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Recovery of Bioactive Compounds from Exhausted Olive Pomace †

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Abstract: Exhausted olive pomace (EOP) is a residue derived from the olive pomace oil industry. One of the main components of this agro-industrial residue is the extractive fraction which contains non-structural components such as bioactive compounds. In this work, different extraction methods, including green technologies, have been compared to evaluate the extraction of antioxidants from EOP: hydrothermal extraction, aqueous accelerated extraction, organosolv extraction, and extraction with aqueous salt solutions. The extracts obtained were characterized regarding the content of total phenols by the Folin–Ciocalteu method. After characterization, hydroxytyrosol was found to be one of the potential active compounds in EOP.

Keywords: agro-industrial residues; exhausted olive pomace; natural antioxidants; green technologies; hydroxytyrosol

1. Introduction

Fruit olive trees and olive oil are two of the main food products in the Mediterranean basin [1,2]. The farming practices of olive trees and the industry around olive oil generate several residues or by-products, such as olive pomace, exhausted olive pomace (EOP), olive stones, and olive leaves from olive cleaning operations at olive mills [3]. This crop and its by-products are of great important for the contemporary economy and society [2]. In particular, EOP is the residual solid biomass obtained after the extraction of the olive pomace oil, generally, with hexane. It presents a high content of non-structural components, around 41% [4], and for this reason, it constitutes promising source of feedstock bioactive compound extraction, such as phenolic compounds [5].

Numerous studies have shown that phenolic compounds exhibit several human health benefits, such as protection against cardiovascular disease, anti-cancer effects [6], prevention of age-related neurodegeneration and cognitive impairment [7], reduction in the risk of suffering type 2 diabetes and Parkinson's disease [8], as well as anti-inflammatory, anti-microbial, and anti-thrombotic effects [9]. Among other olive phenolic compounds, some studies suggest that hydroxytyrosol and its derivatives also have beneficial properties [10–12]. These compounds are not only present in the olive leaves and olive oil, but also in the aforementioned olive-derived biomasses. Nevertheless, the type of phenolic compound depends on several factors, including the biomass type [13].

EOP has rarely been explored; therefore, the aim of this work was to obtain antioxidants from EOP, using different extraction methods, including green technologies. Both conventional techniques, such as hydrothermal extraction, organosolv extraction and aqueous extraction using saline conditions, and a new extraction technique, accelerated solvent extraction, were applied. This method has shorter extraction times and lower solvent consumption than conventional methods [14]. Then, all the extracts obtained were

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characterized regarding the content of total phenols (TPC) by the Folin–Ciocalteu method. The phenolic extract profiles were also identified by high performance liquid chromatography (HPLC)-UV.

2. Methods

2.1. Chemical and Standards

All the chemicals and reagents were of analytical grade and supplied by Sigma-Aldrich (St. Louis, MO, USA): Folin and Ciocalteu's phenol reagent, sodium carbonate, orthophosphoric acid, methanol, acetonitrile sodium chloride, and the standards of gallic acid and hydroxytyrosol. Ethanol (96%) was obtained from VWR Inc. (West Chester, PA, USA). Ultrapure water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Raw Material

EOP was obtained from a local olive pomace industry "Spuny SA" in the province of Jaén, Spain. It was partially pitted and pelletized, with an average length of 14.5 mm and an average diameter of 4.6 mm.

2.3. Conditions Applied in the Extraction Methods

Different extraction methods, including green technologies, have been compared to evaluate the extraction of antioxidant compounds from EOP. Table 1 shows the conditions applied, including the type of solvent, concentration of the solvent, temperature, time, and solid load.

Briefly, hydrothermal extraction was applied at medium thermal conditions (85 °C) in a thermostatic water bath provided with mechanical agitation and magnetic control at 200 rpm. Additionally, it was carried out at high thermal conditions (200 °C) using a laboratory-scale 1 L stirred tank reactor (Parr Instrument Company, Moline, IL, USA). Aqueous accelerated solvent extraction at different temperatures (55 and 190 °C) and extraction times (30 and 10 min) were applied, and two sequential extraction cycles were carried out using ASE™ 350 equipment (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Likewise, the effect of organosolv extraction (50% ethanol at 200 °C) in the Parr reactor and aqueous salt extraction at 55 °C and different concentrations of sodium chloride (3–9% w/v) were also tested, in a Medline Scientific™ SI-600R orbital provided with agitation at 200 rpm (Thermo Fisher Scientific, Waltham, MA, USA).

After each extraction, the samples were filtered under vacuum and stored in cold conditions. Before analysis, the samples were filtered through nylon membrane filters (0.45 μm, SinerLab Group, Madrid, Spain).

Table 1. Conditions applied to recover antioxidants from exhausted olive pomace.

Extraction Method	Solvent	Solvent (%, v/v)	Temperature (°C)	Time (min)	Solids (%)
Hydrothermal extraction	Water	100	85	90	10, 15
		100	200	0 [b]	5, 15, 25
Organosolv extraction	Ethanol [a]	50	200	0 [b]	5, 10, 15, 20, 25
Accelerated extraction	Water	100	55	30	2
		100	190	10	2
Extraction with aqueous salt solution	Sodium chloride [a]	3, 9	55	90	5, 15, 25
		6	55	120	5, 15, 25

[a] Aqueous solutions. [b] The ramp was 4–5 °C/min.

2.4. Total Phenolic Content

The total phenolic compound (TPC) amount was measured using a UV–VIS spectrophotometer (UV-1800) from Shimadzu Schweiz GmbH (Reinach BL, Switzerland) following the Folin–Ciocalteu method, with some modifications [5]. Briefly, 0.3 mL of diluted

extract was added to 2.5 mL of the Folin–Ciocalteu reagent (1:1, v/v). Subsequently, 2 mL of a solution of Na_2CO_3 (7.5% w/v) was added, and the mixture was homogenized. After 1 h incubation at room temperature, the absorbance was measured at 765 nm. Gallic acid was used as a reference standard compound and the results were expressed as milligrams of gallic acid equivalents (GAE)/g EOP. The measurements were carried out in triplicate.

2.5. Phenolic Profile by HPLC

The phenolic profile was determined at 280 nm by HPLC using a Shimadzu Prominence UFLC chromatograph (Kyoto, Japan) equipped with a C18 reverse-phase column (250 mm × 4.6 mm), type BDS HYPERSIL 5 µm (Thermo Fisher Scientific Inc., Waltham, MA, USA). The mobile phase used was a ternary gradient, composed of an aqueous solution of orthophosphoric acid (0.2%, v/v), methanol, and acetonitrile. The elution flow was 1 mL/min, the oven temperature was set at 30 °C, and the volume of sample injected was 20 µL [4]. Hydroxytyrosol was identified by comparison with its commercial standards, through retention times and UV absorption spectra provided by a diode array detector.

3. Results and Discussion

3.1. Evaluation of the Extraction Conditions

The main component of EOP is the extractive fraction, which accounts for 41% on a dry basis [4]. Therefore, the valorization of this agro-industrial residue implies the utilization of this fraction, which contains bioactive compounds. For that reason, in this work, different techniques have been evaluated to recover antioxidants from EOP using preliminary conditions.

Figure 1 shows the TPC results of the extracts of EOP obtained after hydrothermal extraction at medium (85 °C) and high (200 °C) thermal conditions, and the organosolv treatment using different solid loadings. Using water as an extractive agent, the values ranged between 26.8 mg GAE/g EOP (200 °C, 0 min, 25% solids) and 50.8 mg GAE/g EOP (200 °C, 0 min, 5% solids). When using 15% solid loading, the values were similar independent of the temperature—37 mg GAE/g EOP (85 °C) and 39.7 mg GAE g EOP (200 °C)—however different extraction times were used. Moreover, the solid loading showed a negative influence on the phenolic compound extraction. Nevertheless, the organosolv treatment was able to recover more phenolic compounds, especially at 200 °C and 5% of solids (82.1 mg GAE/g EOP). Besides the antioxidants present in the extractive fraction, it seems that the use of ethanolic solutions as a solvent can lead to partially solubilized lignin, but it depends on the temperature, ethanol concentration, and treatment time [15,16]. Thus, this may explain our results, at least partially.

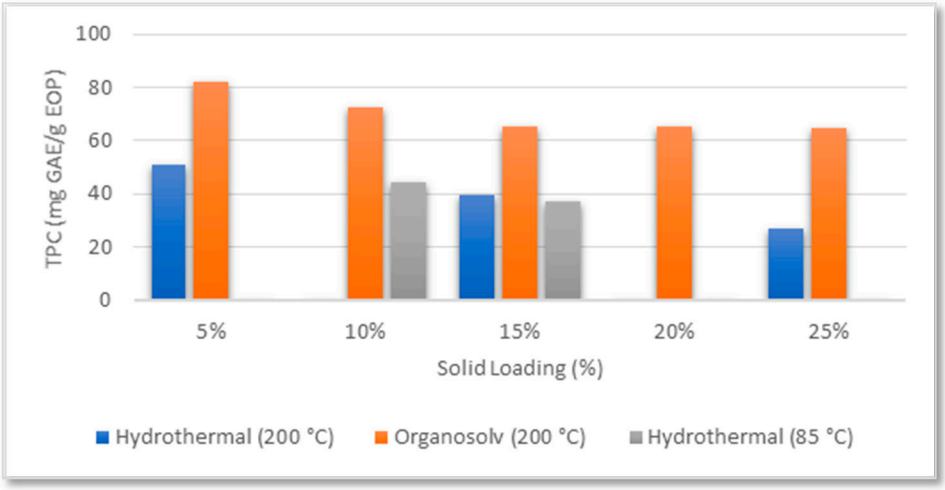

Figure 1. Total phenolic content (TPC) of exhausted olive pomace extracted using hydrothermal and organosolv treatments.

Table 2 shows the TPC values obtained for the aqueous accelerated solvent extraction with different sequential extraction cycles. The temperature showed a clear positive effect on the phenolic compound extraction, and the extraction time was considerably reduced. Some authors such as Pearson et al. [17] and Richter et al. [18] confirmed that this extraction technique shortens the extraction time compared to conventional extraction techniques. Finally, this technique enabled the obtaining of 40.6 mg GAE/g EOP at 190 °C in 10 min, which is higher than that obtained in the aforementioned conditions when the same solvent and solid loading was applied. It was also observed that one cycle was not enough to recover all the phenolic compounds present in EOP, but it can extract more than 80% of the total amount.

Table 2. Total phenolic content (TPC) of the aqueous extracts obtained from exhausted olive pomace (EOP) using accelerated water extraction.

Cycle [a]	Temperature (°C)	Time (min)	Solid Loading (%)	TPC (mg Gallic Acid Equivalents/g EOP)
1	55	30	25	35.6
2	55	30	25	6.3
1	190	10	25	40.6
2	190	10	25	9.3

[a] In the second cycle, the material resulting from the first extraction step was extracted again using the same conditions as in the first cycle.

Figure 2 shows the TPC values for the extracts obtained from EOP using saline conditions. The addition of salts did not favor the extraction of phenolic compounds compared to the conditions applied before, but the temperature applied was lower. As before, the solid loading had a negative influence on the extraction. The best results, 5% solid loading, 3% sodium chloride at 55 °C for 90 min, yielded an extract with a phenolic content of 36.94 mg GAE/g EOP.

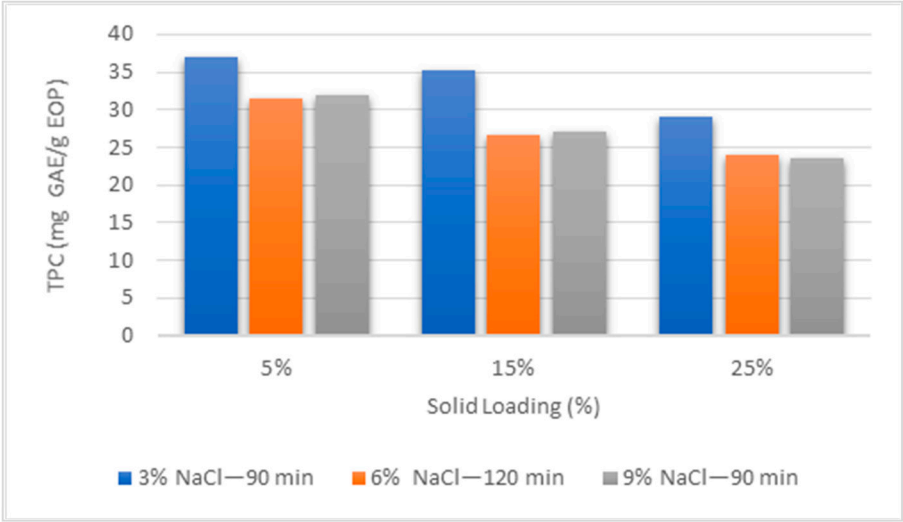

Figure 2. Total phenolic content (TPC) of exhausted olive pomace extracted using saline solutions.

3.2. Phenolic Profiles

Some of the extracts were characterized to determine the phenolic composition through HPLC-UV. As an example, Figure 3a,b show the chromatograms of the extracts obtained using hydrothermal extraction at medium and high thermal conditions, respectively. These chromatograms indicated that hydroxytyrosol (HT) and tyrosol (T) were the main phenolic compounds. The amounts of both phenolic compounds in these extracts

for medium and high thermal conditions were 6.25 mg HT/g EOP, 1.62 mg T/g EOP and 14.5 mg HT/g EOP and 2.75 mg T/g EOP, respectively. Both compounds are considered to be the most powerful antioxidants in olive products. Largely due to the presence of hydroxytyrosol and its derivatives, olive oil is considered by the European Food Safety Authority (EFSA) as a beneficial product for health [19].

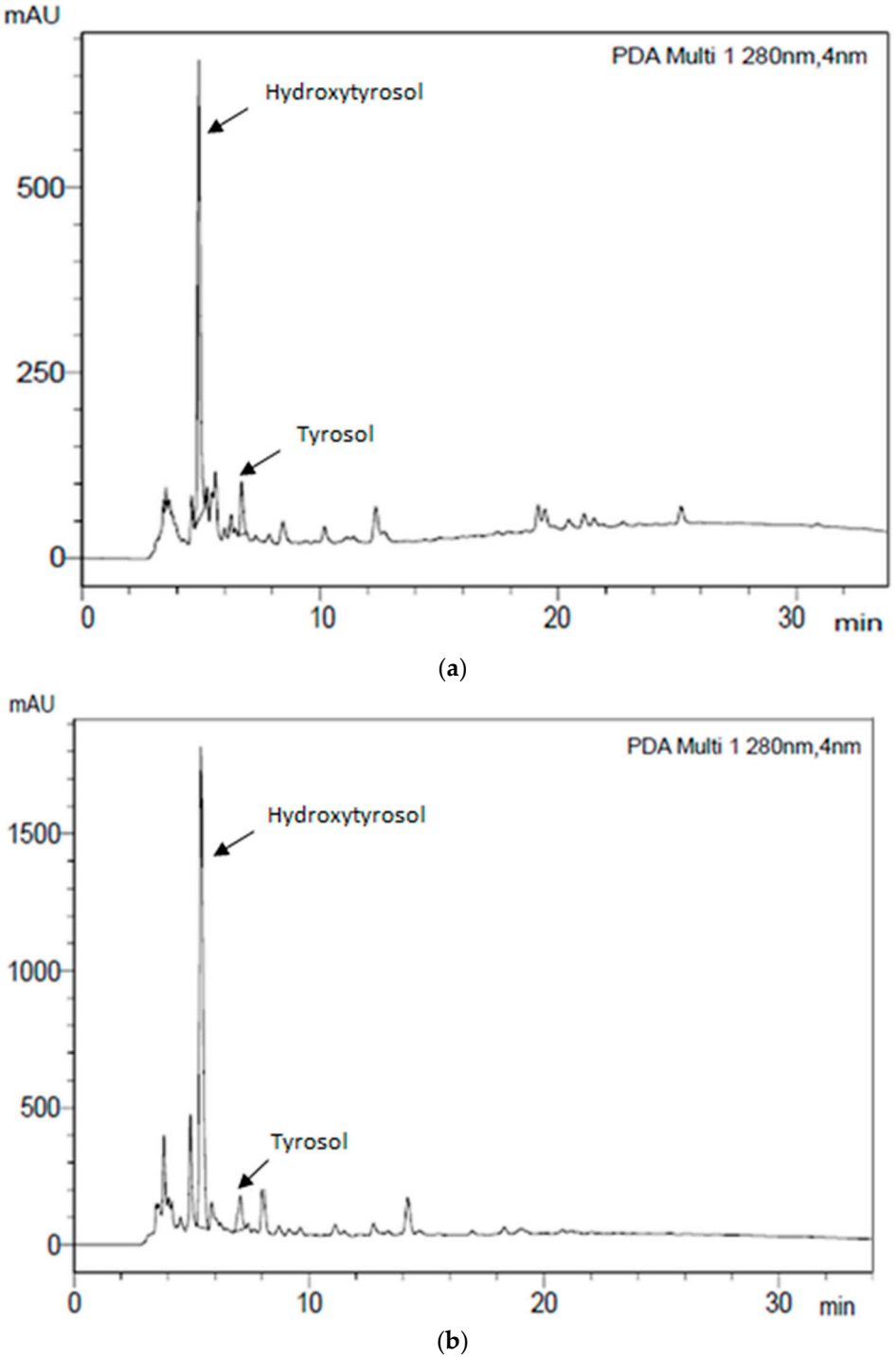

Figure 3. Chromatograms at 280 nm of the aqueous extracts obtained by hydrothermal extraction using (**a**) medium (85 °C) and (**b**) high (200 °C) thermal conditions.

Thus, in this case, because the solvent used was water, the extracts could be applied to promote functional ingredients for food and pharmaceutical applications. Moreover, further studies are required to optimize the accelerated solvent extraction because it can

shorten the extraction time compared to these conventional procedures to provide hydroxytyrosol and tyrosol from EOP using water and aqueous ethanolic solutions.

4. Conclusions

EOP presents a great number of phenolic compounds, including hydroxytyrosol and tyrosol, to promote the production of functional ingredients. These phenolic compounds can be obtained in environmentally friendly conditions, for example, by using water as extractive agent. In this sense, accelerated aqueous extraction can be further optimized to shorten the extraction time. Alternatively, 50% ethanol can be also applied because it showed the best results of TPC using high thermal conditions.

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Data Availability Statement: Data are available on request from the corresponding author.

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