural source of mannitol versus the current industrial production.

To convert the OL into valuable added products, the first step should be the extraction of the bioactive compounds. Different extraction techniques can be applied. The most commonly extraction system used has been the solid–liquid extraction by maceration of the plant biomass in a solvent. Among the different extraction technologies, UAE has been reported as a clean, green, extraction technique that presents several benefits such as the enhance of the extraction yield or the enhance of the extraction of heat-sensitive components (Tiwari, 2015). The industry usually prefers to use powdered extracts instead of phenolic compounds in liquid environments. Furthermore, drying of the extracts with high content of phenolic compounds will help to preserve these bioactive compounds.

The aim of this study was to obtain a freeze dried extract of OL rich in bioactive compounds from the Spanish variety "*Serrana de Espadán*". This variety has been hardly studied for their bioactive compounds and antioxidant capacity. The effect of the freeze drying technology on individual phenolic profile and antioxidant activity, as well as on other important compounds present in the OL, such as mannitol was investigated. First, extraction kinetics of total phenolic compounds,

TPC, from OL were carried out. The effect of different extraction technologies, specifically, conventional solvent extraction and UAE, type of solvent, OL/solvent ratio and the source of olive leaves on TPC extraction rate and yield and other bioactive compounds was studied. In this work as solvent type different ethanolic aqueous mixtures were selected; however, it must be highlighted that the development of new novel green solvents such as the use of deep eutectic solvents combined with different extraction technologies is a promising technology in the recovery of bioactive compounds from different plants and crops (Kurtulbaş et al., 2020; Sahin, 2019; Şahin et al., 2020). TPC extraction kinetics were fitted to different empirical extraction kinetic models. Furthermore, TPC and oleuropein content in OL extracts reported previously in the literature have been summarized for a better comparison between the different data taking into account the solvent type, extraction technology, variety and equilibrium time. The optimum extract was freeze dried to protect and concentrate the easily oxidizable phenolic compounds, as well as other valuable compounds such as mannitol and pigments present in the freeze dried extract.

2. Materials and methods

2.1 Raw material

OL samples were collected from two different sources in three different types: (1) Spanish OL from the pruning of olive trees of the variety "*Serrana de Espadán*", provided by Maicas-Sediles (Teresa, Castellón). (2) Iranian OL from the pruning of olive trees provided by the cooperative "Mamalan" Agriculture & Industry Company Tarom city (Zanjan, Iran) and (3) Iranian OL collected as by product in the oil factory.

OL were dried in a convection oven at 45 ºC to reduce the humidity to values lower than 10 %. This initial drying step by hot-air has resulted in higher oleuropein recovery in previous studies reported in the literature (Difonzo et al., 2017). After drying, OL were grounded by using a cutting mill (Retsch SM100, Retsch Technology GmbH, Germany) to obtain a particle size in the range

from 0.5 to 0.1 mm to increase the interfacial surface and improve extraction. Samples were stored in the freezer at -18ºC until extraction studies.

2.2 Determination of chemical composition of olive leaves

Spanish OL were characterized according to the Standard Biomass Analytical Methods provided by the National Renewable Energy Laboratory (https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html)

The lipid fraction of Spanish OL was analysed by Soxhlet extraction method using a BUCHI 811 extraction system (Buchi Laboratotiums Technik AG, Flawil, Switzerland). The fatty acid profile was determined by the official method of the AOAC. The chromatographic method has been detailed elsewhere (Solaesa et al., 2014). Protein content of OL was determined by elemental analysis by a Shimadzu TOC-V-CSN (Shimadzu, Japan) analyser by applying a conversion factor of 6.25.

The total amount of mannitol in OL was determined by following the same extraction procedure as Oddo *et al.* (Oddo et al., 2002). 300 mg of powdered OL were extracted twice with 10 mL of a hydroalcoholic mixture, 80% ethanol (v/v), and once with 10 mL of H_2O, overnight at room temperature. The combined extracts were subjected to mannitol quantification by HPLC (section 2.6.3).

2.3 Extraction procedures

2.3.1 Conventional solvent extraction.

Extractions were performed in an orbital shaker (Grant instruments-OLS 200, Shepreth Cambridgeshire, England). For Spanish OL, extraction kinetics were carried out for 1 h at 50ºC and samples were taken periodically. Different ethanol aqueous mixtures were selected as extraction solvents due to their food grade features (100 % ethanol, 80 % ethanol, 50 % ethanol, 20 % ethanol and 100% water). The effect of solvent volume to DOL mass ratio was determined

by varying this ratio from 20 mL/g_{DOL} to 3 mL/ g_{DOL} by using an 80 % ethanol aqueous mixture (v/v) as extraction solvent at 50ºC for 1h.

Conventional solvent extractions were also carried out for Iranian OL from two different sources: Iranian OL-T from the pruning of trees and Iranian OL-F from the factory at 50ºC, a ratio of 20 mL:g_{DO}L. Extracts were compared with the results obtained for Spanish OL.

2.3.2 Ultrasound assisted extraction (UAE)

UAE was performed by using water as solvent. UAE was carried out by using a 750 W Sonics Material TM with a 13 mm probe (VibraCell^{TM} 75042, Bioblock Scientific, U.S.A.). Samples were processed at a constant ultrasound frequency of 20 kHz and 79 µm of amplitude and a ratio of 20 mL solvent/ g_{DOL} was used. After a short initial period of time, temperature was kept at 50ºC by using a thermostated vessel where the jacketed water was circulated constant at 30 ºC. OL were introduced in the vessel (Φ = 4.8 cm, V = 199 cm^3) and the probe was submerged in the solution at a constant depth of 2 cm from the bottom of the vessel (Illera et al., 2018). The temperature was continuously recorded during extraction and samples were taking periodically to follow the extraction kinetics.

2.4 Freeze-drying process

A freeze-dried extract was obtained from the liquid extract obtained at 50ºC by using 80 % ethanol aqueous mixture (v/v) at 20 mL solvent/g_{DOL} ratio. Before freeze drying, ethanol was removed by a rotary evaporator and afterwards, the remaining extract was submitted to freeze drying. First, samples were frozen with liquid nitrogen (-196 ºC), equilibrated at -80 ºC for 2 h and then submitted to freeze-drying in a Labconco Freeze Dry System (Labconco Corporation, U.S.A.) at $1.5 \cdot 10^{-4}$ mbar during 48 h. The moisture content of the freeze dried particles was determined

gravimetrically by weighing small amounts of dried particles (around 0.5 g) before and after drying in an oven at 120 ºC until constant weight.

2.5 Characterization of extracts

2.5.1 Total polyphenol and flavonoid content determination

TPC were determined by using the Folin-Ciocalteau reagent (VWR) (Singleton et al., 1999). Briefly, 100 µL of the extract were mixed with 2.8 mL of water and subsequently with 100 µl of the Folin-Ciocalteau reagent. After that, 2 mL of sodium carbonate 7.5 % (w/v) were added to the mixture. Absorbance was measured at 750 nm after 60 min of reaction (spectrophotometer V-750, Jasco, Japan). A calibration curve was prepared with standard solutions of gallic acid and results were expressed as mg gallic acid equivalent (GAE)/g_{DOL}.

The total flavonoids content (TFC) was determined following the procedure described by Chang *et al.* (Chang et al., 2002). Briefly, 0.5 mL of the OL extract were mixed with 2.8 mL of distilled water and 0.1 mL of $AlCl_3$ (10%, w/v). Finally, 0.1 mL of CH_3 COOK (1M) and 1.5 mL of ethanol were added. Absorbance at 415 nm was measured after 30 minutes in darkness (spectrophotometer V-750, Jasco, Japan). A quercetin standard curve in ethanol was determined and results were expressed as mg Quercetin Equivalent (QE)/g_{DOL}.

To determine the TPC and TFC in the freeze-dried extract (FDE), a solution of 1 mg/mL of the FDE in 80 % ethanol was used for the analytical procedures.

2.5.2 Antioxidant capacity

Three assays were used to determine the antioxidant capacity of the liquid extracts and the FDE: the ABTS assay, the DPPH assay and the FRAP method.

The $ABTS^+$ radical was prepared according to Re *et al*. (Re et al., 1999). 3 mL of a previously prepared ABTS·+ solution were added to 100 µL of the liquid extracts. After 1 h in the dark, the

absorbance was measured with a spectrofotometer (V-750, Jasco, Japan) at 734 nm using ethanol as a blank.

The method DPPH was performed as described by Brand-Williams *et al.* (Brand-Williams et al., 1995). 60 µL of the liquid extract were mixed with 2940 µL of the reagent DPHH, absorbance was measured after 60 min of reaction in darkness at 517 nm.

For both methods, a Trolox standard curve in ethanol was used to express the antioxidant capacity of the samples as mg Trolox Equivalent (TE) per g DOL.

The FRAP method was performed according to Benzie and Strain (Benzie and Strain, 1996). 2850 µL of the working FRAP reagent was added to 150 µL of the OL extract and incubated at 37 °C for 30 min. Absorbance was read at 593 nm. As standard, a solution of $FeSO_4 \cdot 7H_2O$ (0.1 M) was used. Results were expressed in mg of $FeSO_4$ per gram of dry OL.

To determine the antioxidant capacity of the FDE, a solution of 1 mg/ml of the FDE in 80 %ethanol (v/v) was used for the antioxidant tests.

2.5.3 Identification and quantification of extract components by HPLC/DAD

Chromatographic separation was performed on a HPLC/DAD Agilent 1110 (Agilent Technologies, Inc., U.S.A.) with Kinetex® 5µm Biphenyl 100 Å, 250 x 4.6 mm column. Phenolic compounds have been analyzed by using a linear gradient of two solvents: solvent A (ammonium acetate 5mM with 1% acetic acid in water) and solvent B (ammonium acetate 5mM with 1% acetic acid in acetonitrile). The gradient profile is presented in Table 1 with a post time of 10 min. The flow rate was set to 0.8 mL/min and temperature column was 25 ºC. UV-VIS detection was done at 240, 280, 330, 340, 350, and 370 nm. Before injection, extracts were filtered through 0.45µm pore size. Identification of individual polyphenol compounds was carried out by comparing retention times and spectral data with those of authentic standards. Oleuropein, luteolin-7-O-glucoside, verbascoside, hydroxytirosol, catequin, luteolin and rutin were purchased from Sigma-

Aldrich. Standard solutions were prepared by dissolution of the compounds in methanol. Results were expressed as mg phenolic compound per g of DOL.

Identification and quantification of soluble carbohydrate was performed by HPLC-RID Agilent 1260 with an Aminex HPX-87H column (300 x 7.8 mm) using H_2SO_4 10 mM as mobile phase with a flow rate of 0.6 $mL \cdot min^{-1}$. The column and detector were maintained at 40 ºC. Pure soluble carbohydrates were used for calibration (xylose, galactose, arabinose, glucose, sucrose and mannitol were purchased from Sigma-Aldrich). Results were expressed as mg carbohydrate per g of DOL.

2.5.4 Pigments in the freeze dried extract

The amount of chlorophylls and carotenoids in the FDE were determined by dissolving 0.5 g of the FDE in 10 mL of diethyl ether according to Sumanta *et al.* (Sumanta et al., 2014). The sample was homogenized and was centrifuged for 10.000 rpm for 15 min at 4 ºC. The supernatant was separated and 0.5 mL of it were mixed with 4.5 mL of diethyl ether. The equations used for Chlorophyll-a (Ch-a), Chlorophyll-b (Ch-b) and carotenoids (Cx+c) quantification were the ones collected by Sumanta *et al.* (Sumanta et al., 2014):

$$\text{Ch-a} = 10.05A_{660.6} - 0.97A_{642.2} \quad [1]$$

$$\text{Ch-b} = 16.36A_{642.2} - 2.43A_{660.6} \quad [2]$$

$$\text{Cx+c} = (1000A_{470} - 1.43\text{Ch-a} - 35.87\text{Ch-b})/205 \quad [3]$$

2.6 Modeling of extraction kinetics and statistical analysis

The knowledge of the kinetics of the extraction of the selected compounds is important to reduce the consumption of energy and work-up and to know the time needed to obtained a certain extraction yield (Galván D'Alessandro et al., 2014). In this work, TPC data along extraction were fitted to two different kinetic models, the power law and the Weibull models. The power law model can be represented as:

$$Y\ (mg\ GAE/gDOL) = B \cdot t^n \quad [4]$$

where B is a constant incorporating the characteristics of the carrier active agent system, and n is the diffusional exponent. This value is lower than 1 for plant material (Kitanović et al., 2008). The Weibull model was expressed as:

$$Y\ (mg\ GAE/gDOL) = A \cdot (1 - exp(kt^n)) \quad [5]$$

The exponent n indicates the shape of the extraction curve. If n > 1, the curve is sigmoid and if n < 1, the curve is parabolic. A represents the maximum extraction yield.

The estimation of the kinetics parameters was carried out by non-linear regression by using the Marquardt algorithm (Statgraphics X64). The quality of the fitting was presented by evaluating the root mean square deviation (RMSD):

$$RMS = \sqrt{\frac{\sum_{i=1}^{n}\left(Y_{i,exp} - Y_{i,calc}\right)^2}{n}} \quad [6]$$

where $Y_{i,exp}$ and $Y_{i,calc}$ are the experimental and calculated extraction yield and n is the number of experimental data points in each extraction.

Statistical differences were obtained using the software Statgraphics X64. The results were presented as the mean ± standard deviation of at least three replicates. The significance of the differences was determined based on an analysis of the variance with the Fisher's least significant difference (LSD) procedure at p-value ≤ 0.05.

3. Results and discussion

3.1 Biomass composition

Chemical composition was only performed for the Spanish OL and is presented in Table 2. It must be highlighted the high content of extractives, around 25 % in a dry basis. Therefore, within a

biorefinery concept, valorization of the extractive fraction of OL is a key initial step in this specific feedstock due also to the high content of TPC in the extractive fraction. Table 2 also lists the chemical composition for other olive tree pruning leaves found in the literature, where the presence of extractives was also remarkable reaching values up to 45 % (Manzanares et al., 2017) (Gullón et al., 2018). The total content of mannitol in water (0.63 ± 0.02 %, w/w) and ethanol (3.36 ± 0.01 %, w/w) extractives was a bit lower (3.99 ± 0.03 %, w/w) than the content determined by the procedure described by Oddo *et al.* (Oddo et al., 2002), 5.50 ± 0.05 (% w/w). This value was of the same order as the one reported by Oddo *et al.* (Oddo et al., 2002), with a mean value for Olea leaves samples of 277 µmol mannitol/g_{DOL} (5.05 % of mannitol w/w). These authors also found that there were not significant variations in the mannitol content during the year for Olea samples, either due to rainfall or to temperature.

The total structural carbohydrate fraction accounted for 30% (12.5 % hemicellulose and 17.5 % cellulose). Manzanares *et al*. (Manzanares et al., 2017) reported lower values for total structural carbohydrates, but similar values were reported by Gullón *et al*., (see Table 2) (Gullón et al., 2018). These differences can be attributed to the different olive varieties. Valorization of this carbohydrate fraction is also interesting from a biorefinery point of view, although is not the objective of this work. The content of acid insoluble lignin was 11 %; since lignin in plants burns very effectively it could be used as bio-based alternative to petroleum (Manzanares et al., 2017).

The fatty acid profile of the lipid fraction, 2.7 % (w/w), was presented in Table 3. The most abundant fatty acid was the polyunsaturated acid linolenic acid (> 40 %, w/w) while oleic acid, accounted for 13 % w/w. Similar profile has been reported by Carvalheiro et al. (Cavalheiro et al., 2015) for OL cultivated in Southern Brazil

3.2 Solid liquid extraction of total polyphenol compounds

3.2.1 Comparison of conventional and ultrasound assisted extraction techniques

Figure 1 shows TPC extraction kinetics by conventional solvent extraction and by UAE by using water as extraction solvent, at 50ºC and 20 mL of solvent/$g_{DOL.}$ Other studies on TPC extraction form olive leaves by conventional solvent extraction and UAE have been carried out in the temperature range from 25 to 70ºC (see Table 4). An increase in temperature usually enhances the solubility and diffusivity of the targeted compounds, improving the mass transfer between the matrix and the solvent. However, it could also cause phenolic compounds degradation. Therefore, in this work, 50ºC was chosen as operating temperature to propose also a more cost-effective process.

Figure 1 also shows the temperature profile for the 15 min of UAE. A sharp temperature increase was observed during the first 3 min of sonication, where most of the extraction took place, and then, it remained constant at a value of 50 ºC. The acoustic energy density in the UAE experience has been evaluated according to O'Donnell et al. (O'Donnell et al., 2010) resulting in 0.84 J/s·g. For both technologies, a fast initial extraction period was observed followed by a second slow period controlled by diffusion. These type of extraction curves have been also described in the literature for bioactive compounds extraction from different plant materials (Kitanović et al., 2008). Faster extraction kinetic was observed by UAE than by conventional extraction. The final extraction yield was reached at 120 s by UAE while ten times longer were needed by conventional extraction, 1200 s. The faster extraction by UAE was reflected in the higher values of the initial extraction rate, 0.21 mg GAE/g_{DOL}·s vs 0.075 mg GAE/g_{DOL}·s by UAE and conventional extraction, respectively. Cavitation created by the ultrasound waves induced a better penetration of the solvent into the OL and improved the diffusion process. Furthermore, the increase in the extraction rates can be attributed to the increased contact surface between the solid and the liquid phase by particle disruption (Shirsath et al., 2012). Despite the faster extraction, similar final

extraction yields were reached by both extraction technologies. In any case, fast extraction of TPC was observed by both technologies probably due to the easy extraction of compounds weakly linked to the cell walls (Amendola et al., 2010). Kemdhakem *et al.* (Khemakhem et al., 2017) also reported faster extraction kinetic of TPC from OL from Tunissia by UAE than by conventional extraction, using water as solvent with similar extraction yields by both technologies, 11 mg GAE/g_{DOL} (at 2.5 % w/v and 50 ºC, see also Table 4). These authors also observed a fast extraction of TPC since 80 % of TPC were extracted in only 1 min.

Table 4 lists other values of equilibrium extraction times reported in the literature regarding TPC from OL for comparison, observing a great variety of the extraction times reported in the literature. For instance, Lafka *et al.* (Lafka et al., 2013) reported equilibrium time longer than 180 min for different extraction solvents including pure ethanol and ethanol:water mixtures (1/1) when extraction was performed in an orbital shaker (5:1 v/w), while 10 min were reported by Khemakhem et al. (Khemakhem et al., 2017) by using water as extraction solvent.

Based on the results obtained in this work, and taking into account the fast extraction kinetics obtained by both technologies, with similar TPC extraction yields, conventional extraction was considered a suitable technology for TPC extraction from OL and the effect of other extraction variables was only determined for conventional extraction.

3.2.2. Effect of type of solvent

The effect of type of solvent was studied by conventional solvent extraction by varying the composition of water-ethanol mixtures. Five different mixtures were employed as solvents, water, ethanol and different ethanol/water mixtures: 80/20, 50/50, 20/80 (v/v). The results are presented in Figure 2. The highest extraction yield was obtained for an 80 % ethanol aqueous mixture (v/v) with a value of 38 ± 1 mg GAE/g_{DOL}). This result has been also found for other plant matrixes that reported that ethanol alone was less effective than hydroalcoholic mixtures, showing that water

plays an important role on phenolic compounds extraction (Alonso-Riaño et al., 2020). The better efficiency of water-ethanol mixtures to improve TPC extraction compared to pure ethanol and water solvents has been explained considering the double effect of water and ethanol mixtures, since water swells the plant matrix, while ethanol could disrupt the bonding between the solute and the plant matrices. The addition of water to organic solvents, such as ethanol, help to create a more polar medium that facilitate the extraction of compounds that are soluble in organic solvents and/or water (Socaci et al., 2018) Therefore, the ratio between ethanol and water is a key factor to be considered to obtain high extraction yields on phenolic compounds due to their relative polarity.

Other values of TPC extraction yields found in the literature by using ethanol aqueous mixtures as solvent have been collected in Table 4. In general, solvent mixtures with ethanol content higher than 50 % (v/v) yielded good results for the different olive cultivars in terms of TPC and the most abundant phenolic compound in OL, oleuropein. Regarding the different extraction methods, those based on the use of pressurized water have not been included in Table 4. In general, conventional extraction resulted in good extraction yields compared to other methods more energetically costly, such as UAE. Different TPC was determined for the different olive cultivars, but in most cases values ranged from 30 to 40 mg GAE/g_{DOL} for extraction times between 10 and 120 min, similar to the values obtained in this work.

3.2.2.1 Influence of extraction solvent on phenolic profile

The influence of the type of solvent was also evaluated on the individual phenolic compounds profile in the extracts and on other compounds such as soluble carbohydrates. The extract color by using different ethanol aqueous mixtures can be appreciated in Figure 3. The extraction of different pigments can be clearly observed in the variation of the color of the liquid extract from light orange, by using water as solvent, to a more greenish color by using pure ethanol as solvent. The

increase in the green color by increasing the amount of ethanol in the extraction solvent indicates the presence of chlorophylls in these extracts, as it will be later described in section 3.4.

The individual phenolic compounds that could be identified by HPLC-DAD have been listed in Table 5. For all the solvents essayed, oleuropein was the major phenolic component in the OL. It was observed a similar trend for oleuropein content as for TPC, with a maximum content of 31 ±2 mg oleuropein/g_{DOL} (3.1 % w/w) for an 80 % ethanol aqueous solution (v/v). This result agrees with other literature data that showed that mixtures of organic solvents can lead to higher oleuropein extraction compared to pure solvents (Cifá et al., 2018).

In Table 4, the content in oleuropein obtained in this work can be compared with other values reported in the literature. Values ranged between 13.7 up to 87 mg oleuropein/g_{DOL} for different ethanol aqueous mixtures and different OL cultivars. Oleuropein was also determined as the major phenolic compound by using other extraction solvents. Bouaziz *et al.* (Bouaziz and Sayadi, 2005) reported a value of 6.8 % (w/w) of oleuropein for OL from Tunisia when using a methanol aqueous mixture (4:1, v/v, 60 g OL/300mL). Regarding the differences reported in oleuropein content, Erbay *et al.* reviewed that the most important variables affecting the oleuropein content was the type of cultivar and the different geographical origin, with higher oleuropein content in younger greener leaves (Erbay and Icier, 2010).

Other important phenolic compounds determined in this work for OL extracts were luteolin-7-O-glucoside with 4.2 ± 0.2 mg /g_{DOL} and verbascoside, 1.2 ± 0.1 mg / g_{DOL}. Lama *et al.* (Lama-Muñoz et al., 2020) reported values between 4.65 and 1.31 mg luteoling7-O-glucoside/g_{DOL} and 0.80 to 2.23 mg verbascoside/g_{DOL} for commercial OL samples from Arbequina and Picual cultivars, respectively. The amount of hydroxytyrosol determined in this work in OL extracts was not very high 0.7 ± 0.2 mg/g_{DOL}, but it was within the range of other varieties (0.08-3.4 mg/g) and

was much higher than the amount of hydroxytyrosol found in olive oil of Spanish olive varieties (0.93 mg/kg) (Wani et al., 2018).

The phenolic profile of OL is known to be affected by several agronomical and technological factors such as leaf age, degree of ripeness, geographical origin, cultivar, phonological stage during sampling, proportion of brunches on the tree, moisture content, degree of contamination with soil and industrial processes employed for extraction (El and Karakaya, 2009).

The co-extraction of other valuable compounds from OL such as mannitol has been also determined for the different extraction solvents. The amount of mannitol and other soluble saccharides has been listed in Table 6. The mannitol content in the extracts decreased as the amount of ethanol in the extraction solvent increased with no significant difference for water and ethanol aqueous solutions with ethanol content lower than 50 % (v/v). The content of mannitol agrees with the recently reported data of solubility of the different polymorphs of D-mannitol in ethanol-water mixtures that determined that the solubility of the three polymorphs decreased with the rise of the ethanol ratio in the binary solvent mixture (Su et al., 2020). The decrease in the amount of the other soluble saccharides identified in the liquid extracts with the increase of ethanol in the solvent mixture also agreed with the solubility order of glucose and sucrose in ethanol aqueous mixtures (Alves et al., 2007; Galvão et al., 2016). Guinda *et al.* (Guinda et al., 2015) found that molasses ethanol 96 %, was an adequate solvent for a selective extraction of mannitol, with values between 2.58 to 3.97 % (w/w) for different Spanish Olea europea cultivars with lower values of sucrose and glucose in the ethanolic extracts, from 0.62 to 1.03 % and from 0.22 to 0.46 % (w/w), respectively. In this work, the relative percentage of mannitol considering only the three soluble carbohydrates determined in this work, varied from 60 to 70 % for pure water and ethanol, respectively, with no significant difference for ethanol concentrations higher than 50 % in the extraction medium. According to Oddo *et al.* (Oddo et al., 2002) mannitol was the main constituent

of the soluble carbohydrates in the OL of four species of Oleaceae analysed. This was also confirmed in this work for the different hydroalcoholic mixtures essayed in this work.

Other values of sugars and mannitol of OL from commercial varieties reported in the literature ranged between the following values in a dry basis: mannitol, 3.7 -8.2 %, glucose, 0.6 -1.8 % and arabinose 0.1 – 0.2 % when using a mixture 60/40 ethanol water (v/v) as solvent (Lama-Muñoz et al., 2020). These authors also reported galactose, xylose and mannose as soluble sugars, but in this work these sugars could not be detected when comparing the retention times to those of authentic standards.

3.2.2.2 Extraction kinetics modelling

Based on the extraction curve shapes, a high initial slope and a slower second period, the power law and the Weibull models were used to fit the experimental extraction kinetic data. Parameters for both models are listed in Table 7. Both models can be considered suitable to describe the extraction curves of TPC from OL. The best model was the Weibull with a medium value of the RMS of 0.63 for all the extraction kinetics. The good fitting can be observed in Figures 1 and 2 where the Weibull model has been represented.

For the power law model, the diffusion coefficient, n, presented values lower than 0.2, that showed that Fickian diffusion controlled TPC extraction from OL. Similar results have been reported by Lafka *et al.* (Lafka et al., 2013) for the extraction of phenolic compounds from wild olive leaves using different extraction solvents (methanol, ethanol, ethanol:water, n-propanol, isopropanol and ethyl acetate) and for other plant matrixes (Alonso-Riaño et al., 2020). The lowest values of n were found for the TPC extraction curves for pure water and ethanol aqueous mixtures with high water content. Regarding the Weibull model, the extraction parameter, A, that can be considered as the maximum extraction yield of TPC, reached a maximum for an 80% ethanol aqueous solution as solvent while the values of k, reached a minimum at this solvent mixture.

3.2.3 Effect of solvent/biomass ratio

The effect of solvent:OL ratio was studied for water as solvent and the optimum extraction solvent found in previous section, 80% ethanol (v/v) aqueous solution. For these two solvents, extractions were performed for 1 h at 50ºC at different solvent:OL ratios: 3, 5, 8, 10, 15, 20 and 25 mL/g_{DOL}. The extraction yield, expressed as mg GAE/g_{DOL}, increased by increasing the solvent/OL ratio, reaching a plateau at 15-20 mL of solvent:g_{DOL} for both solvents (Figure 4). This behavior can be explained taking into account the higher driving force of the process by increasing the solvent:OL ratio, leading to higher diffusion rate. The concentration gradient between the solid and the solvent, is higher when a lower amount of solid is used. However, the optimum solvent:OL ratio should also take into account economic factors to provide high recoveries with minimum solvent consumption. Figures 4 also shows that the total polyphenol concentration, mg GAE/L, in the extraction medium continuously decreased by increasing this ratio due to the dilution effect. Therefore, 15 -20 mL/g_{DOL} can be considered as the optimum solvent:OL ratio for TPC from OL. Lafka *et al.* (Lafka et al., 2013) found similar results observing an increase in the extraction efficiency by increasing the ratio solvent:OL due to larger concentration gradients. These authors found an optimum solvent/sample ratio of 5:1(v/w) as the most suitable ratio for TPC extraction. Cifá *et al.* (Cifá et al., 2018) reported that the ratio 10:1 (v/w) provided the greatest oleuropein extraction yield (29.7 ± 1.7 mg/g) observing no significant differences for the other ratios (3:1, 5:1 and 7:1, 2 h and 25ºC). However, Sifaoui *et al.* (Sifaoui et al., 2016) reported a much higher value as the optimum solvent/OL ratio for TPC from OL, 77 mL:g by using water as solvent at 58 °C, for an extraction time of 54 min, at pH of 8 and an agitation speed of 246 rpm.

3.2.4 Effect of olive leaves source

Extractions were carried out by using an 80 % ethanol aqueous solution (v/v) as solvent at 50 ºC and 20 mL solvent/g_{DOL} ratio. Extracts were characterized by determining TPC, TFC and antioxidant capacity. Results are collected in Table 8. The highest content of total bioactive compounds corresponds to Iranian OL collected as waste at the factory, then the Spanish OL and finally the same Iranian OL collected at the tree pruning. The higher content of bioactive compounds led also to higher antioxidant activity for the different tests assayed in this work, DPPH, ABTS and FRAP assays. The high concentration of TPC and TFC of OL collected at the factory could be due to the fact that these OL were in contact for a few days with the olives which could led to higher TPC. Therefore, due to the high antioxidant activity of olive mill leaves, its valorisation could make more profitable the olive sector and reduce the environmental impact, and more attention should be paid to this by-product, specially taking account that the transport cost would be reduced (Contreras et al., 2020).

The variety of OL used in different studies found in the literature was also listed in Table 4. For instance in the study of Bilgin & Sahin (Bilgin and Şahin, 2013) different cultivars from Turkey were employed in the extraction of TPC by UAE reporting values in the range from 7.35 to 38.66 mg GAE/g_{DOL} by using methanol as solvent. The highest value corresponded to Bursa cultivar, located at high altitude with terrestrial and Mediterranean climate. Ben *et al.* (Ben et al., 2018) determined TPC for 21 OL cultivars from the diff erent geographical regions of South-Eastern Tunisia. The highest amount was found in Zalmati Zarzis cultivar (20.7 mg GAE/g_{DOL}) while the lowest was registered in Chemlali ontha cultivar (1.35 mg GAE/g_{DOL}). The different TPC reported proved the influence of geographical and climate conditions on TPC.

3.3 Freeze dried extract (FDE)

A solid extract from Spanish OL was obtained from the liquid extract obtained by using an 80% (v/v) ethanol aqueous mixture at 50ºC and 20 mL solvent/g_{DOL} ratio This liquid extract was freeze dried previous removal of the ethanol by a rotatory evaporator. The freeze dried extract (FDE) presented a characteristic green color and the humidity content was 7 %. The total yield of the FDE respect to the DOL was 24 %. A complete characterization of the FDE is presented in Table 9. FDE presented a high content of bioactive compounds as determined by the TPC and TFC, 113 ± 1 mg GAE/g FDE and 13.2 ± 0.7 mg QE/g FDE, respectively, with a high antioxidant capacity as determined by ABTS, DPPH and FRAP tests, respectively.

According to the individual phenolic profile identified in the liquid extract (Table 4), the major phenolic compound that could be identified in the FDE was oleuropein with a content of 117 ± 7 mg oleuropein/g FDE, more than 11 % (w/w) of the FDE. Other important phenolic compounds in the FDE were luteolin 7-O-glucoside and verbascoside, 13.8 ± 0.5 mg/gFDE and 5.74 ± 0.08 mg/gFDE, respectively. Rutin was determined in the liquid extract but it could not be quantified in the FDE. Just opposite to luteolin that was determined in the FDE but could not be determined in the liquid extract.

The soluble carbohydrate fraction in the FDE was also determined. Mannitol accounted for 17.37 % (w/w) of the FDE, while sucrose and glucose represented 7.8 and 3.9 % (w/w) of the FDE. The green color of the extract was due to the presence of relative important amounts of chlorophylls as reflected in the content of Chlorophyll-a, followed by Chlorophyll-b and lower amounts of carotenoids.

TPC and TFC were determined after two months of storage at refrigerated conditions, 4 ºC, with values of 118 ± 6 mg GAE/g FDE and 15 ± 2 mg QE/g FDE, respectively. Therefore, it can be

concluded that TPC and TFC were kept after two months of storage. This could be also observed in the antioxidant activity, as determined by the FRAP assay after two months, with values of 505 ± 10 mg Fe^{2+}/ g_{FDE}.

Conclusions

Chemical composition of OL makes this by-product an attractive raw material to be incorporated into a biorefinery context. Extractives valorisation has been proposed as the first step by using different ethanol aqueous mixtures as solvents. UAE led to faster TPC extraction kinetics but similar extraction yield was reached as for conventional extraction. A 80% ethanol aqueous mixture (v/v) was found to be the best extraction solvent with a TPC yield of 37.6 ± 0.8 mg GAE/g_{DOL} and a oleuropein content of 31 ± 1 mg oleuropein/g_{DOL}. A ratio of 20:1 (v/w) was selected as an optimum ratio due to an increase in extraction driving force.

A freeze dried extract was obtained with a high content of bioactive compounds, more than 11 % (w/w) of oleuropein and 1.4 % (w/w) of luteolin-7-O-glucoside. This FDE contain also important amounts of mannitol, more than 17 % (w/w).

Scaling-up of the process will be feasible since a cost-effective extraction technology was proposed based on the use of green solvents and the use of an inexpensive and abundant raw material obtaining an extract with good antioxidant properties. As future trends, it can be concluded that phenolic rich food products could be obtained by using freeze-drying process and it is suggested to use the FDE as an additive for food, pharmaceutical or cosmetic industries.

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The authors declare no Conflict of Interest

Reference

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Table 1. HPLC-DAD solvent gradient used for polyphenols analysis

% B	2	2	8	10	18	38	65	80
t, min	0	7	20	35	55	65	75	80

Table 2. Chemical composition of "Serrana de Espadán" OL expressed as g/100 g_{DOL}

Compound	This work	(Gullón et al., 2018)	(Manzanares et al., 2017)
Cellulose	17.5 ± 0.6	21.6 ± 0.2	9.3 ± 0.4
Hemicellulose	12.5 ± 0.5	14.5 ± 0.2	9.5 ± 0.2
Xylose	*7.8 ± 0.3*	*9.0 ± 0.0*	*4.5 ± 0.1*
Galactose	--	*2.0 ± 0.0*	*2.0 ± 0.1*
Arabinose	*4.7 ± 0.2*	*2.8 ± 0.2*	*4.0 ± 0.4*
Mannose	--	*0.5 ±0.1*	*0.3 ±0.0*
Extractives	24.6 ± 2	28.6 ± 1.4	45.2 ± 1.5
Water soluble extractives	16.4 ± 0.9	23.5 ± 1.4	
Glucose	*2.2 ± 0.2*	*7.3 ± 0.1*	
Sacarose	*0.28 ± 0.04*		
Phenolics	*1.4 ± 0.1*	*2.9 ± 0.0*	
*Mannitol**	*0.63 ± 0.02*		*Glucose 7.1 ± 0.1*
Ethanol soluble extractives	8 ± 1	5.1 ± 0.2	*Phenolics 4.4 ± 0.2*
Glucose	*0.37 ± 0.01*		
Sacarose	*0.11 ± 0.01*		
Phenolics	*1.7 ± 0.1*		
*Mannitol**	*3.36 ± 0.01*		
Acid-insoluble lignin	10.76 ± 0.02	15.4 ± 0.4	15.1 ± 0.5
Acid-soluble lignin	6.2 ± 0.3	2.3 ± 0.1	2.6 ± 0.2
Ash	4.69 ± 0.02	3.9 ± 0.6	8.3 ± 0.2
Lipid	2.7 ± 0.2	nr	nr
Proteins	10.3 ± 0.4	3.1 ± 0.2	nr

nr: non reported, phenolics are expressed as mg GAE/ g_{DOL}, proteins determined as 6.25×N;
*Total mannitol content as determined by Oddo *et al.* (Oddo et al., 2002) = 5.50 ± 0.5 % (w/w)

Table 3. Fatty acid profile (%, w/w) for the Serrana de Espadán OL

Fatty acid	Percentage	
Myristic acid (C14:0)	2.50 ± 0.01	
Palmitic acid (C16:0)	22.8 ± 0.1	
Palmitoleic acid (C16:1n-7)	1.90 ± 0.05	
Stearic acid (C18:0)	3.50 ± 0.06	
Oleic acid (C18:1n-9)	12.7 ± 0.4	
Linoleic acid (C18:2n-6)	15.9 ± 0.1	
Linolenic acid (C18:3n-3)	40.2 ± 0.4	
SFA: 28.8 ± 0.2	MUFA: 14.6 ± 0.5	PUFA: 56.1 ± 0.5

Table 4. Comparison of TPC and oleuropein content of OL extracts from different OL cultivars by using different extraction solvents.

Solvent	Extraction method	Variety	solvent:gOL (mL:g)	TPC mg GAE/g_{DOL}	T, ºC	t, min	Oleuropein, mg/g_{DOL}	Reference
80 % EtOH	Conventional	"Serrana de Espadán"	20:1	37.6	50	60	31± 2	This work
70 % EtOH	Conventional UAE	Istrsk belica (Slovenia)	5:1	32.7 138.4^{10min}	25	120 --	27.3 38.1^{120min}	(Cifá et al., 2018)
EtOH 50 % EtOH	Conventional (pH =2)	Agrielia (Greece)	5:1	≈ 36 ≈ 30	25	180	--	(Lafka et al., 2013)
47 % EtOH EtOH	UAE	Picual (Jaen)	17:1 20:1	22.3 ± 0.8 14.1 ± 0.9	room	50 30	4.2 ± 0.2 13.7 ± 6	(Contreras et al., 2020)
Molasses alcohol	Omni Mixer	Arbequina Hojiblanca Lechin Picual	20:1	--	--	3	45.3 ± 3.4 65.7 ± 6.5 61.3 ± 4.3 87.2 ± 8.2	(Guinda et al., 2015)
60 % EtOH	Dynamic maceration	Picual	12:1	41.1	55ºC		31.8	(Lama-Muñoz et al., 2019a)
60 % EtOH	Soxhelt UAE	Picual	15:1 13:1	42.9 ± 0.4 35.77 ± 0.6	-- --	240 17.9	65.77 69.91	(Lama-Muñoz et al., 2019b)
60 % EtOH	Conventional	Picual	--	42.5		240	49.1	(Lama-Muñoz et al., 2020)
Water	Conventional UAE	Chemchali	40:1	12	70	10	6.5	(Khemakhem et al., 2017)
MeOH	Ultrasonic bath HAE	Bursa (Anatolia, Tk) Canakkale (Anatolia, Tk) Bursa (Anatolia, Tk) Canakkale (Anatolia, Tk)	20:1	38.66 7.35 64.66 10.11	25	60 25000xg (3 times)	--	(Bilgin and Şahin, 2013)

HAE: homogenizer assisted extraction

Table 5. Phenolic profile of OL extract by using different hydroalcoholic mixtures (mg/g_{DOL})

Solvent	Hydroxytirosol	Catequine	Rutin	Verbascoside	Luteolin	Luteolin-7-O-glucoside	Oleuropein
Water	0.13 ± 0.01^{a}	0.067 ± 0.001^{a}	n.d.	n.d	n.d.	0.09 ± 0.01^{a}	0.24 ± 0.03^{a}
20 % EtOH	$0.27 \pm 0.08^{a,b}$	2.85 ± 0.06^{c}	0.28 ± 0.03^{b}	n.d	n.d.	2.07 ± 0.05^{c}	3.7 ± 0.5^{b}
50 % EtOH	0.39 ± 0.09^{b}	3.1 ± 0.3^{c}	0.5 ± 0.1^{c}	1.0 ± 0.2^{b}	n.d.	4.2 ± 0.2^{d}	15 ± 2^{c}
80 % EtOH	0.7 ± 0.2^{c}	1.4 ± 0.5^{b}	0.22 ± 0.01^{b}	1.2 ± 0.1^{c}	n.d.	4.1 ± 0.2^{d}	31 ± 2^{e}
100 % EtOH	$0.17 \pm 0.01^{a,b}$	0.46 ± 0.06^{a}	0.026 ± 0.004^{a}	0.022 ± 0.002^{a}	n.d.	0.9 ± 0.2^{b}	26 ± 1^{d}

Values with different letters in each column are significantly different when applying the Fisher's least significant difference (LSD) method at p-value ≤0.05.

Table 6. Saccharides and polyols, g/100g_{DOL}, in the liquid extracts by using different hydroalocholic mixtures as solvent.

Solvent	Mannitol	Glucose	Sucrose
Water	4.8 ± 0.1[c]	2.21 ± 0.04[d]	0.96 ± 0.09[b]
20 % EtOH	4.9 ± 0.1[c]	1.28 ± 0.05[c]	0.98 ± 0.08[b]
50 % EtOH	5.06 ± 0.09[c]	1.08 ± 0.06[b]	1.05 ± 0.06[b]
80 % EtOH	4.48 ± 0.09[b]	0.99 ± 0.05[b]	1.01 ± 0.06[b]
100 % EtOH	2.89 ± 0.05[a]	0.72 ± 0.06[a]	0.6 ± 0.1[a]

Values with different letters in each column are significantly different when applying the Fisher's least significant difference (LSD) method at p-value ≤0.05.

Table 7. Kinetic model parameters for TPC extraction at 50ºC for the power law and the Weibull models.

	Power law			Weibull			
Extraction	B	n	R^2/RMS	A	k	n	R^2/RMS
CE-Water	12.5 ± 1.5	0.091 ± 0.02	0.982 / 1.25	24.8 ± 0.4	0.34 ± 0.03	0.33 ± 0.03	0.995 / 0.21
UAE-Water	16.7 ± 1.8	0.067 ± 0.02	0.980 / 1.30	25.8 ± 0.7	0.23 ± 0.13	0.53 ± 0.15	0.914 / 0.70
20 % EtOH	12.6 ± 0.7	0.082 ± 0.01	0.995 / 0.62	36 ± 30	0.41 ± 0.10	0.13 ± 0.08	0.930 / 0.73
50% EtOH	9.1 ± 0.9	0.161 ± 0.01	0.991 / 1.22	58 ± 41	0.14 ± 0.07	0.22 ± 0.10	0.955 / 1.11
80% EtOh	7.9 ± 0.6	0.191 ± 0.01	0.997 / 0.74	66 ± 37	0.11 ± 0.05	0.25 ± 0.06	0.986 / 0.77
100% EtOH	7.2 ± 0.7	0.178 ± 0.01	0.993 / 0.89	35 ± 2	0.15 ± 0.01	0.31 ± 0.02	0.986 / 0.26

CE: conventional extraction

Table 8. Characterization of OL extracts from different sources, Spanish and Iranian OL from the tree pruning and Iranian OL collected as wastes at the olive oil factory (T = 50ºC, 80 % ethanol aqueous mixture, 20 mL solvent:g_{DOL})

Parameter	Spanish OL	Iranian OL	Iranian OL from factory
TPC, mg GAE/g_{DOL}	37.6 ± 0.8^{b}	29.1 ± 0.7^{a}	61 ± 2^{c}
TFC, mg QE/g_{DOL}	4.4 ± 0.3^{b}	2.3 ± 0.3^{a}	6.9 ± 0.7^{c}
FRAP mg Fe^{2+}/g_{DOL}	70 ± 1^{b}	60 ± 4^{a}	134 ± 3^{c}
DPPH, mg Trolox/g_{DOL}	62.2 ± 0.2^{a}	60.4 ± 0.1^{a}	73 ± 1^{b}
ABTS, mg Trolox/g_{DOL}	24 ± 1^{a}	21.8 ± 0.2^{a}	37.6 ± 0.7^{b}

Values with different letters in each raw are significantly different when applying the Fisher's least significant difference (LSD) method at p-value ≤0.05.

Table 9. Characterization of the freeze dried extract (FDE) from "Serrana de Espadán" OL

Total antioxidants	TPC, mg GAE/g_{FDE}	113 ± 1
	TFC, mg QE/g_{FDE}	13.2 ± 0.7
Antioxidant Activity	FRAP, mg Fe^{2+}/g_{FDE}	435 ±7
	DPPH, mg Trolox/g_{FDE}	694.2 ± 0.9
	ABTS, mg Trolox/g_{FDE}	230.4 ± 0.3
Individual phenolic compounds	Hydroxytyrosol, mg/g_{FDE}	2.96 ± 0.07
	Catechin, mg/g_{FDE}	6.2 ± 0.1
	Verbascoside, mg/g_{FDE}	5.74 ± 0.08
	Luteolin-7-O-glucoside, mg/g_{FDE}	13.8 ± 0.5
	Oleuropein, mg/g_{FDE}	118 ± 7
	Luteolin, mg/g_{FDE}	0.51 ± 0.08
	Rutin, mg/g_{FDE}	n.d.
Other components	Mannitol, mg/g_{FDE}	173.7 ± 0.2

	Sucrose, mg/g_{FDE}	78 ± 1
	Glucose, mg/g_{FDE}	39 ± 4
	Ch-a, mg/g_{FDE}	1.3 ± 0.1
	Ch-b, mg/g_{FDE}	0.54 ± 0.05
	Cx+c, mg/g_{FDE}	0.49 ± 0.04
Total antioxidants and antioxidant activity after 2 months	TPC, mg GAE/g_{FDE}	118 ± 6
	TFC, mg QE/g_{FDE}	15 ± 2
	FRAP, mg Fe^{2+}/g_{FDE}	505 ± 10

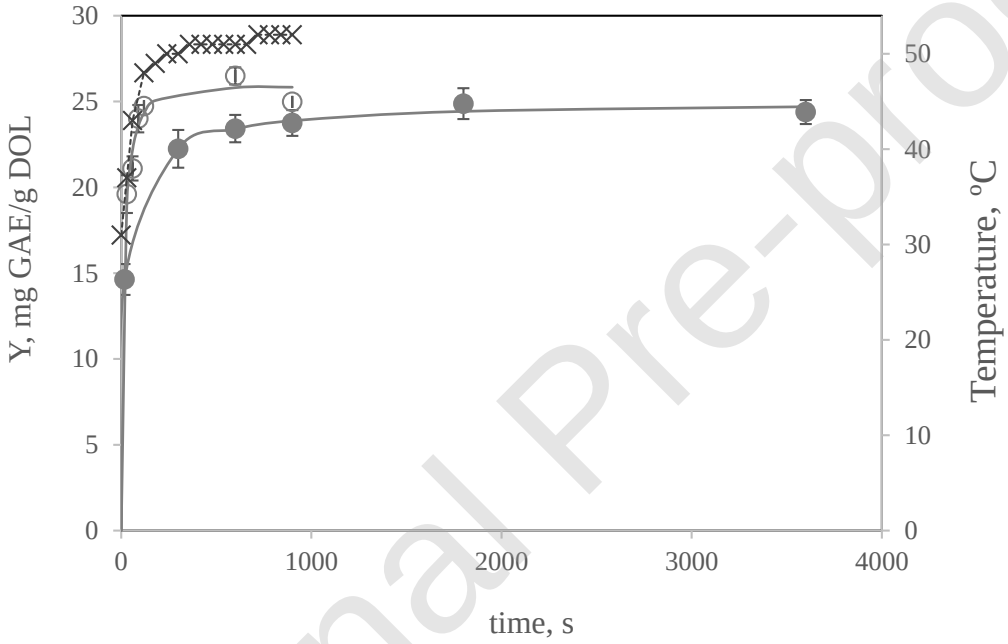

Figure 1. TPC extraction kinetics by using water as solvent at 50ºC, 20 mL of solvent/g_{DOL} (●: conventional solvent extraction, ○ ultrasound assisted extraction, × temperature profile in the UAE). Continuous lines represent the Weibull model.

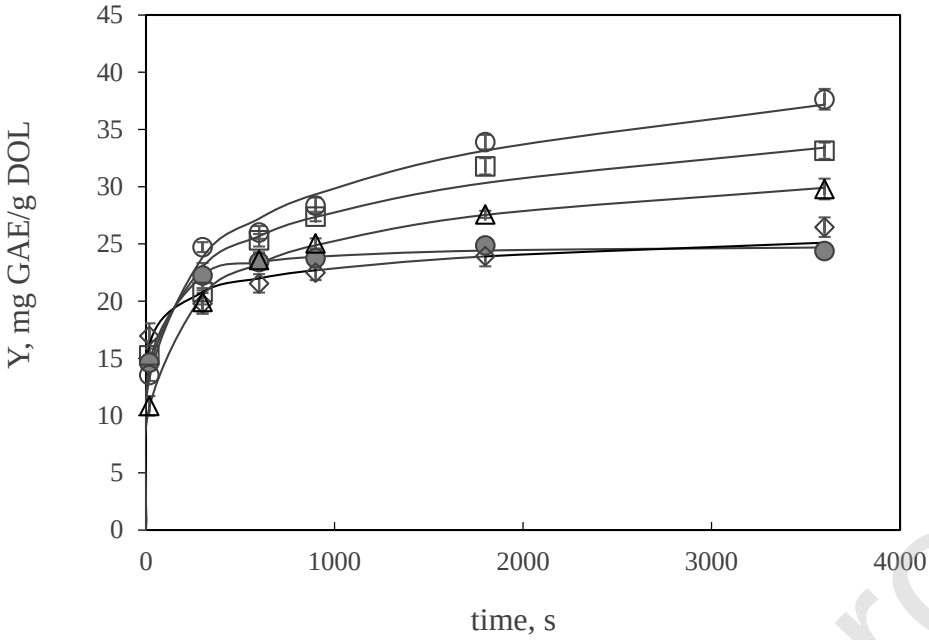

Figure 2. Effect of type of solvent on TPC extraction kinetics from Spanish OL (T = 50ºC, 20 mL solvent/g dry OL): ● 100% water, ◇ 20 % ethanol, □ 50 % ethanol, ○ 80 % ethanol, △ 100 % ethanol. Continuous lines represent the Weibull model.

Figure 3. Olive leave extracts by using different solvents. From left to right 100 % water, 20 % ethanol, 50 % ethanol, 80 % ethanol and 100 % ethanol.

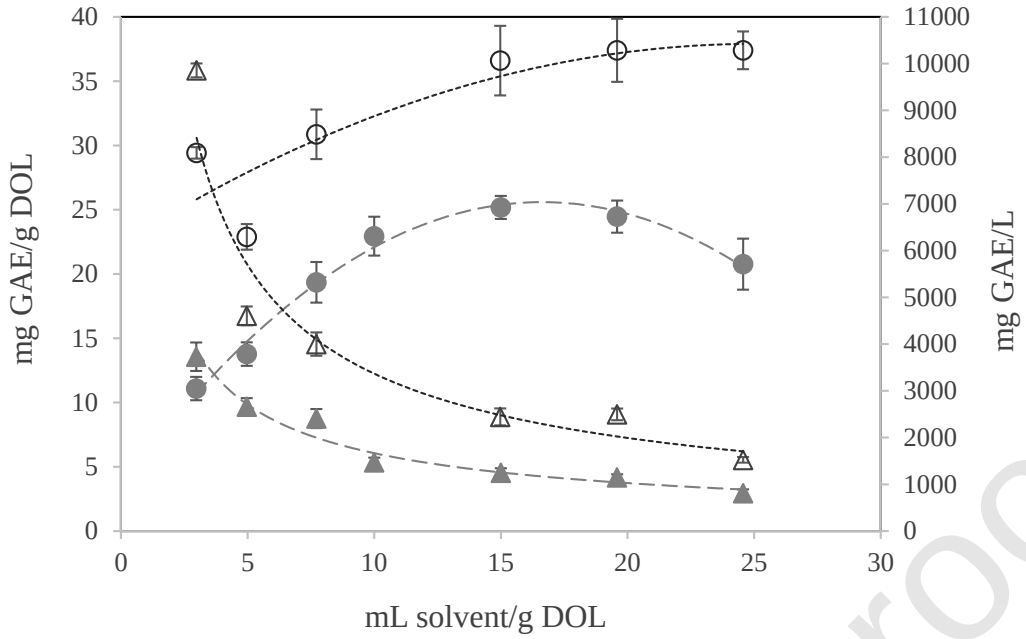

Figure 4. Effect of the solvent:DOL ratio on TPC extraction yield (mg GAE/g_{DOL} ○, ●) and total polyphenol concentration in the extraction medium (mg GAE/L, △▲) 80 % ethanol (v/v) (open symbols) 100 % water (filled symbols). Lines are to guide the eye.