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Combined Extraction and Ethanol Organosolv Fractionation of Exhausted Olive Pomace for Bioactive Compounds

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The olive pomace oil extracting industry generates large amounts of exhausted olive pomace (EOP), a lignocellulosic waste that needs to be managed according to sustainable criteria. The aim of this work is to devise an integrated strategy to valorize EOP by applying two-step extraction, and to evaluate the effect of an ethanol organosolv pretreatment on the delignification and enzymatic hydrolysis of the extracted EOP. Once the extraction and organosolv pretreatment conditions are selected, solubilized lignin is recovered from the pretreatment liquor using different methods. In addition to those organosolv lignin samples, a lignin-rich solid is obtained after enzymatic saccharification of the pretreated solid. All the lignin samples are fully characterized aiming at further valorization. The selected two-step aqueous extraction (85 °C, 90 min, 10% biomass) removes 89% of the extractives content in raw EOP and achieves the full recovery of phenols and mannitol content in that fraction, 4.7 mg gallic acid equivalents per g EOP and 4.5 mg g^{-1} EOP, respectively. The organosolv pretreatment (50% ethanol catalyzed with 1% H_2SO_4, 140 °C, 60 min, 15% biomass) results in a delignified solid with 81% of enzymatic digestibility and a high purity organosolv lignin (>71%), rich in guaiacyl units.

1. Introduction

Biomass has been traditionally used as a renewable resource for energetic use. Currently, the necessity of replacing fossil raw materials for renewable sources is growing and this converts biomass resources, including agro-food wastes, in attractive feedstock for multiproduct processes in a biorefinery system. The implementation of versatile biorefineries able to produce renewable chemicals and energy carriers requires the use of different lignocellulosic biomass sources with low cost and high availability.[1] Currently, several investigations are focused on the utilization of plants and oil wastes to generate antioxidant and antimicrobial extracts. These can be used as natural ingredients with health-promoting properties in the food and pharmaceutical industries.[2–4] In this context, olive oil sector generates every year huge amounts of wastes such as olive tree pruning, olive leaves or exhausted olive pomace that must be properly managed. Currently, their applications are still scarce although their lignocellulosic nature makes them interesting raw materials for biorefinery processes but also their non-structural fraction can be exploited.[5–7] Furthermore, the valorization of these residues through bioconversion processes would also contribute to the development of the circular economy model in the rural areas where they are generated.[8]

Exhausted olive pomace (EOP) is the final residue after extracting with hexane the oil that remains in the olive pomace. Like olive pomace, EOP contains fragments of olive skin, pulp, and stone but its oil content is lower than 2% and its moisture is also much lower than olive pomace, about 10%.[9] EOP is the main residue generated in the olive pomace extracting industry and its main application is as fuel in the same industries where it is generated. The presence of stones in the composition of EOP contributes to its high calorific value (about 3755 kcal kg^{-1}). Nevertheless, the amount of stones in EOP is variable and depends on the upstream depitting because the stones are removed from olive paste in the mills and/or from olive pomace in the extracting industries.[10] In addition, organic compounds highly polluting have been identified in the gas emissions generated during the combustion of this waste.[11] Therefore, EOP could be considered residual biomass with great potential for the production of bioactive ingredients, such as the phenolic compound hydroxytyrosol, which has been reported as a compound with antioxidant, chemopreventive and anti-inflammatory properties.[9]

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Bioactive compounds in biomass, such as the antioxidant hydroxytyrosol, are usually found in the non-structural fraction.[12] Nevertheless, antioxidants can also be generated from the lignin fraction; for example, after a partial delignification and depolymerization of the biomass[12,13], while the hydrolysis of the phenolic acids ester-linked to the polymers of the cell wall, mainly to lignin, is also a plausible way.[14] As an example, high-purity lignins exhibiting antioxidant properties have been obtained from *Cistus ladanifer* biomass,[15] spruce and eucalyptus,[16] apple tree pruning residues,[17] and sugarcane bagasse.[18]

In fact, lignin is the main renewable source of aromatic compounds that have a wide variety of applications in the biofuel, bioplastic and food sectors.[18,19] Nonetheless, the quality of the lignin and consequently, its applications vary depending on, for example: the biomass type due to its natural heterogeneity, the conditions used for its extraction,[16,17,20] as well as if it is obtained in the first steps of the valorization process or as an end co-product after obtaining other fractions, such as extractives, sugars, etc.[13,21,22] Extractives are non-structural components that do not have a structural function within the cell wall of the lignocellulosic material; this fraction includes fats, phenolic compounds, waxes and inorganics.[23]

The use of organic solvents to extract lignin preserving the reactive cellulose in the solid fraction and the subsequent solvent recovery has been reported in several studies.[17,24] Particularly, ethanol organosolv is a simple process that uses aqueous ethanol and commonly an inorganic acid as catalyst.[25,26] This pretreatment used to be effective enabling to generate lignins with high purity with great potential to produce high value lignins from different lignocellulosic materials.[27]

In the case of olive-derived biomasses, the lignin content ranges from 17% for olive mill leaves up to 43% for olive pomace.[28] Several methods have been applied to extract lignins from them. As an example, Cequier et al.[29] reported 40% lignin extraction from olive pomace after pretreatment with ionic liquids. These authors observed a positive influence of the stone/pulp ratio on the delignification of olive pomace. Rodríguez-Gutierrez et al.[30] reported a sequential pretreatment of steam explosion/alkaline to obtain olive pomace lignins for pharmaceutical applications. Moreover, olive tree pruning biomass was organosolv pretreated and the effect of the temperature and reaction time for obtaining high-purity lignins was studied.[25]

In the case of EOP, lignin fraction represents 22% of its composition and therefore, its valorization is essential.[31] Our previous work[13] reported that antioxidant lignins can be recovered from EOP. Nevertheless, the literature about fractionation options for EOP is scarce. In this work, a fractionation procedure for EOP is proposed to obtain antioxidants from extractives, lignin-derived products and glucose from cellulose fraction. For this purpose, a bioprocess including a two-step extraction followed by organosolv pretreatment and enzymatic hydrolysis was studied. Different strategies for extraction and organosolv pretreatment were applied. Once the best configuration process was selected, the lignin solubilized during the pretreatment was recovered from the liquor using two different methods, acid precipitation and evaporation. The resulting lignin samples were characterized in terms of purity and elemental analysis, thus, molecular weight distribution by gel permeation chromatography (GPC), Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy, and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) were applied. Moreover, these lignin samples were compared to that obtained after the enzymatic hydrolysis of the pretreated biomass. The overall aim of this research is to obtain bioactive compounds capable of replacing synthetic additives in food and medicinal products. This work contributes to sustainable development and especially to achieve the goal 12 of SDG of the United Nations "Responsible production and consumption".

2. Results and Discussion

2.1. Extraction of EOP with Water and Acetone

EOP has been reported as an interesting feedstock for production of sugars and other valuable compounds in a biorefinery system.[8,32] Therefore, taking into account its high extractive content, higher than 40% (**Table 1**), the integral valorization of EOP implies the recovery and characterization of this fraction. For this reason, after the first aqueous extraction of EOP, which decreased its extractive content to 15% (E1-solid),[31] a second extraction step was tested with 70% acetone (E2) or water (E3) to maximize the solubilization of this fraction.

Regardless of the solvent used, the second extraction of EOP solubilized around 10% of biomass, mainly extractives. In general, solids resulting from the second extraction with acetone (E2-solid) and water (E3-solid) showed similar composition (Table 1). Nevertheless, in terms of extractives content in E1-solid, the second aqueous extraction reached a higher extractive removal than acetone extraction, 47.4% versus 41%. Considering the two sequential extraction steps, E1+E2 removed 87.5% of extractives content in the raw material whilst the double extraction E1+E3 removed 89% of them. This indicates that the second aqueous extraction (E3) resulted to be slightly more efficient to solubilize the extractives from EOP.

2.2. Organosolv Pretreatment and Enzymatic Saccharifcation

2.2.1. Delignification by Organosolv Pretreatment

Ethanol organosolv pretreatment has been widely reported as an efficient treatment to solubilize lignin from biomass.[33] One of the objectives of this work is the recovery of the solubilized lignin from EOP to be characterized before its valorization as a source of chemicals and antioxidants. For this purpose, after the second extraction of EOP, resulting solids were pretreated with 50% ethanol at different temperatures using 1% H_2SO_4 as catalyst (**Figure 1**).

As shown in Table 1, the temperature played an important role in the organosolv pretreatment of EOP. Thus, when E2-solid was pretreated at 110 °C (P1), only 24% biomass was solubilized and the delignification yield was lower than 12%. However, using the same extracted solid (E2-solid), when the pretreatment temperature increased up to 130 °C (P2), 52% biomass was solubilized and the delignification yield increased up to 43%. This positive effect of the temperature in the organosolv ethanol process agrees with that reported for other lignocellulosic materials such as *Cistus ladanifer*

Table 1. Chemical composition of the raw exhausted olive pomace (EOP), solids obtained after first (E1) and second extraction (E2 and E3) and delignified solids by organosolv pretreatments (P1–P4).

Component	Raw EOP[26]	E1-solid[8]	E2-solid	E3-solid	P1-solid	P2-solid	P3-solid	P4-solid
Extractives	41.8 ± 1.8^{a}	15.2 ± 0.3^{b}	10.1 ± 0.3^{c}	9.0 ± 0.1^{c}	–	–	–	–
Aqueous	37.9 ± 1.9^{a}	8.2 ± 0.2^{b}	4.4 ± 0.0^{bc}	2.3 ± 0.1^{c}	–	–	–	–
Ethanolic	3.8 ± 0.2^{c}	7.0 ± 0.2^{a}	5.8 ± 0.4^{b}	6.7 ± 0.0^{a}	–	–	–	–
Glucan	9.7 ± 0.8^{e}	12.8 ± 0.5^{de}	13.9 ± 1.3^{d}	13.0 ± 1.4^{de}	19.4 ± 1.2^{c}	26.7 ± 1.7^{b}	32.1 ± 1.4^{a}	29.0 ± 1.2^{ab}
Hemicellulose	10.9 ± 0.5^{d}	17.9 ± 0.8^{a}	14.7 ± 0.9^{b}	18.6 ± 1.2^{a}	19.2 ± 0.8^{a}	12.7 ± 0.4^{bcd}	14.2 ± 0.7^{bc}	11.9 ± 0.3^{cd}
Xylan	9.8 ± 0.5^{c}	13.0 ± 0.5^{b}	10.5 ± 0.7^{c}	14.1 ± 1.1^{ab}	15.9 ± 0.7^{a}	8.6 ± 0.4^{cd}	9.8 ± 0.6^{c}	7.4 ± 0.3^{d}
Galactan	0.3 ± 0.0^{d}	3.3 ± 0.6^{a}	2.4 ± 0.2^{c}	3.1 ± 0.2^{ab}	2.5 ± 0.1^{bc}	2.3 ± 0.1^{c}	2.3 ± 0.1^{c}	1.9 ± 0.0^{c}
Arabinan	1.8 ± 0.0^{b}	2.1 ± 0.1^{ab}	2.4 ± 0.2^{a}	2.3 ± 0.1^{a}	1.7 ± 0.1^{b}	1.8 ± 0.1^{b}	2.2 ± 0.3^{ab}	2.1 ± 0.1^{ab}
Mannan	0.4 ± 0.0^{d}	1.2 ± 0.1^{c}	1.3 ± 0.1^{bc}	1.4 ± 0.1^{abc}	1.4 ± 0.2^{abc}	1.5 ± 0.0^{ab}	1.6 ± 0.1^{a}	1.5 ± 0.0^{ab}
Acetyl groups	1.5 ± 0.2^{ab}	1.0 ± 0.1^{bc}	1.9 ± 0.4^{a}	1.7 ± 0.2^{a}	1.3 ± 0.1^{ab}	0.5 ± 0.0^{cd}	0.5 ± 0.0^{cd}	0.3 ± 0.0^{d}
Lignin	21.8 ± 0.9^{d}	34.5 ± 0.5^{c}	38.6 ± 0.8^{b}	40.6 ± 0.5^{b}	44.8 ± 0.8^{a}	46.0 ± 0.7^{a}	45.4 ± 0.5^{a}	44.1 ± 0.9^{a}
AIL	20.3 ± 0.7^{e}	33.6 ± 0.3^{d}	37.3 ± 0.3^{c}	39.7 ± 0.3^{b}	43.6 ± 0.5^{a}	45.3 ± 0.6^{a}	44.5 ± 0.3^{a}	43.1 ± 0.8^{a}
ASL	1.5 ± 0.5^{a}	0.9 ± 0.4^{ab}	1.3 ± 0.8^{ab}	0.9 ± 0.5^{ab}	1.3 ± 0.7^{ab}	0.7 ± 0.3^{b}	0.9 ± 0.4^{ab}	0.9 ± 0.5^{ab}
Ash	6.4 ± 0.2^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ± 0.0^{b}
Solid recovery	–	59.0^{*}	89.1^{**}	89.0^{**}	$75.9^{\#}$	$47.7^{\#}$	$48.2^{\ddagger}$	$43.5^{\ddagger}$
Delignification	–	$6.6 \pm 1.0^{*d}$	0.0^{**}	0.0^{**}	$11.8 \pm 1.7^{\#c}$	$43.1 \pm 1.0^{\#b}$	$46.2 \pm 0.4^{\ddagger b}$	$52.8 \pm 1.0^{\ddagger a}$

In each row, different letters (superscript) indicate significant differences between the data ($p < 0.05$).
The data are expressed as mean ± standard deviation (n = 3). AIL: Acid insoluble lignin; ASL: Acid soluble lignin. E1-solid (from first aqueous extraction); E2-solid (from second extraction with acetone); E3-solid (from second aqueous extraction); P1-solid (from organosolv pretreatment of E2-solid at 110 °C); P2-solid (from organosolv pretreatment of E2-solid at 130 °C); P3-solid (from organosolv pretreatment of E3-solid at 130 °C); P4-solid (from organosolv pretreatment of E3-solid at 140 °C).
*With respect to raw EOP; **With respect to E1-solid; #With respect to E2-solid; ‡With respect to E3-solid.

biomass,[15] *Eucalyptus nitens*,[34] wheat straw,[35] or sugarcane bagasse.[36]

Nevertheless, comparing the two pretreatments carried out at 130 °C (P2 and P3), when EOP had been previously extracted by two aqueous steps (E1+E3), the subsequent P3 pretreatment reached a higher delignification yield, 46.2% versus 43.2% for E1+E2+P2. At the same time, the combination E3+P3 resulted in a solid richer in carbohydrates, 46.3% versus 39.4% for E2+P2. These results compare favorably with those previously reported for EOP, when only one aqueous extraction step was

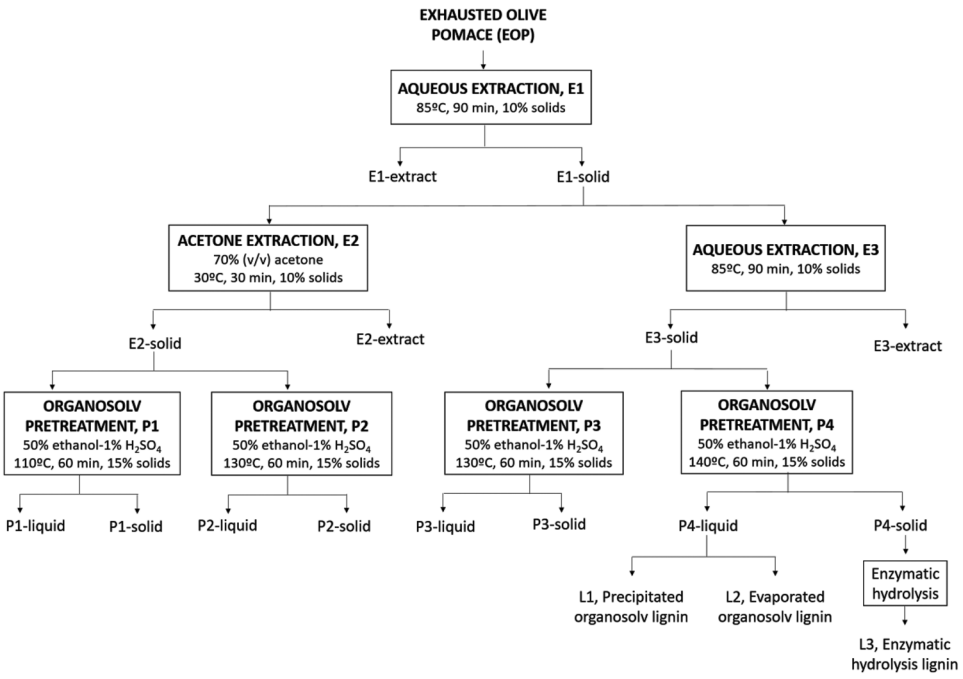

Figure 1. Flow diagram of the experimental procedure for the extraction and organosolvent pretreatment of exhausted olive pomace (EOP).

Table 2. Elemental composition of the solid obtained after first (E1-solid) and second extraction (E2 and E3-solid), organosolv pretreatment (P1, P2, P3, and P4-solid), and lignin samples L1, L2, L3.

Solids	C [%]	H [%]	N [%]	O [%]	S [%]
Raw EOP[a)]	42.42 ± 0.24	5.55 ± 0.08	1.31 ± 0.06	44.31 ± 0.02	n.d.
E1[b)]	45.41 ± 0.68	5.88 ± 0.05	1.85 ± 0.08	42.98 ± 0.04	n.d.
E2	48.66 ± 0.31	5.76 ± 0.05	1.84 ± 0.03	43.06 ± 0.12	0.48 ± 0.14
E3	49.16 ± 0.73	5.77 ± 0.07	1.69 ± 0.26	42.71 ± 0.24	0.49 ± 0.14
P1	48.64 ± 0.53	5.80 ± 0.11	1.55 ± 0.26	43.49 ± 0.27	0.23 ± 0.31
P2	50.21 ± 0.10	6.03 ± 0.03	1.24 ± 0.02	41.74 ± 0.07	0.46 ± 0.12
P3	49.75 ± 0.26	6.10 ± 0.01	1.05 ± 0.01	42.41 ± 0.09	0.42 ± 0.06
P4	49.48 ± 0.32	6.08 ± 0.01	0.86 ± 0.01	42.82 ± 0.07	0.49 ± 0.02
L1	62.97 ± 0.3	8.95 ± 0.22	1.28 ± 0.01	25.48 ± 0.12	1.31 ± 0.04
L2	46.01 ± 0.15	8.34 ± 0.15	1.14 ± 0.02	40.66 ± 0.29	3.86 ± 0.06
L3	57.69 ± 1.26	8.73 ± 0.10	1.15 ± 0.10	31.61 ± 1.47	0.82 ± 0.04

[a)]Gómez-Cruz et al.;[31] [b)]Gómez-Cruz et al.,[13] n.d.: not detected.

applied prior to an organosolv pretreatment at 130 °C, which yielded only 28% of delignification.[13] In this work, the highest delignification yield (53%) was reached when EOP was organosolv pretreated at 140 °C (P4) after a two-step aqueous extraction (E1+E3).

The elemental composition of the solids obtained after each extraction step and the pretreated solids was also analyzed and compared (**Table 2**). Carbon was the major component of EOP as in other olive biomasses,[37,38] regardless if it was or not extracted, as well as in the pretreated solids. The lowest carbon content was found in the raw EOP, which could be explained by the fact that the ash content was diminished after extraction (Table 1). Similarly to olive leaves and olive tree pruning biomass (leaves and small branches), EOP and the extracted solids (E1, E2, and E3) contained relatively high amount of nitrogen, which can lead to the formation of NOx[39] when used as heating fuels. Nonetheless, the organosolv pretreatment solubilized a part of the nitrogenous components, especially the pretreatment P4, and thus the pretreated solids showed lower amounts, which varied from 1.55% (P1) to 0.86% (P4).

E1-solid (from first aqueous extraction); E2-solid (from second extraction with acetone); E3-solid (from second aqueous extraction); P1-solid (from organosolv pretreatment of E2-solid at 110 °C); P2-solid (from organosolv pretreatment of E2-solid at 130 °C); P3-solid (from organosolv pretreatment of E3-solid at 130 °C); P4-solid (from organosolv pretreatment of E3-solid at 140 °C); L1: organosolv lignin precipitated with sulfuric acid; L2: organosolv lignin obtained by evaporation; L3: lignin from enzymatic hydrolysis.

2.2.2. Sugars Recovery by Enzymatic Hydrolysis

In addition to obtain bioactive compounds from both extractive and lignin fractions, the fractionation and integral valorization of EOP implies the recovery of glucose from delignified solids by enzymes. In this sense, ethanol organosolv pretreatment is usually reported to remove both hemicelluloses and lignin and keep most of the cellulose fraction in the substrate,[40] which is crucial for an efficient enzymatic hydrolysis and sugars valorization. Thus, after the delignification, the enzymatic digestibility of the resulting solids was evaluated by subjecting them to enzymatic saccharification tests. Enzymatic digestibility, referred to the ratio of glucose released to the glucose content in the pretreated EOP, ranged from 34% for P1-solid to 81% for P4-solid. The highest value of cellulose conversion achieved in this work (81%) compares favorably to that previously reported for EOP (76.5%) using an unique aqueous extraction step followed by an organosolv pretreatment at 130 °C.[13] Abu Tayeh et al.[8] reached a cellulose conversion of 90% from olive pomace pretreated by microwave at 140 °C with 0.6 M formic acid for 10 min.

2.3. Phenolic Composition and Antioxidant Properties of the Extracts and Liquors

2.3.1. Total Phenolic Composition (TPC) and Antioxidant Activity

In order to achieve an integral valorization of EOP, a high solubilization of the extractives is required, as commented before. For this purpose, after the first aqueous extraction (85 °C for 90 min), in which a phenolic content of 45 mg GAE g^{-1} EOP was obtained from its non-structural fraction,[31] a second extraction was performed to remove more extractives from EOP and recover more antioxidant compounds. As could be expected, the first extraction of EOP yielded the extract richest in phenolic compounds and antioxidant capacity. The second extraction step with 70% acetone (E2) and water (E3) resulted in extracts very similar in terms of TPC, with a phenolic content of around 12 mg GAE g^{-1} E1-solid (**Figure 2**). The antioxidant capacity of these extracts was also evaluated by ABTS and FRAP assays and they showed a similar behavior to TPC. According to these results and taking into account that the second extraction with water achieved a slightly higher solubilization of extractives, it was chosen as better option for the overall recovery of antioxidant compounds from the extractive fraction of EOP. In addition, it is worthy to highlight that the use of water as extraction

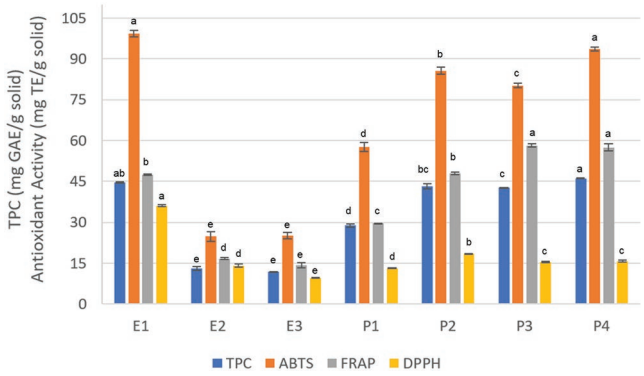

Figure 2. Total phenolic compounds (TPC) and antioxidant activity of the liquids after extraction (E1, E2, and E3) and after pretreatment (P1, P2, P3, P4). Values of phenols and antioxidant activity referred to raw, extracted o pretreated biomass, as applicable. For each parameter, bars with different letters indicate significant differences ($p < 0.05$).

solvent is classified as a safe and environmentally friendly option and an interesting alternative to organic solvents.[41]

As commented before, partial depolymerization of lignin can lead to obtain liquors with antioxidant activities and antioxidant lignins after purification.[12,13] Concerning the pretreatment liquors obtained, the highest TPC content and antioxidant capacity were determined in P4-liquor. This is closely related to the higher delignification produced by this pretreatment, since it is considered that the TPC can be related to the phenolic hydroxyl groups of the solubilized lignin,[42] which can provide antioxidant properties.[12]

2.3.2. Phenolic Profile by CZE and HPLC-RID Analysis

The EOP extracts were also characterized through CZE to determine their phenolic composition. As an example, **Figure 3** shows the electropherogram of the first aqueous extract obtained at 85 °C for 90 min. As can be appreciated, this technique allowed to tentatively identify hydroxytyrosol, tyrosol, catechol, pinoresinol, oleuropein derivative and 3-phenylphenol in E1-extract. The extracts from the second step showed similar profiles although the concentration of phenolic compounds was lower than that for the extract obtained in the first step. It can be noticed that hydroxytyrosol was the major compound identified in all the extracts, which agrees with previous works using olive pomace as raw material.[9,43,44]

P4-liquor, which presented the highest TPC and antioxidant activity values, was also studied by CZE. **Figure 4** shows its electropherogram and the compounds tentatively identified were: hydroxytyrosol, 3-hydroxybenzaldehyde, epicatechin, syringaldehyde, and acetovanillone, being these later two tipically lignin degradation compounds.[45,46] Alves-Ferreira et al.[15] also used CZE to characterize phenolics from depolymerized lignin and found vanillic acid and gallic acid as the major compounds in the organic liquor of *Cistus ladanifer* biomass, confirming that the composition in phenolic compounds depends mainly on the biomass origin.

In addition to antioxidant compounds, sugars and other derived-sugar compounds (e.g. furfural) were detected in the liquors. The effect of the pretreatment, in terms of solubilization of sugars and generation of inhibitor compounds, was determined by HPLC-RID. Glucose concentrations lower than 2 g L^{-1} were measured in all cases whilst the concentration of hemicellulosic sugars ranged from 5.1 g L^{-1} (P1-liquor) to 12.7 g L^{-1} (P4-liquor). This is attributed to the higher harshness of P4, which was performed at the highest temperature, 140 °C. P4 resulted in the highest solubilization of xylans that caused the generation of the highest furfural concentration (2.27 g L^{-1}), which was also detected by CZE at 220 nm (Figure 3). Moreover, the presence of acetic acid from the hydrolysis of hemicelluloses (up to 2.88 g L^{-1} in P4-liquor) and formic acid (up to 1.29 g L^{-1} in P1-liquor) was detected in all liquors. Overall,

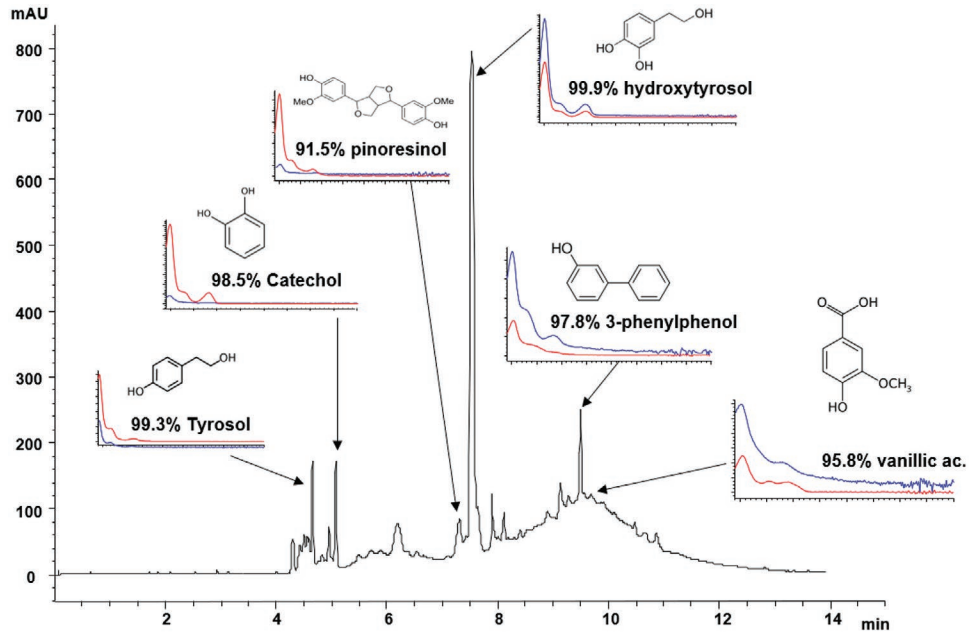

Figure 3. Electropherograms at 200 nm of the aqueous extract obtained in the first extraction step (E1; 85 °C, 90 min, 10% solids).

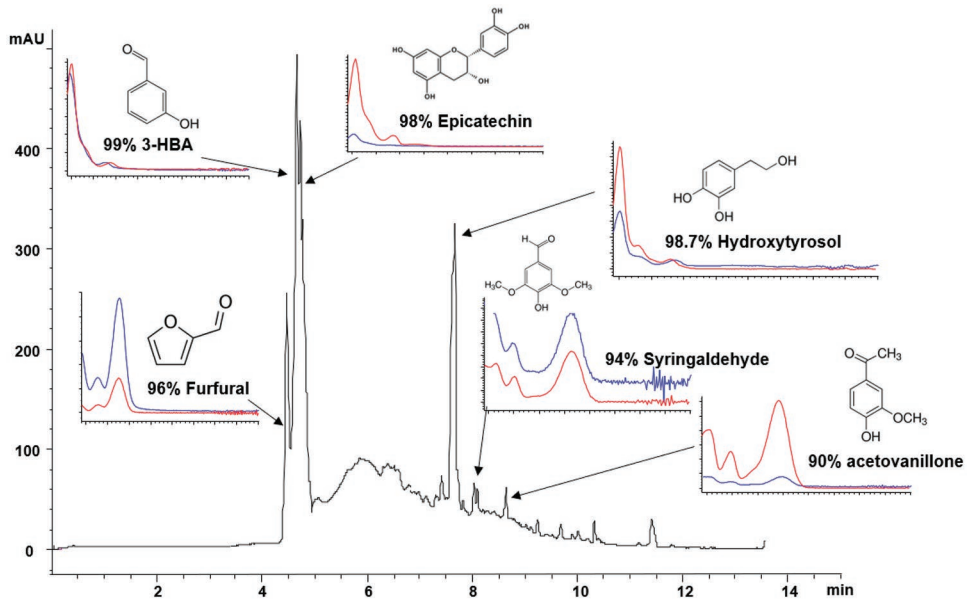

Figure 4. Electropherograms at 200 nm of the organosolv liquor from the pretreatment P4 (140 °C, 60 min, 15% solids).

low inhibitor compound concentrations were determined in the EOP liquors, which is advantageous for a potential valorization of these sugars by a bioconversion process.

2.4. Lignin

Considering the results of delignification and enzymatic hydrolysis, together with the antioxidant properties of the pretreatment liquors, the fractionation process of EOP including a two-step aqueous extraction followed by organosolv pretreatment at 140 °C (E1+ E3 + P4) was selected as the best option. Therefore, lignin was recovered from the P4-liquor to continue the valorization scheme of EOP. Thus, two organosolv lignins were obtained from the P4-liquor: one by precipitation with sulfuric acid (L1) and the other by solvent evaporation (L2). In addition, a final solid rich in lignin was also recovered after enzymatic hydrolysis of P4-solid (L3). All lignin samples were analyzed and compared through different assays.

2.4.1. Elemental Analysis, Purity, and GPC

An elemental analysis was carried out to determine the composition of the different samples and the results are represented in Table 2. Almost the same quantity of hydrogen and nitrogen was found in the three samples. As commented before, the organosolv pretreatment solubilized a part of the nitrogen content contained in the E3-solid together with lignin (Table 2). On the other hand, L1 sample showed to have the highest amount of carbon and L2 the lowest. Although sulfur was not detected in raw EOP by elemental analysis (Table 2), a relatively high sulfur content was determined in the lignin samples (Table 2), even more than in the E3-solid (Table 2). This could be due to the use of sulfuric acid as catalyst during the organosolv process. Furthermore, the presence of sulfur was more remarkable in L2 sample, since the sulfur was retained together with the lignin sample after the evaporation of the organosolv liquor.

According to Pan and Saddler,[27] the extraction and recovery of high purity lignins from biomass is crucial because the generation of added value lignin compounds depends on the quality of the lignin. **Table 3** shows the purity of the EOP lignin samples, that was assessed based on Klason lignin content, as well as the resulting carbohydrates. According to those data, the purity of the lignin obtained through precipitation with sulfuric acid (L1) was the highest one (71.1%). This result was similar to those obtained by other authors with olive tree pruning biomass employing other organosolv extraction methods (acetosolv, formosolv and acetosolv-formosolv) and a subsequent lignin recovering by precipitation with water and centrifugation.[25] On the other hand, L2 lignin was the least pure sample (46.95%), suggesting that probably some impurities that were dissolved in the organosolv liquor were retained

Table 3. Composition (%), average molecular weight (M_w), average molecular number (M_n), and polydispersity index (PI) of the lignin samples (n.d.: not detected; L1: organosolv lignin precipitated with sulfuric acid; L2: organosolv lignin obtained by evaporation; L3: lignin from enzymatic hydrolysis).

	L1	L2	L3
Composition (%)			
Klason lignin	71.10 ± 3.64	46.95 ± 10.58	61.41 ± 1.79
Glucan	1.13 ± 0.13	1.45 ± 0.35	19.54 ± 0.87
Xylan	1.55 ± 0.24	8.59 ± 2.47	n.d.
Arabinan	n.d.	1.54 ± 0.19	n.d.
Characterization			
M_w (Da)	2053	19 986	12 965
M_n	745	1067	879
PI	3.36	18.74	14.74

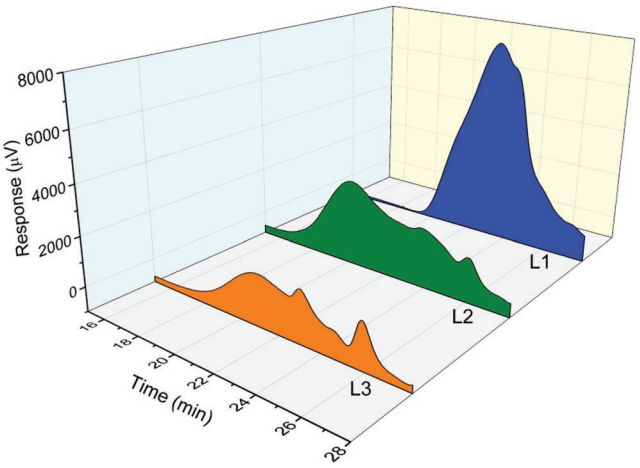

Figure 5. Gel permeation chromatograms of the lignin samples from exhausted olive pomace. L1: organosolv lignin precipitated with sulfuric acid; L2: organosolv lignin obtained by evaporation; L3: lignin from enzymatic hydrolysis.

in the resulting solid after the evaporation process. However, as can be seen, the highest amount of carbohydrates corresponded to L3 sample with 19.54% of glucan. This high glucan content indicates the incomplete hydrolysis of cellulose into glucose during the enzymatic saccharification.

The samples L1, L2, and L3 were analyzed by GPC to determine their M_w, M_n, and PI. The obtained chromatograms are shown in **Figure 5** and the corresponding results of M_w, M_n, and PI are summarized in Table 3. A different behaviour of the lignin samples is observed in Figure 4. While the lignin sample obtained by precipitation with sulfuric acid (L1) presented a low M_w and low PI, L2 and L3 samples showed high molecular weights of 19 986 and 12 968 Da, respectively, and wider molecular weight distribution, i.e. higher polydispersity indexes. The presence of carbohydrates that were not eliminated during the evaporation step (as shown in Table 3), could explain the results obtained in the case of L2 lignin. Similarly, the presence of cellulose that was not converted into glucose during the enzymatic hydrolysis process could explain the results of the L3 sample. However, organosolv lignin obtained by acid precipitation (L1), with less impurities, showed a molecular weight similar to those obtained from organosolv treatment of rice straw.[47]

2.4.2. Spectroscopy Characterization (FTIR and NMR)

The chemical characterization of the lignin samples was determined by Py-GC-MS. **Table 4** summarizes a list of 50 compounds whose area was higher than 1% referred to the total area of the chromatogram in. It showed that three lignins were mainly composed by G units although different percentages can be observed. Thus, L1 lignin presented the largest percentage of G units (44.54%), while sample L3 was the second one with an area of 26.46%. Finally, L2 lignin showed the smallest area of G units with a 15.69% of the total area. These results are in line with the conclusions obtained in the FTIR analysis. Regarding S units, the highest area corresponded to L2 lignin (9.2726%) followed by L1 and L3 with 6.90% and 2.84% of the total area, respectively.

These results enabled to give new insights into the characteristics of EOP lignin for further applications, which presented low S:G ratio; from 1:2 (L2) to 1:9 (L3). In this regard, Sampedro et al.[48] also reported the predominance of G units in lignins from olive pomace, from which EOP is obtained. Other fruits like pear fruit has also revealed low S:G ratios.[49] Alternatively, the higher predominance of G units is not characteristic of other olive biomasses, like olive tree pruning, which has shown similar or higher predominance of S units, depending on the pretreatment method, but including organosolv delignification.[25,50–52] Moreover, although G-lignin is likely to form relatively stable, not easily degraded C=C bonds,[49] the conditions applied lead to a purified lignin L1 with low M_w, as commented before. Although it has been suggested that lignins obtained from organosolv pretreatment generally have low molecular mass, it also depends on the delignification conditions applied.[25]

2.4.3. Chemical Characterization by Py-GC-MS

The chemical structure of the lignin samples was analyzed by FTIR and the obtained spectra are shown in Figure S1a,b (Supporting Information). A list of the vibrational modes which were identified in the FTIR spectra are summarized in Table S1 (Supporting Information).

There were no significant differences in the signals corresponding to the OH stretchings (3362 cm^{-1}) and C-H stretch in methyl and methylene groups (2920 and 2848 cm^{1}), which can be attributed to the lignin molecule as well as to the carbohydrate structure. The most significant differences among the analyzed samples were found in the fingerprint region, which is shown in Figure S1b in the Supporting Information. However, in this region certain distinctive lignin and carbohydrate signals could appear overlapped.

Table S1 (Supporting Information) summarizes the signals that can be attributed to lignin samples. The aromatic structure of the samples was proved since in the three lignin samples the bands related with the aromatic ring were visible (1606, 1511, and 1427 cm^{-1}). However, these signals were less intense for L2 sample and since in the case of L3 sample the band at 1606 cm^{-1} had almost disappear. The presence of syringyl (S) and guaiacyl (G) units were confirmed by the bands at 1114 cm^{-1} (S) and 1267 cm^{-1}, 1214 cm, 1031, and 863 cm^{-1} (G) and both S and G units at 1327 cm^{-1}. However, as it can be observed, bands assigned to G ring at 1327 and 1267 cm^{-1} were not visible in L2 lignin sample. Additionally, the band at 1244 cm^{-1} wavenumber which is attributed to G units was not clearly identified since it could be overlapped by the broad peak at 1186 cm^{-1} which is assigned to hemicelluloses. As mentioned above, in the fingerprint region bands assigned to carbohydrates can be found overlapped with lignin bands. Thus, signals at 1721 cm,1427, 1186–1160, and 1030 cm^{-1} can also be attributed to hemicellulose and cellulose.[53,54]

The functional groups of the different lignin samples were determined by ^{13}C-NMR analysis. A comparison between the different spectra obtained for each sample is shown in

Table 4. Compound identification in lignins from exhausted olive pomace through Py-GC-MS (L1: organosolv lignin precipitated with sulfuric acid; L2: organosolv lignin obtained by evaporation; L3: lignin from enzymatic hydrolysis.G: Guaiacyl, S: Syryngyl, FA: Fatty acid, P: *p*-hydroxyphenyl, CA: Carbohydrate).

		L1		L2		L3		
Retention time	Compound		Area [%]		Area [%]		Area [%]	Origin
3.491	1-Heptene	×	–	×	–	√	3.74	
4.281	Toluene	√	3.24	√	3.78	√	2.68	
5.103	Furfural	√	1.23	×	–	×	–	CA
5.166	Pyrazole, 1,4-dimethyl-	×	–	√	1.29	×	–	
5.212	Furan, 2,5-dimethyl-	×	–	×	–	√	1.20	CA
5.639	Ethylbenzene	×	–	√	1.98	×	–	
5.692	Pyridine, 2-methyl-	√	1.34	×	–	×	–	
6.055	Bicyclo[4,2,0]octa-1,3,5-triene	×	–	√	2.11	×	–	
7.257	Pyridine, 3-ethyl-	√	3.73	√	1.54	√	3.17	
7.355	2,4-Hexadiyne	×	–	×	–	√	1.19	
7.539	Phenol	√	1.02	√	1.86	√	1.54	P
7.661	Acetic acid, phenyl ester	×	–	×	–	√	1.22	
7.782	Hexanoic acid, ethyl ester	√	1.11	×	–	×	–	
8.856	Bicyclo[3,2,0]heptane, cis-	×	–	×	–	√	1.05	
8.845	*o*-Cresol	√	3.36	×	–	×	–	P
9.053	*m*-Cresol	×	–	√	1.76	×	–	P
9.105	*p*-Cresol	√	3.25	×	–	√	3.08	P
9.272	4-Undecene, (E)-	×	–	×	–	√	1.23	
9.388	Guaiacol	√	17.46	√	8.29	√	7.91	G
9.474	Pyridine, 5-ethyl-2-methyl-	×	–	×	–	√	1.54	
10.468	Phenol, 2,4-dimethyl-	√	1.16	×	–	×	–	P
10.733	Phenol, 3-ethyl-	×	–	√	2.71	×	–	P
10.785	Phenol, 4-ethyl-	√	1.49	×	–	√	2.15	P
11.068	3-Methylguaiacol	×	1.91	×	–	√	1.45	G
11.213	Cyclododecane	×	–	×	–	√	1.41	
11.461	4-Methylguaiacol	√	15.21	√	3.51	√	7.76	G
12.044	Cyclodecene	×	–	×	–	√	1.82	
13.419	3-Methoxycatechol	√	2.33	√	3.29	√	1.17	S
13.898	4-Ethylguaiacol	√	5.93	√	1.08	√	2.88	G
15.042	4-Vinylguaiacol	√	2.76	√	1.48	√	2.31	G
16.075	Syringol	√	4.56	√	4.95	√	1.67	S
16.214	4-Allyguaiacol	×	–	×	–	√	1.16	G
16.445	4-Propylguaiacol	√	1.26	×	–	√	1.12	G
18.241	4-Methoxy-2-methyl-1-(methylthio)benzene	√	3.09	×	–	×	–	
18.155	2H-Pyran-2-one, 3-acetyl-4-hydroxy-6-methyl-	×	–	√	2.21	×	–	
18.253	Propenylguaiacol	×	–	×	–	√	1.87	G
18.981	Naphthalene, 1,2,3,4-tetrahydro-2,2,5,7-tetramethyl-	×	–	×	–	√	1.71	
19.697	Homovanillic Acid	×	–	√	1.32	×	–	G
20.205	Naphthalene, 1,4,5-trimethyl-	×	–	√	1.11	×	–	
20.216	Naphthalene, 1,6,7-trimethyl-	×	–	×	–	√	1.46	
20.569	2,4′-Dihydroxy-3′-methoxyacetophenone	×	–	√	1.69	×	–	
21.712	8-Heptadecene	×	–	×	–	√	1.63	
22.105	4-Allylsyringol	×	–	√	1.03	×	–	S

Table 4. Continued.

Retention time	Compound	L1		L2		L3		Origin
			Area [%]		Area [%]		Area [%]	
24.975	*n*-Hexadecanoic acid	×	–	√	5.48	√	2.48	FA
25.027	cis-7,cis-11-Hexadecadien-1-yl acetate	×	–	√	1.69	×	–	
25.258	Hexadecanoic acid, ethyl ester	×	–	×	–	√	1.39	FA
26.644	Oleic acid	×	–	√	1.78	×	–	FA
26.875	Octadecanoic acid	×	–	√	3.59	√	1.26	FA
26.927	Ethyl oleate	×	–	√	4.41	√	3.77	FA
	TOTAL S		6.89		9.27		2.84	
	TOTAL G		44.53		15.69		26.46	

Figure 6. The aromatic carbon region (100–160 ppm) is the most important area in the ^{13}C-NMR lignin analysis since different types of aromatic carbon of lignin can be observed.[55] Notice that the signal of the tertiary aromatic carbons of the S units of lignin (103–110 ppm) is not appreciable in the spectra of L3 sample. This is in agreement with the results obtained by pyrolysis analysis, in which the L3 sample showed the lowest amount of S units (Table 4). On the other hand, signal of the tertiary aromatic carbon of the G units (110–123 ppm) was visible in the three lignin samples. As expected, this signal was more intense for the L1 sample since the amount of G units obtained in the Py-GC-MS analysis was higher than in L2 and L3 samples (Table 4). Furthermore, as expected from the previous results obtained by FTIR and Py-GC-MS, signals corresponding to quaternary (C_{Ar}-O) and non-oxygenated (C_{Ar}-C) carbons in the regions of (156–137 ppm) and (137–123 ppm) respectively, decreased for L2 and L3 samples in comparison to L1. An explanation for this decrease of aromatic structures in L2 and L3 samples could be the high contamination of the samples.

2.5. Overall Process Material Balance

Fractionation scheme selected for EOP includes i) a double aqueous extraction (E1 + E3), ii) organosolv ethanol pretreatment at 140 °C (P4), iii) recovery of organosolv lignin by acid precipitation, and iv) enzymatic hydrolysis of the delignified solid. This processing scheme has shown the best results in terms of obtaining extracts with antioxidant properties, solubilization of lignin with higher antioxidant properties, recovery of lignin with higher purity and glucose recovery by EH. The mass balance of the whole fractionation process for EOP is shown in **Figure 7**.

The two-step aqueous extraction of EOP was carried out using 10% of biomass. This solid loading was chosen as acceptable, because the use of higher values hinders the extraction of phenolic compounds[31] although the production of concentrated extracts is desirable. On the basis of 100 g of raw EOP, 52.5 g solids can be recovered after a two-step aqueous extraction. The two-step aqueous extraction solubilized almost 90% of the extractives in raw EOP and allowed recovering all phenolics content in that fraction, 5.1 g phenolic compounds per 100 g EOP. In addition, the presence of mannitol was detected in the two aqueous extracts, mainly in E1-extract, which accounted for 4.5 g per 100 g of raw EOP. Mannitol is an interesting compound with antioxidant properties and applications in pharmaceutical and chemical industries that has been reported in extracts of olive leaves, olive pomace and olive tree pruning biomass.[28,56,57]

The next process step, organosolv pretreatment, was performed at 15% of solids and yielded 22.8 g delignified solid (in the basis of 100 g raw EOP) and a liquor with sugars, mainly, hemicellulosic sugars. Moreover, phenolic compounds from the solubilized lignin (2.4 g per 100 g of raw EOP) were detected in this liquid stream. This organosolv lignin was recovered by acid precipitation (L1) with a purity of 71% (Table 3), a carbon content of 63% (Table 2), and a higher predominance of aromatic structures compared to the other lignin samples (Table 4).

The cellulose fraction of the delignified solid resulting from the P4-pretreatment was converted into glucose by enzymes, with an efficiency of 81%. The overall mass balance indicated a partial solubilization of glucose during the extraction whilst organosolv pretreatment was able to preserve the cellulose content in biomass. The overall recovery of glucose in the liquid streams was over 86%, confirming that selected process can recover most of the glucose content in raw material. Nevertheless, the greater chemical reactivity of xylose versus glucose resulted in a lower recovery of hemicellulosic sugars in liquid streams, 50.4%.

After enzymatic hydrolysis step, a lignin-rich solid is obtained (L3) although with lower purity than the organosolv lignin due to the presence of residual cellulose that enzymes did not hydrolyze. The selected process scheme allowed a good fractionation of the biomass and the recovery of sugars, biocompounds from both extractive and lignin fractions and high purity lignins.

3. Conclusions

The fractionation scheme proposed in this work means the integral valorization of exhausted olive pomace. This strategy process includes a two-step aqueous extraction at 85 °C followed by organosolv ethanol pretreatment at 140 °C and enzymatic saccharification. The two-step aqueous extraction

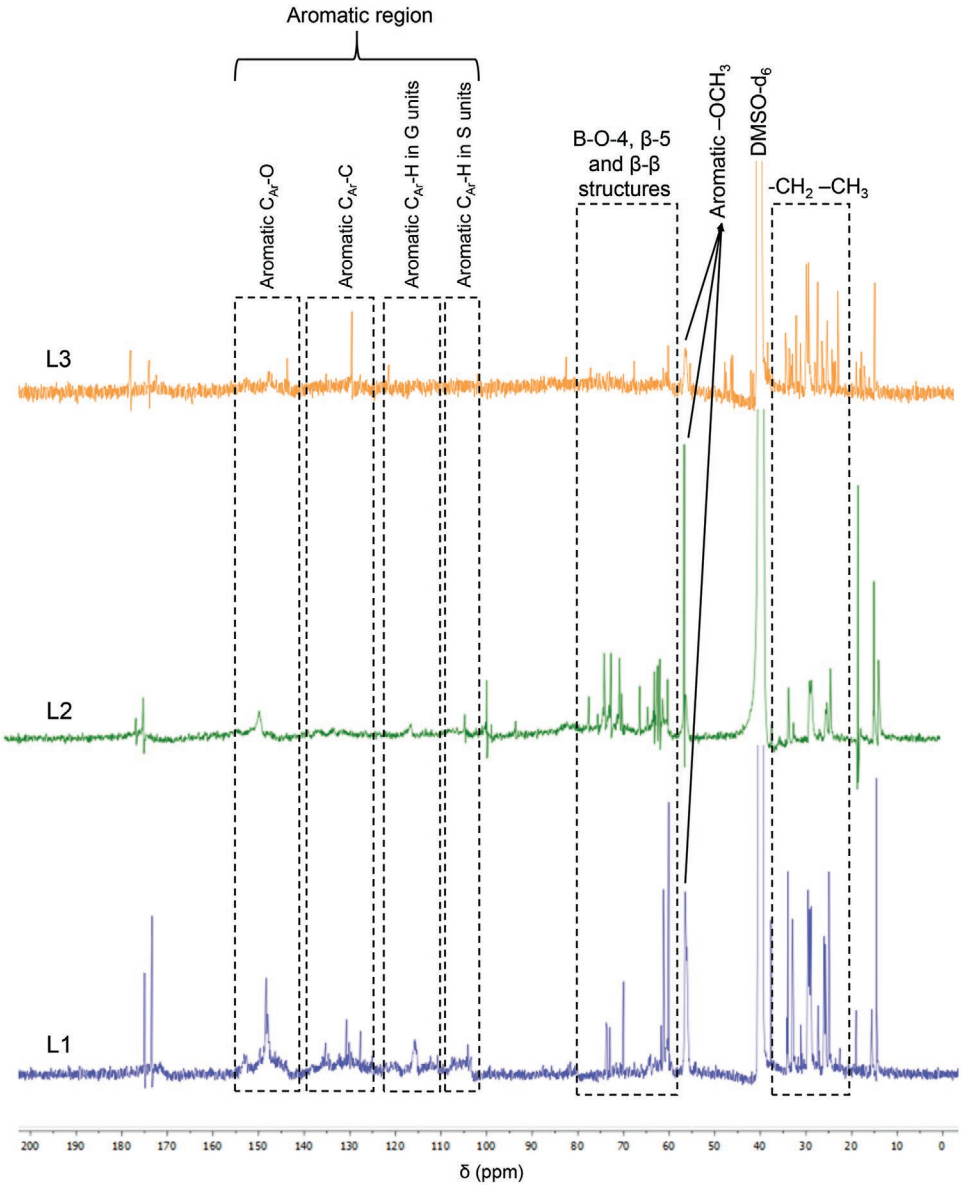

Figure 6. NMR Spectra the functional groups of the lignin samples from exhausted olive pomace. L1: organosolv lignin precipitated with sulfuric acid; L2: organosolv lignin obtained by evaporation; L3: lignin from enzymatic hydrolysis.

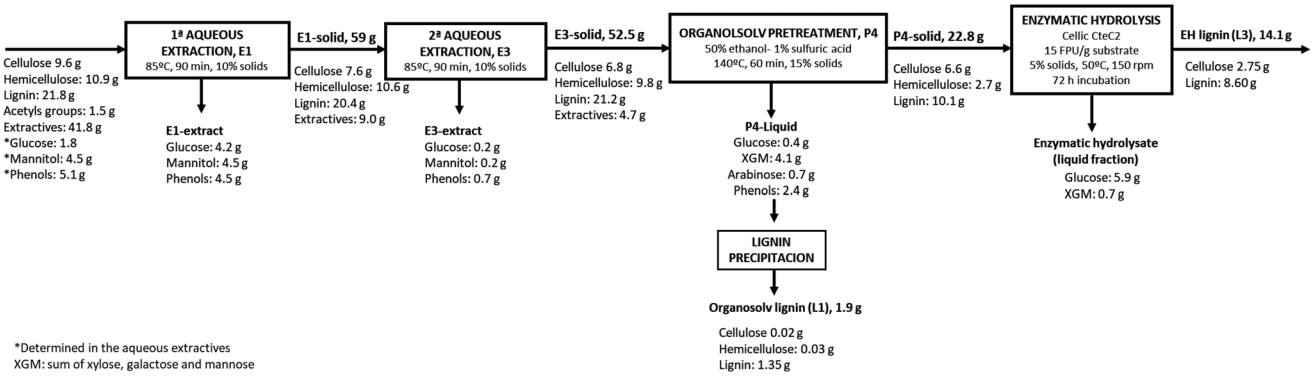

Figure 7. Material balance of the fractionation process of exhausted olive pomace (EOP) for the selected strategy.

removed almost 90% of the extractives in raw EOP, and allowed to recovering all phenolic compounds content in that fraction. Organosolv pretreatment yielded a high purity organosolv lignin (>71%), composed mainly by guaiacyl units, and a delignified solid with high enzymatic digestibility (>81%). Further research should focus on improving the enzymatic hydrolysis step both to recover more glucose and to obtain a lignin with fewer impurities.

4. Experimental Section

Aqueous and Acetone Extraction: The EOP, partially pitted and pelletized, was supplied by the company 'Spuny SA' located in the province of Jaén, Spain. The average pellet length was 14.5 mm, and the average diameter, 4.6 mm.

The EOP used in this work was subjected to two sequential extraction steps (Figure 1). The first previously optimized aqueous extraction step (E1-85 °C, 90 min and 10% solids) was carried according to Gómez-Cruz et al.[31] As a result, two fractions were obtained: an aqueous extract (E1-extract) and a solid fraction (E1-solid). Nevertheless, in order to remove more extractives that may interfere in the subsequent process steps and to recover more antioxidant compounds, E1-solid was subjected to a second extraction step. Two solvents were tested in the second extraction step. A mixture acetone:water (70% v/v) at 30 °C, for 30 min using 10% solid loading (E2), performed in a Medline Scientific™ SI-600R orbital shaker at 200 rpm (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and another E1-solid aliquot was subject to an aqueous extraction in the same conditions of the first extraction step (E3) (Figure 7).

The resulting solids after vacuum filtration were dried at 40 °C to determine the solid recovery and they were preserved and stored at 4 °C together with the liquid extracts for further analysis.

Organosolv Pretreatment and Lignin Recovery: The resulting solids from the second extraction with 70% acetone (E2-solid) and water (E3-solid) were subjected to organosolv pretreatment, in a laboratory scale 1-L stirred tank reactor (Parr Instrument Company, Moline, IL, USA). According to previous works[13], organosolv pretreatment was carried out with 50% ethanol solution, catalyzed with 1% sulfuric acid for 60 min and different temperatures were tested. E2-solid was pretreated at 110 °130 °C and E3-solid was pretreated at 130 and 140 °C (Figure 1). A biomass loading of 15% (w/v) was used in all pretreatment experiments.

Once reached the pretreatment time, the reactor was immediately cooled in an ice bath and the hydrolyzate obtained was separated from the residual solid by vacuum filtration. The pretreated solids were washed with water to remove residual ethanol and acid, dried at 40 °C. The solid recovery after organosolv pretreatment was determined with respect to the extracted EOP. Pretreated solids and liquors were stored for further analysis.

The best extraction and pretreatment conditions were selected and the lignin solubilized in the pretreatment liquor under those conditions was recovered. Two methods were used for recovering the solubilized lignin, one by acid precipitation and the other by evaporation. The lignin recovery by acid precipitation (L1) was performed according to the procedure described by Gómez-Cruz et al.[13] For recovering lignin by evaporation (L2), the pretreatment liquor was concentrated about twice in a rotavapor. Then, the remaining liquid in the flask was decanted and the residual solid on the flask walls was washed with pure ethanol using a volume equal to the initial amount of the liquor. Ethanol was removed from the suspension using a rotary evaporator and finally, the resulting solid was dried in an oven at 40 °C.

Characterization of Extracted and Pretreated Solids: The chemical composition of solids from the second extraction step (E2-solid and E3-solid) and the delignified solids resulting from the pretreatment (P1, P2, P3 and P4-solids) was determined according to National Renewable Energy Laboratory (NREL) analytical methods for biomass.[58] All analytical determinations were carried out in triplicate.

The elemental composition of extracted and pretreated solids (C, H, N, and S) was analyzed using a TruSpec Micro device (Leco, St. Joseph, MI, USA). The lignins obtained in section 4.2 and after enzymatic hydrolysis were also subjected to elemental analysis.

Enzymatic Saccharification: The solids resulting from the organosolv pretreatment were enzymatically hydrolyzed to know the influence of the experimental pretreatment conditions on their enzymatic digestibility. Enzymatic hydrolysis (EH) experiments were carried according to the procedure described in Martínez-Patiño et al.[59] The glucose concentration in the enzymatic hydrolyzates (liquid fractions) was determined by high performance liquid chromatography (HPLC) provided with a refractive index detector (RID). The results of the enzymatic hydrolysis were expressed referred to glucose in the delignified solids (enzymatic digestibility).

Characterization of Extracts and Pretreatment Liquors—Total Phenols Content and Capillary Zone Electrophoresis Analysis: The total phenolic content (TPC) in the extracts and the pretreatment liquors was determined by Folin–Ciocalteu assay[60] with some modifications[31] using a Bio-Rad iMarkTM microplate absorbance reader (Hercules, CA, USA). Gallic acid was used as standard and the results expressed as milligrams of gallic acid equivalent (GAE) per gram of EOP. All the measurements were carried out by triplicate.

Capillary zone electrophoresis (CZE) from Agilent Technologies (Waldbronn, Germany), was employed to identify the phenolic profile of EOP extracts.[61] Finally, by comparing the migration time and UV spectra with those of the standards stored in the internal library of the Agilent 3D-CE ChemStation software (Rev B.04.01), the different phenolic compounds in the electropherograms obtained were identified.

Characterization of Extracts and Pretreatment Liquors—Antioxidant Capacity Assays: Antioxidant activity of the EOP extracts and the pretreatment liquors was determined using the ABTS (2,20-azino-di(3-ethylbenzothiazoline-6-sulfonic acid) and ferric reducing power assays (FRAP). The measurements were carried out by triplicate at 734 nm for ABTS and 593 nm for FRAP[31] in a Bio-Rad iMarkTM microplate absorbance reader, as before. Trolox was used as standard for both assays and the results were expressed in milligrams of Trolox equivalent (TE) per gram of EOP.

Characterization of Extracts and Pretreatment Liquors—HPLC-RID Analysis of Sugars and Derived-Sugar Compounds: The content of the pretreatment liquors in sugars, acetic acid, furfural, 5-hydroxymethylfurfural, and formic acid was analyzed by HPLC-RID, using a ICSep ICE–COREGEL 87H3 column (Transgenomic, Inc., Omaha, NE, USA) operating at 65 °C with 5 mM sulfuric acid as the mobile phase (0.6 mL min^{-1}). The same equipment and operational conditions were used to determine the glucose content in the enzymatic hydrolyzates.

Characterization of Extracts and Pretreatment Liquors—Lignin Characterization: The purity of lignin samples was determined according to TAPPI T-249-cm-09 standard. The lignin samples were subjected to a two-step acid hydrolysis according to Prado et al.[62]

Average molecular weight (M_w), average molecular number (M_n) and polydispersity index (PI) of lignins were analyzed through GPC using a JASCO instrument equipped with an interface (LC-NetII/ADC) and a reflex index detector (RI-2031Plus). Two serial columns PolarGel (300 × 7.5 mm) (Agilent Technologies, California, EE.UU) were employed and the eluent of the samples and the mobile phase was *N*,*N*-dimethylformamide with 1% of lithium bromide. The flow rate was 0.7 mL min^{-1} and the analyses were carried out at 40 °C. Calibration was carried out using polystyrene standards (Sigma-Aldrich) in the range of 70 000 to 266 g mol^{-1}.[52,63]

The chemical structure of lignins was analyzed by FTIR characterization employing a Perking Elmer Spectrum Two FT-IR spectrometer, which is equipped with a Universal Attenuated Total Reflectance accessory with an internal diamond crystal lens. 18 scans were accumulated in transmission mode employing a resolution of

4 cm^{-1} from 4000 to 600 cm^{-1}. The functional groups of the different lignin samples were determined by ^{13}C-NMR analysis. NMR spectra were recorded at 30 °C on a Bruker Avance 500 MHz equipped with a z-gradient BBI probe. Typically, 40 mg of sample were dissolved in DMSO-d6.[64]

The chemical composition of the lignin samples was determined by analytic pyrolysis (Py-GC-MS) according to Sequeiros and Labidi[52] with some modifications. A CDS analytical Pyroprobe 5150 was employed at 600 °C for 15 s with a heating rate of 2 °C ms^{-1}. A GC (7890A)-MS (5975C inert MSD with Triple-Axis Detector) Agilent provided with a capillary column HP-%MS (5%-Phenyl-methylpolysiloxane, 30 m × 0.25 mm) with Helium as carrier gas was used for the identification of the products obtained after pyrolysis. The temperature programme was started at 50 °C and held for 2 min, then raised to 120 °C at 10 °C min^{-1}, kept for 5 min, increased again to 280 °C at 10 °C min^{-1}, held for 8 min and finally, elevated to 300 °C at 10 °C min^{-1} holding for 10 min.

Statistical Analysis: An ANOVA analysis was performed using the post hoc test Bonferroni using Statgraphics Centurion XVII (StatPoint Technologies, Inc., Warrenton, VA, USA).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords

capillary zone electrophoresis, exhausted olive pomace, lignin, organosolv pretreatment, phenolic compounds

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