An integrated olive stone biorefinery based on a two-step fractionation strategy

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Abstract

Olive stones (OS) constitute a waste lignocellulosic material produced by the olive oil industry in great amounts, that currently is only used as a low-value energy source for industrial or domestic boilers. Having in view its full valorization, this work proposes and validates an integrated strategy aiming to obtain three different streams of sugars / lignin-derived compounds. Dilute acid hydrolysis was used to obtain a xylose-rich hydroysate that was chemically converted into furfural with a 48.7 % yield. The resulting acid-pretreated solid biomass that consisted mainly of lignin and cellulose, was subjected to a catalyzed ethanol-based organosolv delignification. Temperature, time, and sulphuric acid concentration were optimized in order to recover added-value lignin products and digestible cellulose.

At the optimal conditions (190 °C and 30 min), a 50 % delignification was reached, together with the highest enzymatic hydrolysis yields (190 g glucose/kg of OS). Phenolic compounds content in organosolv liquors reached 41.6 mg GAE/g OS. This extract presented an antioxidant capacity up to 10.9 mg TE/g OS. The pretreated solid fraction was used as a substrate for ethanol production by a pre-saccharification and simultaneous saccharification and fermentation process, enabling to obtain an ethanol concentration of 47 g/L, with a fermentation yield of 61.4% of the theoretical maximum. Globally, from 100 kg of OS processed according to this experimental scheme, 6.9 kg of furfural, 6.2 kg of ethanol, 7.4 kg of lignin, and 4.2 kg of phenolics compounds can be obtained as main products, thus constituting a way of valorization of renewable material in a multiproduct biorefinery strategy.

Keywords

Bioethanol, Biomass pretreatment, Ethanol-water organosolv, Furfural, Lignin-derived products, Olive oil industrial waste

1. Introduction

Lignocellulosic biomass (LCB) has a high potential for the production of fuels and value-added chemicals, in a biorefinery concept (Scopel and Rezende, 2021). The production of bioethanol and petroleum-derived products from residual LCB is a sustainable way to reduce dependence on fossil fuels, due to their abundance, renewability, and non-competition with food (Ferreira and Taherzadeh, 2020). One example of such lignocellulosic biomass are the olive stones.

Olive stones (OS) are a LCB generated in the olive oil production process, where other olive-derived residual biomass is produced, making this agroindustrial sector an interesting source of raw materials in a biorefinery context (Ruiz et al., 2017). To obtain olive oil, the whole olive is subjected to milling as a first step, then a malaxing process, and finally the separation of the oil from the rest of the mixture (olive pomace). The olive pomace is further conducted to an extraction facility to obtain olive pomace oil by solvent extraction and a final solid residue called exhausted olive pomace (EOP).

In recent years, the separation of the OS from the milled olives or from the final olive pomace has become frequent practice, due to its use as fuel in small industrial and domestic boilers (Manzanares et al., 2017), but still, this is a niche market. In order to illustrate the volume of available olive stones, and considering only Spain, as the main olive-producing country in the world, an estimation of 750,000 tonnes of OS is considered, based on the fact that it produces about 7.5 million tonnes of olive fruit in 2021 (https://www.fao.org/faostat/en/), and assuming that OS accounts for approximately 10 % by weight of the olive fruit (Romero-García et al., 2014). Moreover, OS has the advantage of not needing to be milled for its use and of being located in the olive mills and olive pomace extracting industry, reducing costs and facilitating operational logistics.

Chemically, OS mainly contains lignin (35.6 %), followed by hemicellulose (26 %), and cellulose (20.9 %) (Padilla-Rascón et al., 2020b), which makes it suitable for valorization in the context of

66 a LCB biorefinery. However, its complex structure and recalcitrance makes it difficult to take
67 full advantage of the different fractions contained in OS.

68 Multiple pre-treatments have been reported to overcome the recalcitrant structure of LCB by
69 breaking the polymeric bonds (Bhatia et al., 2020). In general, more severe conditions are
70 required for the recovery of cellulose and lignin than those used for the recovery of the
71 hemicellulosic fraction, since it is a more labile fraction and degrades more easily (Huijgen et
72 al., 2012; Romero et al., 2015; Zhao et al., 2009). Therefore, a two-stage pre-treatment is
73 preferred to recover both hemicellulose and cellulose sugars with adequate yields (Padilla-
74 Rascón et al., 2020b).

75 Dilute acid and liquid hot water pre-treatments are two widely used pre-treatment methods
76 for the hydrolysis of the hemicellulosic fraction (Yang et al., 2020). In previous work on OS, the
77 conditions to maximise the recovery of hemicellulosic sugars in the liquid fraction were
78 optimized (128 °C, 10.5 g acid/100 g OS and 33 % solids) leaving a pretreated solid that
79 consists mainly of cellulose and lignin (Padilla-Rascón et al., 2020b). Lignin is often considered
80 a physical barrier blocking the access of enzymes to cellulose hydrolysis which binds to
81 cellulase complex in a non-productive way and deactivates it (Miliotti et al., 2019). The use of
82 organosolv pre-treatments aims at delignification together with partial hydrolysis of
83 hemicellulose and a reduction in the degree of polymerisation and crystallinity of the cellulose
84 fraction (Mosier, 2005; Taherzadeh and Karimi, 2008). This makes the cellulose fibrils more
85 accessible to enzymes and increases the yields of enzymatic saccharification (Huijgen et al.,
86 2012; Pan et al., 2005). In addition for increasing enzymatic saccharification, organosolv pre-
87 treatment has the advantage of producing high purity lignin streams (Huijgen et al., 2012),
88 which is of relevance, as lignin is a potential source for obtaining aromatic chemicals, such as
89 phenolic compounds with antioxidant properties (Zakzeski et al., 2010). The most promising
90 organosolv solvents are ethanol, formic acid, and acetic acid. In particular, the most studied

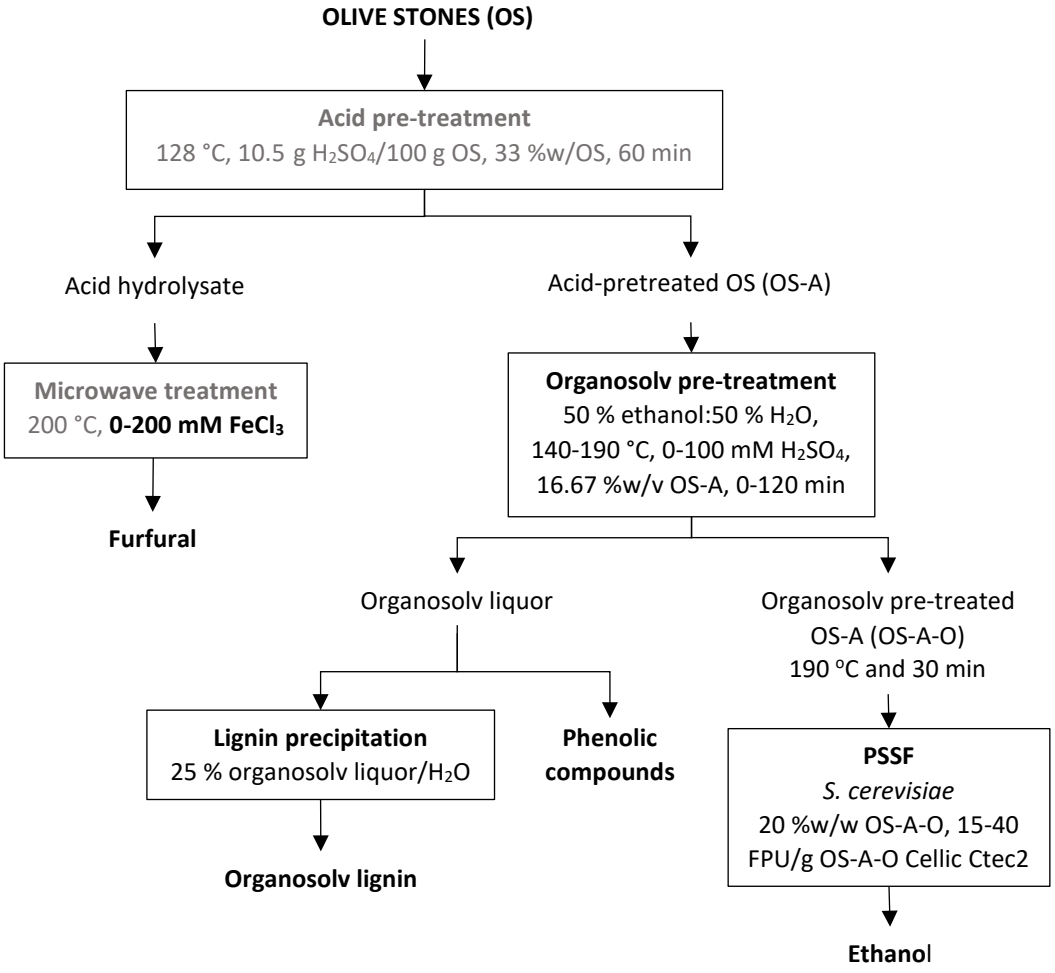
and used is ethanol, as it can solubilise lignin, has low toxicity, high volatility, is easily recyclable, cheap and green (Chen et al., 2015).

However, due to the high recalcitrance of OS, the delignification of this biomass has been scarcely studied as the data obtained were not very promising. Thus, to avoid biomass recalcitrance and to take full advantage of biomass composition, process configurations involving a two-stage pre-treatment, in which a second organosolv stage is employed have been studied for different LCB, such as rice straw (Moniz et al., 2015), wheat straw (Huijgen et al., 2012) and grape stalks (Amendola et al., 2012).

The aim of this work was to study the effectiveness of organosolv pre-treatment in an integrated OS fractionation strategy, in order to optimise the recovery conditions of sugars (mainly glucose and xylose) and lignin. The proposed scheme involved a first dilute acid pre-treatment step for the recovery of xylose, which was used for the production of furfural. The second organosolv pre-treatment step aimed to improve the cellulose saccharification potential by lignin solubilization. The recovery of lignin and bioactive phenolic compounds in the liquid fraction has also been addressed. Ethanol production from the cellulose enriched solid by a pre-sacchararification and simultaneous saccharification and fermentation process (PSSF) has been evaluated.

2. Material and methods

The strategy to upgrade OS-A here developed is detailed in Figure 1. It comprises a two-step fractionation approach to enable the full upgrade of both the polysaccharides and the lignin fractions.



113

114 **Figure 1**. Scheme summarising the strategy used for the valorisation of the main OS fractions.
115 In grey, is the experimental procedures optimised in previous works (Padilla-Rascón et al.,
116 2020b, 2021).

117

118 ## 2.1. Raw material

119 Shattered olive stones (OS) were kindly provided by a local company in Jaén (Andalucía, Spain).
120 They had a moisture content of 8 % and a particle size between 0.1-0.32 cm. OS were
121 subjected to acid hydrolysis pre-treatment in an autoclave, under previously optimized
122 conditions for efficient solubilisation of the hemicellulosic fraction (128 °C, solid/liquid ratio of
123 33 % and 10.5 g H_2SO_4/100 g OS, for 60 min) (Padilla-Rascón et al., 2020b). The process was
124 performed in 1 L-ISO bottles filled with 500 mL solution and 165 g dry OS, and multiple
125 repetitions were performed to obtain enough amount of pre-treated material. The resulting

slurry was vacuum filtered to separate the liquid from solid fractions. The xylose enriched liquid fraction was chemically characterised as described in section 2.7.2 and stored at 4 °C, then subsequently used to obtain furfural. The solid fraction was washed with distilled water, dried at 40 °C, mixed to homogenise, chemically characterised (according to section 2.7.1) and stored at room temperature for further use. The solid fraction obtained in this acid pre-treatment (OS-A) formed mainly by cellulose and lignin, was the biomass used as raw material to be fractionated by organosolv pre-treatments in the present work.

2.2. Organosolv pre-treatment of OS-A

The organosolv treatment was carried out in a 600 mL Parr reactor (482 model), using OS-A as feedstock, and an ethanol/water mixture (50:50, w/w) as usually described before, e.g. (Alves-Ferreira et al., 2021; Wildschut et al., 2013). The solid/liquid ratio used was 16.7 %. The previous conditions were fixed for all experiments, but the effects of temperature (140, 165, and 190 ^{o}C), sulphuric acid concentration (0, 50 or 100 mM), and reaction time (0-120 min) were studied.

Upon the reaction completion, the reactor was rapidly cooled down to 50 °C and the solid and liquid fractions were separated by vacuum filtration. The solid fraction (OS-A-O) was washed with the same volume of ethanol/water solution used in the organosolv pre-treatment and subsequently, with twice the volume of distilled water. When required, the solid pretreated biomass was dried at 40 °C and stored at room temperature. Pretreated biomass was analysed according to section 2.7.1. The liquid fraction was analysed according to section 2.7.2 and stored at 4 °C.

2.3. Lignin recovery

The delignification yield in the solid fraction after organosolv pre-treatment was calculated from the acid-insoluble lignin (AIL) content in the pretreated OS before (OS-A) and after

organosolv pre-treatment (OS-A-O). AIL is considered to be the acid-insoluble fraction obtained after the solid characterization (Section 2.7.1) (Eq. (1)). Calculations were performed according to (Moniz et al., 2015).

$$\text{Delignification yield (\%)} = 100 - \text{organosolv solid yield (\%)} \cdot \frac{\text{AIL OS A O (g)}}{\text{AIL OS A (g)}} \quad (1)$$

Solubilised lignin in the liquor obtained from the organosolv treatment was precipitated by diluting the hydrolysates with water (4:1, water:liquor ratio w/w), incubated in an orbital shaker (Comecta, Spain) for 2 h at 30 °C, and then the mixture was separated by centrifugation (6000 rpm) for 20 min at room temperature (centrifuge Ortoalresa, Digicen 21R, Spain) and the supernatant was separated from the precipitated material by decanting. The recovered lignin precipitate was dried at 40°C for 48 h and quantified by weighing (Eq. (*2*). This procedure was carried out in triplicate. An overall recovered lignin yield after organosolv pre-treatment was calculated (Eq. (*3*) taking into account the lignin recovered in the solid fraction, the precipitated lignin, and the total phenolic compounds determined as described in section 2.7.3:

$$\text{Precipitated lignin yield (\%)} = \frac{\text{Precipited AIL OS A O (g)}}{\text{AIL OS A (g)}} \text{x100} \quad (2)$$

$$\text{Overall recovered lignin yield (\%)} = \frac{\text{AIL OS A O (g)} + \text{Precipited AIL OS A O (g)} + \text{Total phenols (g)}}{\text{AIL OS A (g)}} \text{x100} \quad (3)$$

2.4. Enzymatic hydrolysis

The solids obtained in each pre-treatment (OS-A and OS-A-O) were used as a substrate for enzymatic hydrolysis (EH) to establish the effect of pre-treatment conditions on enzymatic saccharification, using a method described before (Padilla-Rascón et al., 2020b). Briefly, in a 100 mL Erlenmeyer flask the substrate (5 %w/v), was mixed with sodium citrate buffer 0.05 M pH = 4.8 and Cellic® CTec2 (Novozymes A/S, Bagsværd, Denmark), with a concentration of 15 FPU/g of dry substrate supplemented with β-glucosidase (Novozymes 50010), 10 % of Cellic® CTec2. The mixture was stirred at 150 rpm in an orbital shaker at 50 °C for 72 h. Each assay was performed in triplicate. Samples of EH liquid were taken every 24 h and analysed by HPLC as detailed in section 2.7.2. The saccharification yield, and the yield of the EH are calculated according to Eq. (4) and Eq. (5), respectively:

$$\text{Saccharification yield } (\%) = \frac{\text{g glucose by EH}}{\text{g glucose in OS A O}} x\ 100 \quad (4)$$

$$\text{EH yield}(\%) = \frac{\text{g glucose by EH}}{\text{g glucose in OS A}}\ x\ 100 \quad (5)$$

2.5. Pre-sacchararification and simultaneous saccharification and fermentation (PSSF) of OS-A-O

After selecting the organosolv condition with the highest EH yield (190 °C and 30 min), the OS-A-O obtained was subjected to a pre-saccharification followed by simultaneous saccharification and fermentation (PSSF) process. The fermenting microorganism was *Saccharomyces cerevisiae* (Fermentis ethanol red, France). The inoculum was grown in an orbital shaker at 30 °C and 150 rpm for 24 h in a culture medium composed of: 2 g/L NH_4Cl; 1 g/L KH_2PO_4; 0.3 g/L $MgSO_4 \cdot 7\ H_2O$ and 30 g/L glucose. PSSF experiments were performed in 100 mL Erlenmeyer flasks, with 20 mL of 0.05 M sodium citrate buffer and 20 %w/w OS-A-O. The enzyme Cellic® CTec2 loadings were 15, 30 and 40 FPU/g OS-A-O (Novozymes A/S, Bagsværd,

189 Denmark) and supplemented with β-glucosidase (Novozymes 50010) at a 10 % of Cellic® CTec2
190 loading.

191 The pre-saccharification step lasted 4 h at 50 °C and 150 rpm. After that, the inoculum was
192 added at a cell concentration of 0.25 g/L, the temperature was adjusted to 35 °C and shaken at
193 150 rpm for 72 h. Samples were taken for analysis in 24 h periods to determine glucose and
194 ethanol concentrations, as described in section 2.7.2. All assays were performed, at least, in
195 triplicate, and average results are shown.

196 **2.6. Production of furfural from acid hydrolysate**

197 The xylose-rich hydrolysate obtained in the first acid pre-treatment step under the optimal
198 conditions previously described (128 °C, 10.5 g H_2SO_4/100 g OS, and solid/liquid ratio of 33 %,
199 according to Padilla-Rascón et al., 2020b), was used for the production of furfural.

200 The experimental procedure was carried out according to the conditions optimised before
201 (Padilla-Rascón et al., 2021). The experiments were carried out in a microwave reactor (Anton
202 Paar Monowave 400, Graz, Austria), using 10 mL capped glass vessels loaded with 4 mL of
203 hydrolysate, and the temperature was set at 200 °C. The catalysts used for the dehydration of
204 xylose into furfural were H_2SO_4 and $FeCl_3$. Sulphuric acid is already present in the hydrolysate
205 due to the acid pre-treatment, hence, no further addition is required. Iron chloride was added
206 in different concentrations (0-200 mM) to determine the amount required to maximize
207 furfural yields. The liquors obtained were analysed according to section 2.7.2. The experiments
208 were performed in triplicate and average results are shown.

2.7. Analytical methods

2.7.1. Chemical characterisation of solids

Raw OS and the solids obtained in the pre-treatments (OS-A and OS-A-O) were characterised according to the National Renewable Energy Laboratory (NREL) methodology (Sluiter et al., 2012). Briefly, for the characterization of the solids, two-stage acid hydrolysis was performed, the first stage at low temperature (30 ^{o}C) and high concentration of H_2SO_4 (72 %), followed by another acid stage at high temperature (120 ^{o}C) and low concentration of H_2SO_4 (4 %). Solid and liquid fractions were separated by vacuum filtration, and the liquid fraction was analysed by HPLC (section 2.7.2) and measured spectrophotometrically to determine its acid soluble lignin concentration (ASL). The resulting solid fraction was washed, dried and weighed, and muffled (550 ^{o}C, for at least 5 h) to evaluate acid insoluble lignin (AIL). Ash content of OS, OS-A and OS-A-O was also determined using the same procedure.

The raw OS was subjected to two previous extractions, first with water (24 h) and then with ethanol (24 h) for extractives. Replicates of all determinations were carried out in triplicate, at least.

2.7.2. Chemical characterisation of liquors

All the liquid fractions obtained from pre-treatments, enzymatic hydrolysis, furfural and ethanol production processes, as well as the hydrolysates generated during OS-A and OS-A-O characterization, were analysed by high performance liquid chromatography (HPLC). For the determination of the monomeric sugars (glucose, xylose, galactose, and arabinose) an HPLC in a Waters Prostar liquid chromatograph with a refractive index detector (Waters 2414), equipped with a CARBOSep CHO-782 Pb column was used, operating at 70 °C with ultrapure water as mobile phase (0.6 mL/min), being the samples previously neutralized with $CaCO_3$. Ethanol, furfural, 5-hydroxymethylfurfural (HMF), acetic, levulinic and formic acid were determined by HPLC using an Agilent Technologies liquid chromatograph (1260 model) with

234 the ICSep ICE-COREGEL 87H3 column operating at 65 °C with 5 mM sulphuric acid as mobile
235 phase (0.6 mL/min). Liquors obtained in organosolv pre-treatment and ethanol and furfural
236 production processes were analysed completely with the ICSep ICE-COREGEL 87H3 column. All
237 samples were filtered before analysis through 0.22 µm nylon filters.

238 2.7.3. Quantification and identification of total phenolic compounds

239 Total phenolic compounds (TPC) were measured spectrophotometrically, following the Folin-
240 Ciocalteu method (Singleton and Rossi, 1965). Some modifications were made, namely, 0.3 mL
241 of diluted extract was added to 2.5 mL of Folin-Ciocalteu solution (10 %). Subsequently, 2 mL
242 of Na_2CO_3 solution (7.5 %w/v) was added and the mixture was homogenised, and stored in the
243 dark for 1 h after which the absorbance was measured at 750 nm in a Bio-Rad iMarkTM
244 microplate absorbance reader (Hercules, CA, USA). Gallic acid was used as a reference
245 standard compound. Results are expressed as Gallic Acid Equivalents (GAE). Analyses were
246 performed in triplicate and average results are shown.

247 The determination of PVPP (polyvinylpolypyrrolidone) precipitable phenolic compounds was
248 performed by their adsorption on the insoluble PVPP matrix. The experimental procedure was
249 carried out according to Roseiro et al. (2013). Briefly, 100 mg of PVPP, 1 mL of acidified
250 distilled water (pH 3) and 1 mL of organosolv liquor were added to Eppendorf tubes. They
251 were allowed to stand for 15 min at 4 °C and then centrifuged for 10 min. The supernatant was
252 used to determine the non-adsorbed compounds following the method described above. The
253 values obtained were subtracted from the total phenol content to obtain the total value of
254 phenolics precipitated by PVPP. Experiments were carried out in triplicate.

255 The liquors obtained from the organosolv treatment were analysed for their phenolic profile
256 using a capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany)
257 equipped with a diode array detector, according to the methodology described in Roseiro et al.
258 (2013). Detection was performed at 320 nm and compounds were identified by comparison

with UV spectra and migration times of phenolic standards analysed under the same conditions as liquors.

2.7.4. Determination of antioxidants activity

The antioxidant activity of the liquors was quantified by a spectrophotometric method using DPPH (2,2-diphenyl-1-picrylhydrazyl) as described in (Gómez-Cruz et al., 2021). Briefly, 2 mL of a solution containing methanol and 6×10^{-5} M DPPH (2,2-diphenyl-1-picrylhydrazyl) was mixed to 200 µL of the diluted sample, shaken, and left to stand for 15 min in the dark. Absorbance was then measured at 490 nm in a Bio-Rad iMarkTM microplate absorbance reader (Hercules, CA, USA). Trolox was used as a reference standard compound. Analyses were performed in triplicate and average results are shown.

The antioxidant activity of the liquors was expressed as Trolox equivalents (TE) and described by the DPPH percentage inhibition, determined as reported before (Roseiro et al., 2013).

3. Results and discussion

3.1. Feedstock composition

3.1.1. Raw olive stones (OS)

Original OS have a significant content of polysaccharides, 21.0 % cellulose and 24.8 % hemicellulose, with xylose being 86 % of the total hemicellulose. Compared with other olive-derived biomass (Contreras et al., 2020), OS are the olive by-product with the highest polysaccharide content, i.e., 46 % *vs* 36 % in olive tree pruning (OTP) or 21 and 16 % extracted olive pomace (EOP) and olive leaves (OL), respectively. Most noteworthy is also the OS lignin content, which reaches 32.9 %, considerably higher than the reported for OTP (18 %), OL (19 %), and EOP (22 %). This high lignin content may explain the high recalcitrancy of OS that makes it one of the most difficult industrial olive derived wastes to hydrolyse (Doménech et

al., 2020) In contrast to other materials, OS extracts content (4.1 %) is much lower than the values found in the rest of olive-derived biomasses (29, 39 and 49 % for OTP, OL, and EOP, respectively). In fact, previous works on valorization processes of these olive-derived biomasses usually involved a first extraction step to remove most of the extractive fraction before applying different pre-treatment strategies to release sugars from the hemicellulose and cellulose fractions (López-Linares et al., 2020, 2018; Martínez-Patiño et al., 2017). Therefore, in the case of OS, no extraction is needed before pre-treatment.

3.1.2. Acid pre-treated olive stones (OS-A)

In this work, a fractionation strategy was applied involving a first acid pre-treatment step previously optimized to solubilize hemicellusoses followed by an organosolv delignification process, as described in section 2.1. The first acid stage allows the hydrolysis of the hemicellulosic fraction and the recovery of xylose-rich hydrolysate. If the organosolv pre-treatment were carried out directly on biomass, this fraction would be lost by degradation as it would undergo the more severe conditions necessary to solubilise lignin and obtain a solid susceptible to saccharification (Huijgen et al., 2012).

Table 1 also shows the composition of OS-A. The acid pre-treatment solid yield was 63.1 %. This biomass is mainly composed of cellulose and lignin, as both fractions were retained in OS-A in a percentage of 95 %, while more than 80 % of the hemicellulosic fraction was solubilized. This solid fraction was the biomass used in this work as raw material for all organosolv pre-treatment experiments described below. The composition of the liquid fraction rich in xylose and its transformation into furfural are described in section 3.7.

Table 1. Composition of raw olive stones (OS) and acid pre-treated olive stones (OS-A). Values are expressed as a percentage of dry weight.

Composition	Raw OS	OS-A

Cellulose	21.0	31.7
Hemicellulose	24.8	7.3
Xylose	24.0	5.2
Galactose	2.0	1.8
Arabinose	2.0	1.3
Acetyl groups	3.3	0.7
Lignin	32.9	49.5
AIL	31.7	48.3
ASL	1.2	0.8
Ash	0.29	0.7
Extracts	4.1	-

AIL: Acid insoluble lignin; ASL: Acid soluble lignin.
Deviations < 5 %.

3.2. Organosolv fractionation of OS-A biomass

3.2.1. Effect on biomass composition

The main goal for the utilization of the organosolv pre-treatment is biomass delignification. Typically, the main used solvent is ethanol, and although ethanol-organosolv delignification can be carried out using ethanol concentrations ranging from 40 to 75% (Zhang et al., 2016). Typical values are usually in the range 50-60% (Huijgen et al., 2011, 2010), with 50% being particularly reported.

Delignification enables also to increase the cellulose content of the pre-treated solids and the enzymatic digestibility of the cellulose fraction, together with the possibility to recover added-value lignin-derived products, as this pre-treatment allows to obtain these in a liquid stream. The organosolv pre-treatment conditions and the composition of the solids obtained (OS-A-O) are shown in Table 2, revealing the composition of OS-A-O solids obtained under the diverse conditions tested. A significant increase in cellulose content was observed as compared to the OS-A composition. Hemicellulose was completely solubilised during organosolv pre-treatment, except for a single condition (190 ^{o}C and 0 min), where a residual xylose content remained. Lignin content was also decreased significantly.

Focusing on the influence of the different experimental conditions tested on biomass fractionation, a significant effect of temperature, acid concentration, and time was observed. In the three first experiments performed at a lower temperature (140 °C), OS-A-O cellulose content was found to increased at a statistical significant confidence level of 95% with the increase in sulphuric acid concentration, while the lignin content decreased (Table 2). Under these conditions, there was apparently no loss of glucose in the solid and the degree of delignification increased with the addition of acid up to 37 % (Table 3). A notable effect of temperature was observed when it was increased from 140 to 165 °C, both in the experiments carried out with and without acid. At 165 °C part of the cellulose is solubilised (up to 20 %), but an increased delignification is achieved, surpassing 40 %.

Table 2. Composition of the solids obtained after organosolv pre-treatment (OS-A-OL) in the different experimental conditions assayed. Values are expressed as a percentage of dry weight.

Sample	Temperature (°C)	H_2SO_4 (mM)	Time (min)	Cellulose	Hemicellulose	Total lignin	AIL	ASL	Ash
OS-A-O_140	140	-	120	46.6	0	47.1	46.7	0.35	0.26
OS-A-O_140-A50	140	50	120	49.6	0	46.1	45.7	0.40	0.15
OS-A-O_140-A100	140	100	120	53.8	0	44.2	43.9	0.26	0.42
OS-A-O_165	165	-	120	48.7	0	42.8	42.6	0.27	0.07
OS-A-O_165-A50	165	50	120	46.8	0	46.3	46.0	0.38	0.26
OS-A-O_190-0	190	-	0	50.7	1.49	44.5	44.1	0.46	0.09
OS-A-O_190-30	190	-	30	54.5	0	44.5	44.0	0.49	0.19
OS-A-O_190-60	190	-	60	46.4	0	44.8	44.5	0.27	0.05
OS-A-O_190-90	190	-	90	45.9	0	57.4	57.0	0.37	0.09
OS-A-O_190-105	190	-	105	31.9	0	64.5	64.3	0.18	0.02

AIL: Acid insoluble lignin; ASL: Acid soluble lignin. Deviations < 5 %.

Table 3. Cellulose and lignin yields obtained in the solids after organosolv pre-treatment (OS-A-O).

Sample	Solid yield[a] (%)	Cellulose yield[b] (%)	Delignification yield[c] (%)
OS-A-O_140	79.2	100	26.3
OS-A-O_140-A50	72.3	99.1	26.7
OS-A-O_140-A100	69.6	98.9	37.0

OS-A-O_165	64.4	86.7	43.6
OS-A-O_165-A50	61.4	79.4	41.9
OS-A-O_190-0	74.2	100	32.6
OS-A-O_190-30	52.4	78.9	49.8
OS-A-O_190-60	51.8	66.4	52.5
OS-A-O_190-90	48.3	61.2	43.4
OS-A-O_190-105	45.7	40.3	39.5

Deviations < 5 %.

[a] Solid yield: expressed as g OS-A-O /100 g OS-A.

[b] Cellulose yield: cellulose contained in OS-A-O, as a percentage of cellulose contained in OS-A.

[c] Delignification yield: AIL removal in OS-A-O, as a percentage of AIL contained in OS-A.

In the experiments conducted at the higher temperature (190 ^{o}C) no acid was added, as at lower temperatures cellulose degradation was already observed with the addition of acid. The effect of different pre-treatment times was studied at 190 ^{o}C. The cellulose content in OS-A-O rose with increasing time up to 30 min (maximum of 54.5 % cellulose). At longer times (60, 90, and 105 min), the cellulose content decreased with time while the lignin content increased. Glucose recovery in the solid decreased with increasing time, with losses of more than one-third of the cellulose after 60 min. The highest levels of delignification were obtained at 30 and 60 min, reaching delignification percentages of around 50 %. This value is comparable to the same obtained by (Gómez-Cruz et al., 2022) with another olive by-product, EOP, reaching a delignification of 53 % with an organosolv pre-treatment at 140 ^{o}C, 60 min and 1 % H_2SO_4 after a two-step aqueous extraction. Slightly higher delignification (66 %) was achieved with olive tree pruning after organosolv pre-treatment at 66 % ethanol, 210 ^{o}C, 60 min (Díaz et al., 2011). Other biomasses, such as wheat straw, have been pre-treated with the same strategy as in this work, (an acid pre-treatment followed by an organosolv pre-treatment), obtaining a maximum delignification of 69 % (Huijgen et al., 2012).

To the best of our knowledge, this is the first work describing ethanol organosolv delignification of OS, with a relevant delignification yield (up to 52.5 %) and no catalyst added.

368

369 3.2.2. Effect on hydrolysate composition

370 The composition of the organosolv liquors obtained is shown in Table 4. Sugar concentration
371 detected, ranged from low values (3.1 g/L) to moderate values (13.3 g/L). Xylose and arabinose
372 come from the solubilization of the remaining hemicellulosic fraction still present in OS-A and
373 are the most relevant sugars. Glucose come from cellulose losses during pre-treatment (as can
374 be seen in Table 3), especially in experiments performed at 190 ^{o}C for longer reaction times.
375 Besides sugars, other compounds such as acetic, formic and levulinic acids, or furanic
376 compounds, were also detected (Table 4). Apart from acetic acid, this group of compounds
377 comes from sugar degradation (Jönsson et al., 2013), mainly under more severe conditions.
378 For example, the addition of 50 mM of sulphuric acid at 165 ^{o}C generated higher furan
379 concentrations (2.1 g/L of HMF and 4.1 g/L of furfural) than in the experiment carried out at
380 the same temperature without acid and then in the experiment performed at 140 ^{o}C and 50
381 mM acid. On the other hand, longer pre-treatment times at 190 ^{o}C generated higher
382 concentrations of degradation products, reaching 10.4 g/L of furanic compounds and 5.9 g/L of
383 acids at 105 minutes.

384 Due to the low sugars concentrations and high concentrations of degradation products that
385 can act as fermentation inhibitors, these liquors are not suitable for ethanol production (Kim,
386 2018). As such, the valorisation of these liquid streams was preferably considered for the
387 recovery of lignin-derived compounds, as described in the following subsections.

388

389 **Table 4.** Composition of the liquors obtained in the organosolv pre-treatment.

Sample	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Formic acid (g/L)	Acetic acid (g/L)	Levulinic acid (g/L)	HMF (g/L)	Furfural (g/L)
OS-A-O_140	0.2	4.8	0.37	2.9	0.01	0.00	0.01	0.68
OS-A-O_140-A50	1.1	4.7	1.2	3.3	0.56	0.02	0.11	1.6

OS-A-O_140-A100	1.5	4.3	1.6	5.1	0.48	0.01	0.21	2.4
OS-A-O_165	1.8	3.7	1.9	1.1	0.52	0.05	0.57	3.2
OS-A-O_165-A50	0.58	1.2	1.3	1.7	1.2	0.48	2.1	4.1
OS-A-O_190-0	1.1	5.5	1.0	1.7	0.42	0.00	0.08	0.94
OS-A-O_190-30	3.7	3.3	1.8	1.8	0.93	0.13	1.8	4.1
OS-A-O_190-60	2.7	2.2	2.2	1.8	1.3	0.28	2.8	4.4
OS-A-O_190-90	4.8	2.8	2.6	2.3	1.1	0.83	4.7	5.1
OS-A-O_190-105	6.4	3.2	3.8	2.4	1.2	2.3	5.1	5.3

Deviations < 5 %.

3.2.2.1. Phenolic compounds and antioxidant activity

Liquid fractions obtained from organosolv pre-treatment have also been analysed to determine the concentration of total phenolic compounds (TPC), phenolic compounds precipitable by PVPP (PP PVPP), and antioxidant capacity (Table 5).

As can be seen in Table 5, TPC and PP PVPP showed the same trend at 140 ^{o}C, both increasing with the addition of acid. The highest values at this temperature were obtained with the addition of 100 mM H_2SO_4 (11.3 g GAE / 100 g OS-A lignin of TPC and 6.0 g GAE / 100 g OS-A lignin of PP PVPP). This trend was also observed in the concentration of phenols obtained in the tests carried out at 165 ^{o}C, reaching the highest concentrations with the addition of 50 mM H_2SO_4 (15.6 g GAE / 100 g OS-A lignin of TPC and 7.3 g GAE / 100 g OS-A lignin of PP PVPP). At 190 ^{o}C, no notable differences were detected at the different pre-treatment times, with TPC ranging from 9.4 to 14.3 g GAE / 100 g OS-A lignin and PP PVPP ranging from 3.5 to 6.8 g GAE / 100 g OS-A lignin. The maximum concentration of TPC obtained was 4.7 kg GAE/ 100 kg OS, exceeding the value was obtained from organosolv delignification of EOP, 2.4 kg GAE/ 100 kg OS (Gómez-Cruz et al., 2022).

Table 5 also shows the antioxidant capacity of the liquors. The results are in the range 2.7 to 3.6 g TE / 100 g OS-A lignin, without notable variability with the experimental conditions used during organosolv pre-treatment. DPPH inhibition was below 75 %.

410

411 **Table 5.** Total concentrations of phenolic compounds (TPC) (g GAE/100 g OS-A lignin), phenols
412 precipitated by PPVP (PP PVPP) (g GAE/100 g OS-A lignin), antioxidants capacity (g TE/100 g OS-
413 A lignin) and DPPH inhibition (%) of the liquid streams obtained in the first acid pre-treatment
414 and the second organosolv pre-treatment at the different experimental conditions assayed.

Sample	TPC	PP PVPP	g TE/100 g	% DDPH inhibition
Acid-pretreated OS	2.8	1.4	2.1	50.9
OS-A-O_140	6.1	3.0	2.7	51.3
OS-A-O_140-A50	9.1	4.3	3.4	63.3
OS-A-O_140-A100	11.3	6.0	3.5	64.3
OS-A-O_165	12.5	5.4	3.4	63.5
OS-A-O_165-A50	15.6	7.3	3.5	64.1
OS-A-O_190-0	9.4	3.7	2.8	53.2
OS-A-O_190-30	13.6	6.7	3.6	73.5
OS-A-O_190-60	14.1	4.7	3.6	68.2
OS-A-O_190-90	14.3	6.8	3.6	65.7
OS-A-O_190-105	13.6	3.5	3.5	64.7

415 Deviations < 5 %.

416

417 The liquors generated in the organosolv pre-treatment were also analysed by capillary
418 electrophoresis to determine their phenolic profile and identify the main phenolic compounds
419 present. Complex matrices were obtained, as can be seen in **Figure 2** where the
420 electropherogram of the organosolv pre-treatment performed at 190 ^{o}C and 30 min is shown.
421 As can be seen, the identified compounds are putatively derived from the lignin fractionation,
422 except for furfural, also identified by HPLC in the organosolv liquors (Table 4), that is derived
423 from sugars (pentoses). Besides, the identified compounds there are several other compounds
424 that were not yet identified. These are probably also lignin-derived compounds, for instances,
425 catechol or its derivatives, as indicated by several putative/partial (lower matching)
426 identification of some of the peaks (data not shown).

427

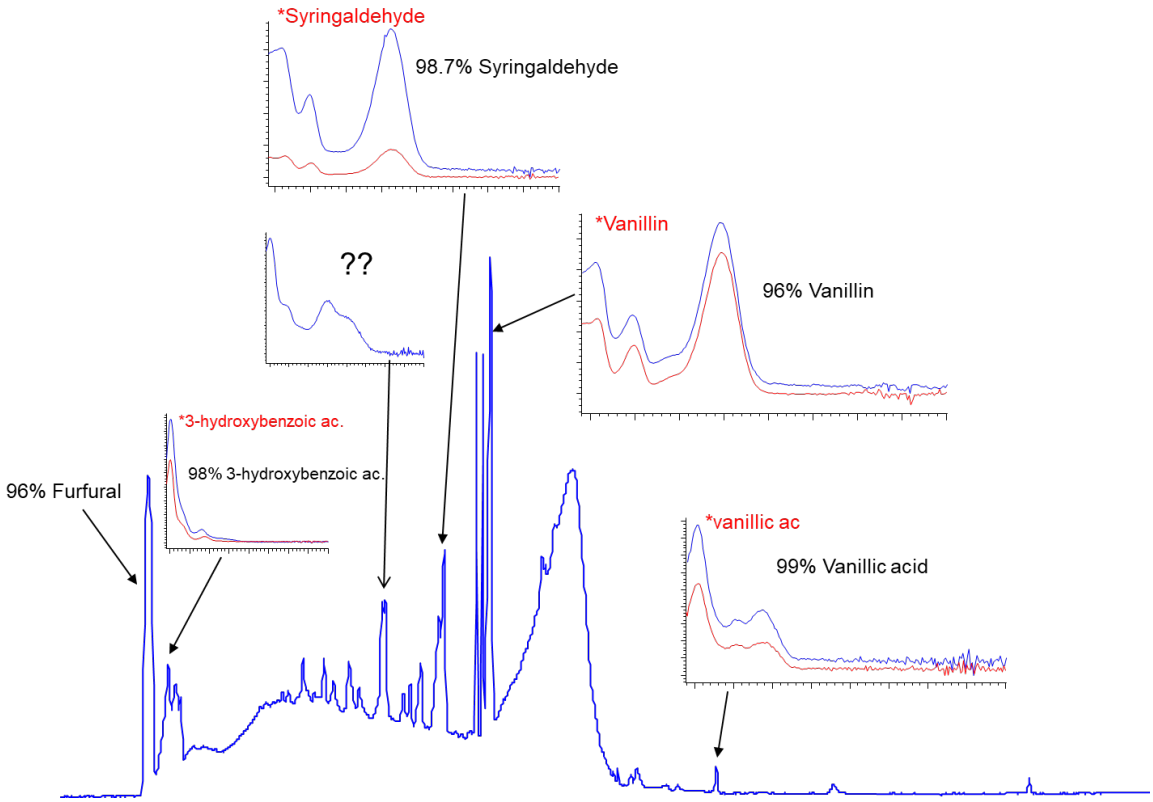


Figure 2. Electropherogram (320 nm) showing the phenolic profile of the soluble lignin products obtained after organosolv pre-treatment. Match percentage was obtained by comparison with the authentic standards analysed under the same conditions.

3.2.2.2. Lignin precipitation

The Lignin solubilized during organosolv pre-treatment was precipitated with water as described in Section 2.3. Precipitated lignin yield (Eq. (2)) was determined taking into account the lignin content in the raw material (OS-A) and the lignin recovered after organosolv pre-treatment at different experimental conditions (Eq. (3)). Results are shown in Table 6. In the experiments performed at 140 ^{o}C the use of sulphuric acid during pre-treatment had a notable effect on the lignin recovered by precipitation, increasing the yield from 7.8 to 21.7 g / 100 g OS-A lignin with the addition of 100 mM sulphuric acid. In contrast, at 165 ^{o}C, no significant difference was detected with the addition of acid. The precipitated lignin yield obtained in the experiment performed at 165 ^{o}C without acid (20.5 g / 100 g OS-A lignin) was in the range of those obtained at 140 ^{o}C with the addition of acid. In the experiments carried out at 190 ^{o}C, an

increase in precipitated lignin yield was observed when the reaction time rose from 0 to 30 min (from 14.4 to 19.48 g / 100g OS-A lignin, corresponding from 46.93 g/100 of dissolved lignin). At longer pre-treatment times this yield did not change significantly, with values around 19 g / 100g OS-A lignin. No direct correlation was found between precipitated lignin yields and the degree of delignification obtained by organosolv pre-treatment (Table **3**), possibly, this is because the increased severity of the pre-treatment causes the formation of smaller lignin fragments, which are more difficult to precipitate (Huijgen et al., 2012).

Table 6 also shows the overall lignin recovery, calculated according to Eq. (3) (2.3. section). The results were in the range of 81-97 %. Lower values were obtained for the experiments performed at 190 ^{o}C for 30 and 60 minutes, which were the ones in which higher delignification yields were achieved (Table 3).

Table 6. Lignin recovered and lignin precipitated after organosolv delignification.

Sample	Precipitated lignin yield[a]	Overall recovered lignin [b]
OS-A-O_140	7.8	90.5
OS-A-O_140-A50	19.5	97.0
OS-A-O_140-A100	21.7	96.4
OS-A-O_165	20.5	89.8
OS-A-O_165-A50	18.0	92.1
OS-A-O_190-0	14.4	91.6
OS-A-O_190-30	19.5	80.9
OS-A-O_190-60	19.7	81.5
OS-A-O_190-90	20.0	87.6
OS-A-O_190-105	20.0	93.3

Deviations < 5 %.

[a] g lignin/100 g OS-A lignin.

[b] g lignin (precipitated + retained in pre-treated solid (OS-A-O) + phenolics)/100 g OS-A lignin.

These data show that the solubilised lignin recovered by precipitation is not enough to compensate for the lignin loss in the solid and thus corroborate the difficulty to precipitate all

465 the solubilised lignin. A possible alternative in order to try to improve these yields is to distill
466 the ethanol used as a solvent in the pre-treatment. The distillation of the solvent is necessary
467 to recover and reuse it, thus improving the economy of the process. Lignin precipitation would
468 also occur as the ethanolic concentration of the liquor decreases. However, the main drawback
469 of this methodology is that the purity of the precipitated lignin is usually lower since residual
470 sugars remain mixed with the lignin in the precipitate obtained. Depending on the form of
471 lignin valorization, the presence of carbohydrates may not be a problem. Previous studies have
472 shown suitable valorization routes for lignins precipitated from organosolvents to produce
473 activated carbon to be used as a water pollutant adsorbent material (Martín-Sampedro et al.,
474 2019). However, for obtaining other compounds such as adhesives, films and biodegradable
475 polymers, it is necessary to use lignins that present higher purity, which are practically
476 unaltered and less condensed, being partially soluble in many organic solvents (Mesa et al.,
477 2011).

478 To compare the organosolv delignification yield obtained with conventional delignification
479 methods, an alkaline treatment with NaOH (2 % and 4 %) at 130 ^{o}C was also tested.
480 Surprisingly the delignification yields obtained with this treatment were much lower (data not
481 shown), than those obtained with organosolv process. Furthermore, no increase in enzymatic
482 saccharification was observed compared to that obtained without this pre-treatment, showing
483 not only that this methodology was not effective for the biomass here studied, but also that
484 organosolv delignification obtained here with OS-A is very interesting. These data contrast
485 with the previously obtained for other biomasses such as *Cistus ladanifer,* which shows higher
486 delignification with NaOH alkaline pre-treatment than with organosolv, achieving a
487 delignification of 76 % and an enzymatic yield of 72 % at 4 % NaOH and 130 ^{o}C (Alves-Ferreira
488 et al., 2021).

3.3. Effect of the organosolv pre-treatment conditions on cellulose saccharification

To evaluate the effectiveness of the organosolv pre-treatment on the enzymatic digestibility of cellulose, enzymatic hydrolysis (EH) of the cellulose-enriched solids obtained in the different experimental conditions tested (OS-A-O) was performed. The results are shown in Table 7. For comparison purposes, EH of OS-A used as the raw material in this work is also presented.

Table 7. Glucose concentration (g/L), enzymatic saccharification yield (%) and enzymatic hydrolysis (HE) yield (%) obtained from OS-A and OS-A-O.

Sample	Solid yield (%)	Glucose (g/L)	Saccharification yield (%)	EH yield (%)
OS-A	63.1	0.39	-	2.0
OS-A-O_140	79.2	0.99	3.9	3.9
OS-A-O_140-A50	72.3	6.6	24.1	23.7
OS-A-O_140-A100	69.6	8.7	28.1	28.1
OS-A-O_165	64.4	16.3	60.8	52.7
OS-A-O_165-A50	61.4	21.8	84.4	67.1
OS-A-O_190-0	74.2	1.9	6.9	6.9
OS-A-O_190-30	52.4	25.4	95.8	75.9
OS-A-O_190-60	51.8	25.5	100.0	66.7
OS-A-O_190-90	48.3	21.8	98.8	52.7
OS-A-O_190-105	45.7	20.2	100.0	46.3

Deviations < 5 %.

The EH yield obtained with OS-A is only 2 %. This EH yield for the non-delignified material is lower than that of other LCB, which is an indication of the high recalcitrance of OS. For example, Martínez-Patiño et al. (2018) obtained an EH yield of 53 % from olive tree biomass after acid pre-treatment.

The low EH yield obtained makes it necessary to perform a second pre-treatment to increase it. In previous work, the steam explosion was used as a second pre-treatment step, saccharification yields increased up to 78 %, and an additional grinding step of the pre-treated solids was necessary (Padilla-Rascón et al., 2020b). In this work, an organosolv pre-treatment has been selected to cause a breakdown of the lignocellulosic structure and swelling of the

biomass, increasing the surface contact and improving the enzymatic digestibility (Alves-Ferreira et al., 2021). Other authors have achieved a maximum enzymatic saccharification yield of 81 % with EOP using an organosolv pretreatment (Gómez-Cruz et al., 2022).

In the organosolv tests performed at a lower temperature (140 ^{o}C), the saccharification yield of cellulose increased slightly with the addition of sulphuric acid, reaching 28 % (8.7 g/L) with the addition of 100 mM H_2SO_4.

By increasing the temperature to 165 ^{o}C the enzymatic digestibility improved, obtaining a saccharification yield of 84.4 % concerning the glucose contained in the OS-A-O g/L for the test carried out with the addition of 50 mM sulphuric acid. However, when the EH yield was calculated, concerning the glucose contained in the OS-A, the result decreased to 67 %, due to the solubilisation of more than 20 % of the cellulose produced under these pre-treatment conditions (Table 3).

In the series of experiments carried out at 190 ^{o}C and different pre-treatment times (0, 30, 60, 90 and 105 min), it can be observed that the saccharification yield increased from close to 7 to 95.8 % from 0 to 30 minutes. From 60 minutes of pre-treatment onwards, the saccharification of the cellulose contained in the OS-A-O is complete. On the contrary, a remarkable decrease of the EH yield occurs for times longer than 30 minutes, due to important cellulose losses. At longer pre-treatment times, more than 30 % of the cellulose is solubilised after 60 minutes of pre-treatment, reaching about 60 % solubilisation in the experiment carried out at 105 minutes. A correlation between saccharification and EH yields and solid yields was observed, with EH yields increasing significantly for solid recoveries around 50 % or lower, and saccharification yields decreasing with increasing solid yields from 52 %, which is indicative of higher degradations and solid losses (Table 7).

There is a relationship between the solubilisation of glucose by EH and the degree of delignification of the solid, obtaining higher EH yields from OS-A-O that has had a higher

533 degree of delignification (Table 3). Therefore, we can affirm that the delignification of OS with
534 an organosolv pre-treatment is effective in increasing the solubility of glucose by EH.

535 In the tested conditions, the highest EH yield (75.9 %) was obtained in the experiment carried
536 out at 190 ^{o}C and 30 min. These conditions have therefore been selected for obtaining ethanol
537 from the cellulosic fraction by PSSF, taking into account the recovery of precipitated lignin and
538 the composition of phenolic compounds and their antioxidant capacity (section 3.2.2.1) were
539 also evaluated for this experiment.

540

541 **3.4. Ethanol production**

542 Results of ethanol production from the OS-A-O obtained at 190 ^{o}C and 30 minutes by a PSSF
543 process are shown in Figure 3. It can be seen that the influence of the different enzyme
544 loadings tested (15, 30 and 40 FPU/g OS-A-O) was not very high. Increasing the enzyme dose
545 hardly affects the ethanol concentration at 24 h (they were in the range 29.2-30.3). At 48 h the
546 difference between the ethanol concentrations obtained at the different enzyme loadings was
547 slightly higher (from 38.6 g/L at 15 FPU to 42.5 g/L at 40 FPU). At 72 h, the ethanol
548 concentration with the different enzyme doses tested is 42.3, 47 y 47.2 g/L, con 15, 30 y 40
549 FPU, respectively. These concentrations correspond to ethanol yields of 55.3, 61.4 and 61.7,
550 respectively, calculated as a percentage of the theoretical maximum that could be obtained
551 from the glucose contained in the OS-A-O (Table 2). These values were similar to that reported
552 by Martínez-Patiño et al. (2017) with olive tree biomass subjected to alkaline delignification
553 with 7 % hydrogen peroxide, in which they obtained 46 g/L ethanol and a yield of 59 %, by
554 simultaneous saccharification and fermentation (SSF) at 20 % solids and 15 FPU/g substrate.

555

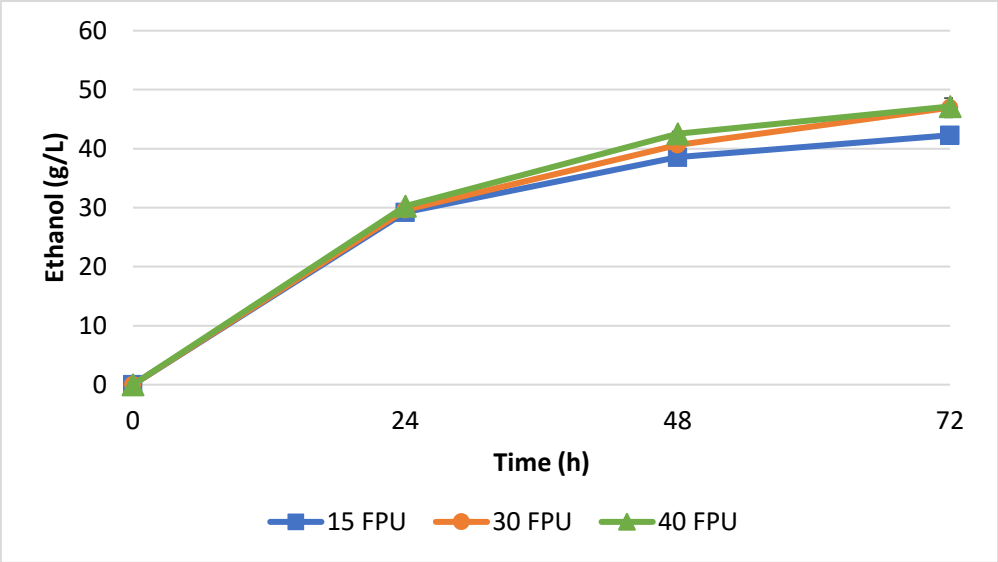


Figure 3. Ethanol production by PSSF from the solid obtained in the organosolv pre-treatment (OS-A-O) at 190 °C and 30 min with different enzyme loadings 15, 30 and 40 FPU/g OS-A-O.

For an ethanol production process to be viable from a distillation efficiency point of view, ethanol concentrations must be above 4 % (w/v). (Koppram et al., 2014). In the present work, this minimum requirement is exceeded at the different enzyme loads tested. The difference obtained at 72 h using 30 or 40 FPU of enzyme is negligible. From the economic point of view, it should be considered the cost of the enzyme versus the benefit obtained in ethanol production, since the cost of enzymes are usually high. Considering profitability, it would be important to determine whether the optimal conditions for obtaining ethanol at 72h would be those with an enzyme load of 30 FPU/g OS-A, with which a maximum ethanol concentration of 47 g/L was obtained, or whether it would be more profitable to reduce the enzyme load to half (15 FPU/g OS-A-O), with an ethanol concentration drop to 42 g/L. A reduction of time to 48 h could also be of interest. The use of high solid loading is recommended in bioethanol production processes to make the process more economically viable, although a drop in the yield usually occurs. Thus, an economic evaluation would be necessary to determine the most appropriate enzyme dose and fermentation time (Joelsson et al., 2016)

574 ### 3.5. Furfural production

575 The xylose-enriched hydrolysate obtained in the acid pretreatment of OS under conditions
576 optimized in previous work (Figure 1) has a high initial xylose concentration, 65.8 g/L, but also
577 contains high concentrations of inhibitory compounds, mainly acetic acid (15 g/L) and furfural
578 (2.3 g/L), as described in Table 8. As ethanolic fermentation of a similar hydrolysate with the
579 xylose-fermenting strain *Escherichia coli* SL10*0* required extensive detoxification methods to
580 remove inhibitors (unpublished results), in this work, furfural production from OS acid
581 hydrolysates was studied as an alternative approach.

582

583 **Table 8**. Composition of the original acid hydrolysate and the liquors
584 obtained from microwave treatment to obtain furfural at different
585 concentrations of $FeCl_3$ catalyst.

	Acid hydrolysate	$FeCl_3$ catalyst concentration (mM)			
		0	50	100	200
Glucose (g/L)	1.3	0.76	0.32	0.16	0.00
Xylose (g/L)	65.8	15.5	5.2	2.2	1.5
Galactose (g/L)	3.5	-	-	-	-
Arabinose (g/L)	3.7	3.1	2.2	2.0	2.0
Formic acid(g/L)	0.80	2.2	2.6	2.9	3.2
Acetic acid(g/L)	15.0	16.8	16.9	16.5	15.9
HMF (g/L)	0.06	0.19	0.17	0.10	0.09
Furfural (g/L)	2.3	20.0	22.1	22.7	19.7

586 Deviations < 5 %.

587

588 The experimental conditions tested were selected based on previous work (Padilla-Rascón et
589 al., 2021). The catalysts tested were $FeCl_3$ with different concentrations (0-200 mM) and
590 sulphuric acid. The latter was not added as the pre-treated solid already contained it. Sulphuric
591 acid and ferric chloride act as Brønsted and Lewis acids, respectively, in the dehydration
592 reaction of xylose to furfural (Kabbour and Luque, 2020; Padilla-Rascón et al., 2020a). In the
593 present case, sulphuric acid has a dual role, firstly, it acts as a catalyst for the production of

xylose from hemicellulose and, subsequently, the acid contained in the hydrolysate acts as Brønsted acid, without the need for its addition to obtain furfural.

The highest furfural concentration produced was 22.7 g/L and it was obtained with 100 mM $FeCl_3$, with a furfural yield of 48.7 % and a xylose conversion of 96.6 % (Figure 4). At $FeCl_3$ concentrations lower than 100 mM, the furfural produced and the xylose conversion were lower, although a very similar concentration was obtained for 50 mM $FeCl_3$. At higher $FeCl_3$ concentrations (200 mM) the conversion of xylose increased slightly but the furfural yield decreased, therefore, furfural losses were detected, due to degradation by autopolymerisation reactions (Köchermann et al., 2018)

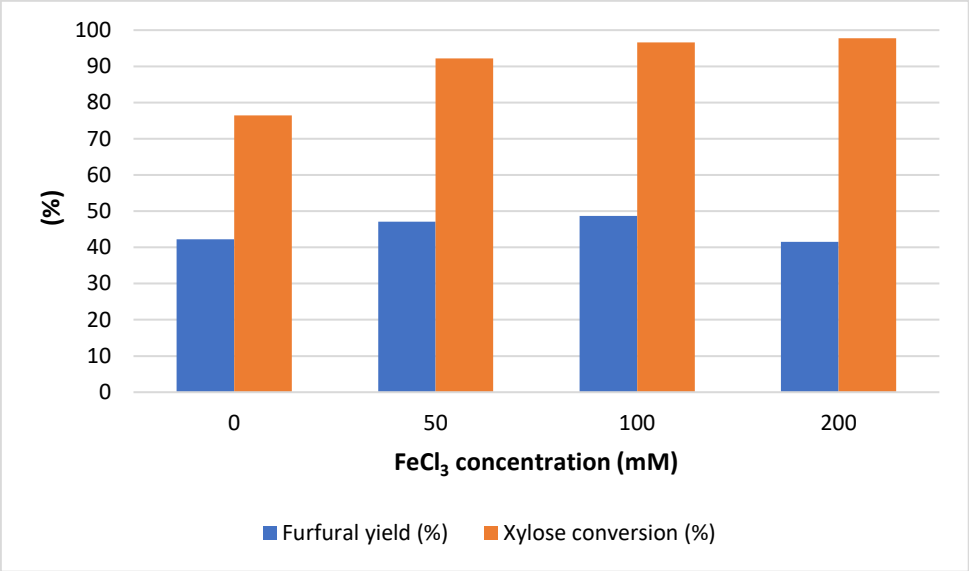


Figure 4. Concentration and yield of furfural produced and xylose conversion.

In the referred previous work, from an OS hydrolysate with 40.3 g/L xylose concentration, 18 g/L of furfural (63.3 % yield) were obtained, using the same experimental conditions (Padilla-Rascón et al., 2021). In the present work, a lower furfural yield was obtained (48.7 %), but furfural concentration was higher, reaching 22.7 g/L. This can be explained because the liquor used in the present work had a higher xylose concentration (65.8 g/L), which may hinder the

612 production of furfural since it increases its degradation by autopolymerisation. This behaviour
613 has already been observed by other authors, where the lower the xylose concentration of the
614 liquor used, the higher the furfural yields obtained (Fúnez-Núñez et al., 2020).

615

616 4. Conclusions

617 The fractionation strategy proposed in this work allows the full valorisation of the olive stones
618 by enabling the full exploitation of both its polysaccharide fractions and its lignin fraction. The
619 mass balance and yields obtained are shown in detail in Figure 5.

620 In the first acid pre-treatment, a hemicellulosic-sugars-rich hydrolysate was obtained, with
621 sugars concentration as high as 73 g/L, equivalent to 22.1 kg hemicellulosic sugars/100 kg OS,
622 the main hemicellulosic sugar being xylose (65.8 g/L, 19.9 kg xylose/100 kg OS). With this acid
623 hydrolysate, a microwave treatment was carried out at 200 ^{o}C with $FeCl_3$ 100 mM as a catalyst,
624 obtaining a liquor with a high concentration of furfural (22.5 g/L, 6.8 kg furfural/100 kg OS).

625 The solid resulting from the first acid pre-treatment was enriched in cellulose and lignin, but
626 still showed low enzymatic saccharification. To increase cellulose digestibility, a organosolv
627 delignification pre-treatment was carried out (190 ^{o}C and 30 min), achieving a 49.8 %
628 delignification yield. The organosolv liquor obtained can be used as a source of phenolic
629 compounds. The concentration of TPC obtained was 4.2 kg GAE/ 100 kg OS. The recovery of
630 lignin by precipitation with water was also addressed, enabling to obtain 7.4 kg/100 kg OS. The
631 delignified solid was mainly composed of cellulose and was used to produce ethanol by PSSF
632 with a solid loading of 20 % w/w and an enzyme dosage of 30 FPU, with *S. cerevisiae* as the
633 fermenting microorganism. Ethanol concentration as high as 47 g/L ethanol was obtained (6.2
634 kg ethanol/100 kg OS) under these conditions.

635 With the proposed strategy, it was possible to recover 90 % of the sugars contained in the raw
636 OS. From the main sugars, xylose, and glucose, it was possible to obtain furfural and ethanol,

637 with yields of 48.7 % and 61.4 % referring to the xylose and glucose content of the OS,
638 respectively.

639 This work shows the advantages of a two-step fractionation process (dilute acid hydrolysis
640 followed by organosolv) to obtain multiple biorefinery products from olive stones, i.e., furfural,
641 lignin, phenolic compounds, sugars, and ethanol. Therefore, the full integrated usage of all
642 structural biomass components of olive stone was achieved, and demonstrated to be
643 technologically feasible. Furthermore, there are several significant points in the overall process
644 that can be explored and further optimized based on energy and mass integration systems that
645 could yield significant savings namely on energy utilization and materials (e.g. ethanol and
646 enzymes recycling), that must be further explored in future research. Thus, the data obtained in
647 this work will be an important contribution to techno-economic studies dealing with olives
648 stones-based biorefineries. Future work would be necessary to assess the economic viability and
649 sustainability of the proposed biorefinery.

650

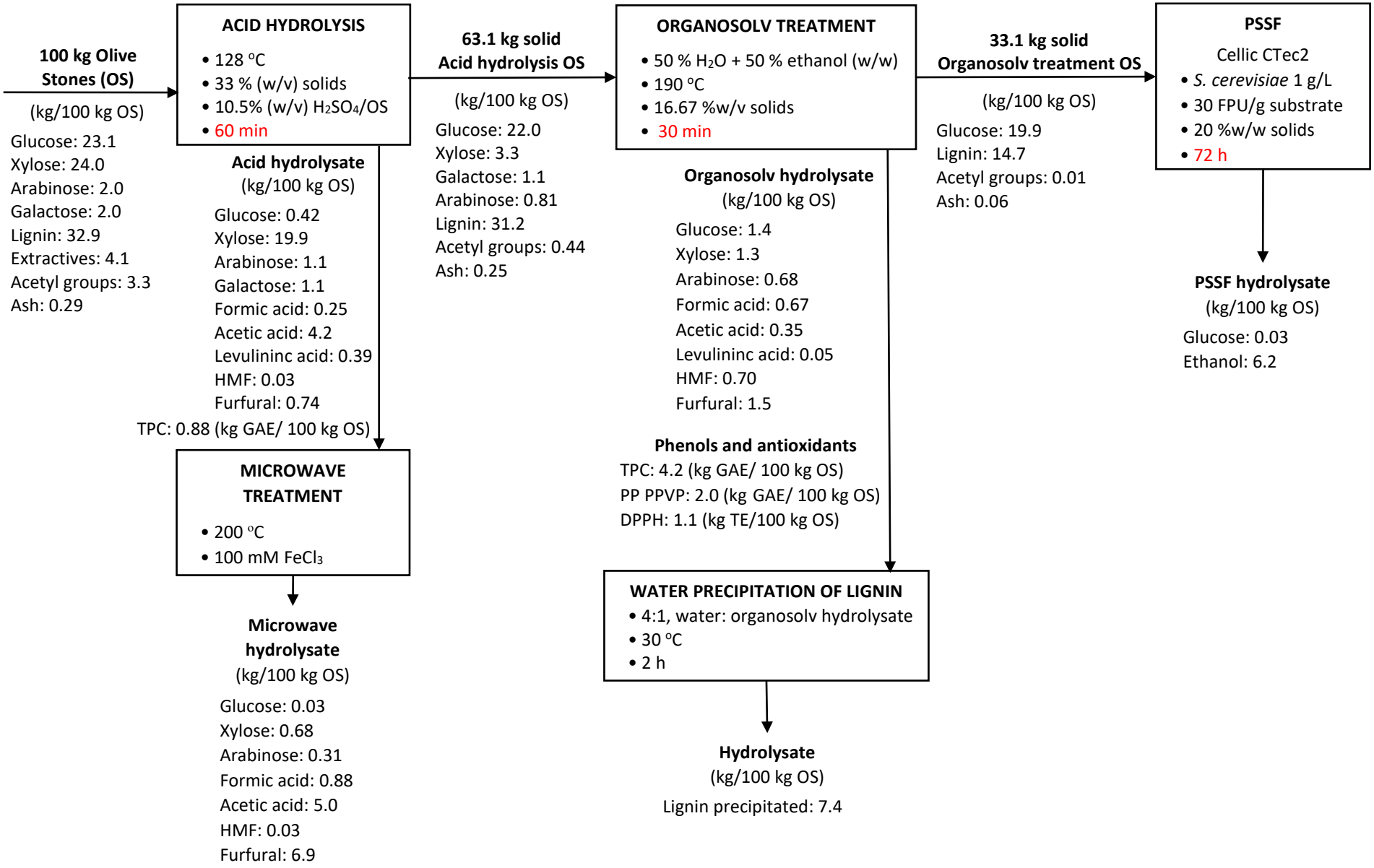


Figure 5. Mass balance obtained through the integrated fractionation strategy proposed for OS valorisation as carried out under the optimized conditions identified in this

work

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