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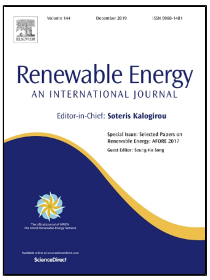

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ABSTRACT

In this work, a residue from olive oil industry, i.e., extracted dry olive pomace (EOP) is studied for valorization into fermentable sugars and other added-value compounds. EOP processing is based on a first water extraction step at 100ºC during 30 min, followed by Liquid Hot Water (170,190ºC and 210ºC) or dilute acid (DA) pretreatment [same temperatures in sulfuric acid 1% and 2% (w/v)], and enzymatic hydrolysis (EH) at laboratory scale using commercial enzymes. The results show that the water-extraction step allows extracting valuable compounds as mannitol and phenols that can contribute significantly to EOP valorization. The pretreatments tested are found to be effective to fractionate EOP biomass and facilitate sugar EH, although DA performs comparatively better, providing the maximum overall process yield considering both glucose and xylose sugars (85 and 62% of sugar content in raw EOP) at 170ºC and 2% acid.

Keywords: lignocellulosic residue; revalorization; olive-oil industry; extraction; pretreatment; fermentable sugar

1. INTRODUCTION

Olive oil production is a key industry in the agroindustrial sector of the Mediterranean countries owing to the high number and capacity of olive processing facilities and the large surface area dedicated to olive tree cultivation. Recent estimations from FAOSTAT [1], show that specifically in Spain, close to 2.5 Mha of the olive crop were cultivated in 2016, representing 24.2 % of total worldwide production, and positioning the country as the leader in olive tree cultivation. The olive oil production process generates several residues or by-products, i.e., olive pomace (OP) and extracted dry olive pomace (EOP), generated in olive oil processing industries, and olive leaves (OL) from cleaning operations in olive mills. Even though some of these residues, particularly EOP, are partially used for energy production in the same or related facilities [2], a promising alternative would be to use them to produce high-added value products in energy and

food markets within a biorefinery concept associated with the olive oil industry. [3]. This concept could be broadened to include the main residue generated by the olive crop, i.e., olive tree pruning (OTP) biomass, thus resulting in an integrated biorefinery around olive cultivation and olive oil production [4,5].

Olive pomace (OP), which represents the main residue of the olive oil extraction process by weight, differs in composition depending on the production process (two or three-phase). In the two-phase separation mode, which is mostly used in Spain, OP is a thick sludge with 55-70% of moisture, compared to 40-45% of the residue from the three-phase operation [6], and pH 4.9-6.8 [7]. Currently, in Spain OP is further processed in extracting industries to remove the residual oil, i.e., the “pomace oil”. This extraction can be carried out with solvents, such as hexane, in the traditional system, or through physical extraction or centrifugation**.** The solid residue is called “extracted or dry pomace” (EOP) and so far has only been used as solid fuel for heat or power generation. A scheme of EOP generation in pomace oil extracting industries following the traditional system is shown in Figure 1.

According to Manzanares et al. [8], EOP generated in Spain was estimated at 1,181,274 tonnes/campaign, as an average of the campaigns 2012/13, 2013/14 and 2014/15, with 89% of national production in Andalusia. EOP is used to some extent as solid fuel to generate heat and/or electricity in the pomace oil extraction industry and/or olive mill. Data provided by the regional government of Andalusia on EOP utilization for energy production shows a surplus of about 25% of the residue generated annually that would be available for other purposes [9]. Therefore, considering this availability and its origin, there is every reason to employ it with its qualities as feedstock to obtain high added value products within a biorefinery concept integrating all residues generated in the olive sector.

To addres this different approach on EOP, firstly, and due to the lignocellulosic nature of this biomass residue, a pretreatment process is necessary to fractionate biomass into its main constituents and facilitate the breakdown of carbohydrates into monomeric sugars. The sugars released could be further converted into biofuels, such as ethanol or other building block

chemicals, by biological processes within an appropiate biorefinery scheme [8]. Fernandes et al. [10] have recently reported on the use of EOP as biomass feedstock focused on ethanol production after acid and alkaline pretreatment and EH of pretreated material. The authors studied several fermentation strategies and found relatively low ethanol production yields, claiming the neccesity to address further studies to improve these results.

There is extensive literature on different pretreatment methods to fractionate lignocellulosic biomass, with different results depending on the characteristics of the biomass and process conditions applied [11, 12, 13]. In this work, hydrothermal pretretament LHW and dilute acid (DA) have been selected for EOP fractionation, in experiments carried out at laboratory scale. Although both hydrothermal pretreatments have been shown to be effective as methods to alter lignocellulosic biomass and favor the carbohydrates breakdown and release of sugars, they present differences in the operation conditions and in the main effects produced that is interesting to consider in the selection of one or another procedure. As a summary, Table 1 below shows the main advantages and disadvantages of both methods, based on information compiled by the authors.

LHW pretreatment, in which pressure is utilized to maintain water in the liquid state at elevated temperatures (150 to 240 ºC), has been reported to successfully breakdown lignocellulose structure in biomass materials, such as, in example, poplar [14], wheat straw [15] and olive pomace [16], among others. This hydrothermal pretreatment method has been reported to solubilize a large portion of hemicellulose while minimizing cellulose hydrolysis [17, 14]. Recently, Capoluco and Faraco [13] have evaluated the most commonly applied “green pretreatment processes” used to fractionate lignocellulosic biomass within a biorefinery concept, including LHW. They highlight the advantages of this method such as high sugar recovery after EH, no need of biomass size reduction, low reactor costs and less inhibitory compounds than other methods.

On the other hand, DA pretreatment, which is generally performed using 0.2-2% (w/w) sulphuric acid at temperatures of 140-210ºC, has been extensively used to pretreat a wide range of

lignocellulosic biomass and proved to be very effective to deconstruct lignocellulose, converting hemicellulose to monosaccharides. According to Gonzales et al. [18], the operating conditions,i.e., reaction time, temperature, acid concentration, and solid/liquid (S/L) ratio of the biomass and acid solution, would affect the efficiency of dilute acid pretreatment significantly. In this work, LHW and DA pretreatment of EOP is studied at laboratory conditions, as a first approach to the processing of EOP for sugars and other valuable compounds production via pretreatment and EH. To assess pretreatment efficacy, the enzymatic digestibility of LHW and DA pretreated EOP is evaluated, together with sugar recovery in the liquid fraction obtained after filtration of the pretreated slurry. Both have been reported to be among the principal determinants in pretreatment efficacy [19]. Moreover, the generation of sugar degradation compounds such as furfural and hydroxymethyl furfural (HMF) and the release of acetic acid and phenolic substances to liquid streams is checked along the process.

Preliminary experiments carried out by the authors on this material have shown the presence of significant amount of water-extractable compounds and thus, a previous aqueous step at mild temperature before pretreatment is envisaged to promote efficient valuable compounds recovery and minimize products degradation. This approach has been proved to be successful to improve overall sugar recovery in similar origin-related residues such as OTP [20].

After testing the different steps proposed to fractionate EOP into main constituents and quantify the amount of sugars and other valuable compounds generated along the process, several parameters are calculated to estimate the overall fractionation process efficiency. As a result, the best processing conditions leading to a maximum recovery of compounds of interest are selected and an overall mass balance calculated.

2. MATERIALS AND METHODS

2.1 Raw and processed EOP composition analysis

Firstly, the study of the chemical composition of EOP was addressed by analyzing a bulk sample from a pomace oil extracting industry (Oleocastellar, Jaen, Spain), following the methodology described by Slutier et al. [21] to quantify extractives, carbohydrates, lignin, ash and minor components. All solid EOP samples generated along processing steps were analyzed for main components following the same methodology than for raw EOP.

2.2 EOP processing

2.2.1. Water extraction

A homogeneous batch of EOP was submitted to aqueous extraction in autoclave at 15% (w/v) solids concentration, 100ºC and 30 minutes. After elapsed time, wet material was vacuum filtered to obtain a fraction of water-extracted EOP (EOP_{WE}) and an aqueous extract (WE). EOP_{WE} was submitted to composition analysis as described above for raw EOP, and WE was analyzed as described below for soluble sugars, mannitol and total phenols content. Solid recovery yield after water extraction (SRY_{WE}) was calculated as dry weight of water-extracted solid EOP referred to 100 g of raw material.

2.2.2 Liquid Hot Water (LHW) and dilute acid (DA) pretreatment

LHW pretreatment was performed on EOP_{WE} in a laboratory-scale stirred autoclave (Model EZE-Seal; Autoclave Engineers, Erie, PA) at 170, 190 and 210ºC (elapsed time until reaching target temperature; heating rate ranged between 2 ºC and 4 ºC/min). DA experiments were carried out in the same reactor using a diluted acid solution at 1 and 2% (w/v) of sulphuric acid as the catalyst. The amount of loaded feedstock corresponded to 100 g EOP_{WE} (dry weight basis, hereinafter dwb) and water/diluted acid was added at 1/5 (w/v) solid/liquid ratio. After reaching the target temperature, the reactor was kept sealed and the slurry was agitated until the reactor was cooled down to about 40ºC (in approximately 7 minutes). Wet material was vacuum filtered to obtain an insoluble solid fraction of LHW or DA-pretreated EOP ($EOP_{LHW\ or\ DA}$) and a liquid fraction or prehydrolyzate ($PH_{LHW\ or\ DA}$). Solid recovery yield ($SRY_{LHW\ or\ DA}$) was

130 calculated as EOP_{WE} dry weight remaining after pretreatment referred to 100 g of EOP_{WE}.
131 Moreover, a combined solid recovery index (CSRY), which considers solid recoveries in both
132 steps, WE and pretreatment, in relation to raw EOP, is calculated multiplying SRY_{WE} by SRY_{LHW}
133 $_{or\ DA}$ (see point 2.2.1 above).
134 A portion of $EOP_{LHW\ or\ DA}$ substrates were dried at 40ºC, milled and submitted to major
135 components analysis as described above for raw biomass. PHs were analyzed for soluble
136 sugars, mannitol, total phenols, acetic acid, furfural and hydroxymethyl furfural (HMF) content
137 as described below in section 2.2.4., Analytical Methods.

138 2.2.3. Enzymatic digestibility of LHW or DA-pretreated substrates

139 $EOP_{LHW\ or\ DA}$ was submitted to EH test at laboratory conditions: 100 ml Erlenmeyer flasks with
140 5% (w/v) dry material load in 0.05 M sodium citrate buffer (pH 4.8). Experiments were
141 performed in on a rotary shaker (Certomat-R B-Braun, Germany) at 50 ºC and 150 rpm for 72 h.
142 Cellulolytic cocktail CellicCtec2, kindly provided by Novozymes A/S (Denmark), was added in a
143 dosage of 15 FPU/g dry $EOP_{LHW\ or\ DA}$. A sample of both raw EOP and EOP_{WE} were also
144 subjected to the same EH test as controls. Samples were withdrawn at 72 hours and sugar
145 concentration in EH media measured by HPLC as described below.

146 2.2.4 Analytical methods

147 The filtrate recovered after water extraction (WE) and $PH_{LHW\ or\ DA}$ were analyzed for its content in
148 monomeric and oligomeric sugars, and WE also for compounds of interest, such as mannitol
149 and total phenols. $PH_{LHW\ or\ DA}$ was also analyzed for furfural, hydroxymethyl furfural (HMF) and
150 acetic acid, as shown below.
151 Sugars and mannitol were quantified by high-performance liquid chromatography (HPLC) in a
152 Waters 2695 liquid chromatograph with refractive index detector, as described by Manzanares
153 et al. [22]. The oligosaccharides ratio was determined as the difference in monomeric sugar
154 concentration before and after mild acid hydrolysis (3% v/v H_2SO_4, 120ºC and 30 min).

Likewise, glucose and xylose concentration in EH tests was measured by HPLC using the same column.

The level of total phenols in the water extract was determined by using Folin-Ciocalteu reagent and external calibration with vanillin following the method of Singleton et al. [23]. Acetic acid, furfural and HMF were analyzed in prehydrolyzates by HPLC (Hewlett Packard, Palo Alto, CA), as described by Negro et al. [20].

2.3. Yield calculations

To evaluate the effect of water extraction step and LHW or DA pretreatment in the fractionation of main carbohydrates (glucan and xylan) and enzymatic digestibility of EOP, several parameters are calculated based on analysis of fractions generated along processing. The formulas, which use the solid recovery yields (SRY) explained above in points 2.2.1 and 2.2.2, are shown below. All solid materials defined in the equations are in dry weight basis. A summary of abbreviations used is shown at the end of this section to facilitate understanding of the formulas.

Sugar recovery yield in water extraction step and LHW (SY)

The yields explained below refer to the amount of carbohydrate/sugar in the solid and liquid fractions after WE and LHW in relation to the initial content in feedstock used in each step.

For water-extraction step, the following yields are calculated:

$$SY\ (EOPWE\ ) = \frac{g\ of\ glucan\ or\ xylan\ in\ EOP\ WE\ x\ \%\ SRY\ (WE)}{g\ of\ glucan\ or\ xylan\ in\ raw\ EOP}\ x\ 100 \quad (1)$$

$$SYWE\ = \frac{g\ of\ glucose\ or\ xylose\ in\ water\ extract}{g\ of\ glucose\ or\ xylose\ in\ raw\ EOP}\ x\ 100 \quad (2)$$

After LHW or DA pretreatment, a solid $(EOP)_{LHW\ or\ DA}$ and a liquid fraction $(PH_{LHW\ or\ DA})$ are generated, and the recovery yields are calculated as follows:

178 $$SY\ (EOPLHW\ orDA) = \frac{g\ of\ glucan\ or\ xylan\ in\ EOP\ LHW\ or\ DAx\ \%SRY\ LHWor\ DA}{g\ of\ glucan\ or\ xylan\ in\ \ EOPWE}\ x\ 100 \quad (3)$$

179 $$SY(PHLHW\ or\ DA) = \frac{g\ of\ glucose\ or\ xylose\ in\ PH\ LHWor\ DA}{of\ glucose\ or\ xylose\ in\ \ EOPWE}\ x\ 100 \quad (4)$$

180 Finally, a total sugar recovery yield (TSRY) for each sugar for the LHW or DA step is calculated
181 summing up the values from equations (3) and (4). All SY values are shown as percentage on
182 dwb.

183 *Enzymatic hydrolysis yield (EHY)*

184 This variable refers to the yield of sugars released by EH of $EOP_{LH\ or\ DA}$ materials and shows the
185 susceptibility to the enzymatic attack of pretreated substrates. It is expressed as a percentage
186 of the maximum theoretical yield that could be achieved if glucan and xylan carbohydrates
187 present in $EOP_{LHW\ or\ DA}$ were fully hydrolyzed (hereinafter % of theoretical) and is calculated as
188 follows:

189 $$EHY = \frac{g\ of\ sugar\ in\ EH}{g\ of\ sugar\ in\ EOPLHWor\ DA}\ x100 \quad (5)$$

190 The values obtained from the formula above allow the evaluation the potential of sugar
191 production from the pretreated substrate. However, it is interesting to calculate the sugar
192 production yield in EH step in relation to raw EOP (SP_{EH}), thus giving information on how the
193 release and recovery of soluble sugars in EH contribute to the overall sugar recovery in the
194 process. The formula to calculate this variable, in percentage of the content of each sugar in
195 raw material, is the following:

196 $$SP\ EH = \frac{[\%\ EHY\ (as\ decimal) * \%\ sugar\ content\ in\ EOP\ LWH\ orDA] * \%CSRY}{\%\ sugar\ content\ in\ EOP}\ x100 \quad (6)$$

197

198

Overall sugar yield (OSY)

Overall sugar yield, calculated for main sugars glucose and xylose, refers to the amount of sugar released in both pretreatment and EH in relation to the amount of sugar in raw EOP, expressed in percentage. It is calculated summing up the production of sugars in EH step and the recovery of sugars in water extraction and prehydrolyzate by the following formula:

$$OSY = EHY\ raw\ EOP\ + \frac{g\ of\ sugar\ in\ WE + (g\ sugar\ in\ PH\ *\ \%SRY\ WE)}{\%\ sugar\ content\ in\ EOP}\ x100 \quad (7)$$

Abbreviations:

EOP_{WE} = solid fraction after water extraction of EOP
WE = water extraction step
SRY_{WE}= sold recovery yield after water extraction
$EOP_{LHW\ or\ DA}$= solid fraction obtained after LHW or DA pretreatment of EOP_{WE}
$SRY_{LHW\ or\ DA}$= solid recovery yield after LHW or DA pretreatment of EOP_{WE}
$PH_{LHW\ or\ DA}$ = liquid fraction or prehydrolyzate obtained after LHW or DA pretreatment of EOP_{WE}
CSR = combined sugar recovery yield

3. RESULTS AND DISCUSSION

3.1. Raw EOP composition

Results in Table 2, first column, show that raw EOP is mostly composed of 25 % structural carbohydrates, 24% lignin and close to 10% ash, dwb. Xylan is the main polymer of the hemicellulose fraction, present in a similar percentage to glucan, around 10%. The relatively high lignin percentage is consistent with the results reported by Sadeghi et al. [24] on lignin content of various olive oil industry byproducts such as partly destoned olive cake, which varies from 22 to 32% (dwb). Fernandes et al. [10] have quantified even higher Klason lignin (equivalent to acid insoluble lignin) content values for EOP, close to 34% dwb. Remarkably, this residue has a very high content of extractives of 42% (dwb), of which close to

90% consist of water extractable compounds and 10% extractives in ethanol. This high extractives content is a common feature of other olive biomass derived residues such as olive tree pruning (OTP) and olive leaves (OL), which have been reported to contain up to 25% and 45%, respectively [8, 25].

3.2. Water extraction step

The presence of soluble valuable compounds reported in water extractives from OL and OTP reported by Romero-Garcia et al. and Ballesteros et al. [26, 27] led to a preliminary analysis of EOP water extract that confirmed the presence of products that otherwise could be lost in subsequent steps in the fractionation process. Moreover, as discussed above, this approach has been demonstrated to be successful to improve overall sugar recovery in OTP [20, 27]. Also in softwood barks, Franko et al. [28] have recently demonstrated that a hot-water extraction step prior to steam explosion pretreatment improves the enzymatic digestibility of pretreated material.

As shown in Table 2, column 2, water extraction step leads to the removal of a great part of extractives, about 65% content of the initial amount, resulting in an extracted material (EOP_{WE}) with roughly 15% of extractives content. The quantification of non-structural sugars contained in water extract shows that glucose is the major component of this fraction (8% of EOP dry weight), with one third of this amount being in oligomeric form. Other non-structural sugars (fructose, arabinose, galactose and to a minor extent xylose and mannose) are also released adding up to a total over 12% of EOP dwb. Moreover, the analysis of extractives has shown the presence of significant amounts of compounds of interest such as phenols and mannitol, accounting for 5% and close to 4% of EOP dry weight and supporting the convenience of the water extraction approach. In agreement with these findings, Clemente et al. [29], studying the composition of EOP derived from two and three phases production processes, found similar compositions than those reported herein, describing also the presence of mannitol and glucose as the major components of polyalcohols and soluble sugars fraction of EOP.

The solid recovery yield in water extraction step (SRY_{WE}) was 64%. As a consequence of weight loss of soluble compounds, structural carbohydrates content in EOP_{WE} increases in comparison to raw EOP, reaching 30%. As a result of water extraction, soluble ash content also diminishes to 6.1% in $EOP_{WE.}$ The material resulting from WE step was used as feedstock for the subsequent fractionation step, i.e., LHW or DA pretreatment.

Regarding the sugar recovery yields in this step, values for glucan and xylan in the EOP_{WE} material [SY (EOP_{WE})], (see formula 1) account for 55.3 and 97.0 % of the content in raw EOP, respectively. In the water extract, SY_{WE}, (formula 3), these figures are 41.6 and 2.7%. Adding up solid and liquid extract, total recovery values of glucose and xylose in water extraction step reach 96.7 and 99.7 % dwb, respectively.

3.2 Effect of LHW or DA pretreatment on EOP fractionation and enzymatic digestibility

3.2.1. Chemical composition of LHW or DA-pretreated EOP and prehydrolyzates

Table 3 below summarizes the composition of the solid EOP_{WE} materials recovered from LHW or DA pretreatment tests ($EOP_{LHW\ or\ DA}$) at different temperatures and acid concentrations, as well as the SRY values. The results show an increase in glucan content as temperature rises in LHW experiments, while the effect of increasing temperature in DA trials shows a different tendency, clearly influenced by the acid concentration in the media. Glucan content in EOP_{DA} slightly increases as temperature is elevated in 1% experiments, while in 2% trials, the increase up to 210ºC leads to a significant decline to 11%, indicating structural glucan solubilization. This finding has been frequently reported in DA pretreatment of lignocellulosic feedstocks at increasing temperature pointing to a threshold that must not be exceeded to avoid structural glucose-based carbohydrates loss in the solid fraction [13]. Particularly for OTP, Cara et al. [30] describe a similar phenomenon when treating the material in the range 170-210ºC and 0.2-1.4% acid.

Xylan, the major hemicellulose polymer of EOP, solubilizes increasingly as temperature raises in both LHW and DA trials, resulting in significantly low, or even lack of, xylan content in almost all EOP_{DA} substrates compared to EOP_{WE} (15.2% dwb). A significant "concentration" effect is found in the acid-insoluble lignin present in pretreated solids as temperature in LHW, and also acid concentration in DA for each temperature value, rises. High lignin contents close to, and even over 70% dwb, are measured in LHW and DA at 190 and 210ºC. These values, referring to the raw EOP considering the CSRY, are in the interval of 23-28% dwb, which are relatively comparable or slightly higher than the value in raw EOP (22.1%, see Table 2). This is consistent with the knowledge that hydrothermal and diluted acid pretreatments can provoke lignin fragmentation that generally results in minor delignification, depending on pretreatment severity [31, 32, 33]. According to Pu et al. [31], the comparable or slightly higher lignin content values found in DA and hydrothermal pretreated biomasses can be attributed to pseudolignin formation, which could impact the enzymatic digestibility of pretreated substrates by re-depositing on the surface of cell walls and limiting enzyme access to cellulose. More particularly, Zhuang et al. [34], state that the cooling step after LHW pretreatment can also result in the deposit onto the surface of pretreated wood of lignin-like materials, which hinders the EH of cellulose. The effect of pseudo-lignin on cellulose EH has been extensively studied in the last years [33, 35], but a consensus on the mechanism underlying the cellulase inhibition caused has not yet been reached.

The analysis of $SRY_{LHW\ or\ DA}$ shows an increasing solubilization of components from EOP_{WE} as the severity raises (temperature in LHW, temperature and acid concentration in DA), with values of insoluble solids recoveries ranging from 77% to about 52% at the most severe conditions. When the solid recovery is calculated in relation to raw EOP, including solid losses in water extraction, these figures drop down to values from 50 to 34% of initial EOP dry weight.

In brief, by pretreating EOP_{WE} by LHW or DA, xylan, arabinan and galactan are partially solubilized, having the conditions of the pretreatment (temperature and aqueous or acidic media) a great impact in the hemicellulose solubilization and in the resulting concentration of

carbohydrates in the pretreated solids. Consistently, glucan content increments at different degrees reaching a maximum value around 25% at 170ºC 2% acid, compared to 15% in EPO_{WE}.

The analysis of prehydrolyzates from LHW or DA runs was aimed at evaluating the effectiveness of the pretreatment method to fractionate hemicellulose polymers and release sugars into the prehydrolyzate, while minimizing sugar losses through generation of inhibitory compounds. The recovered xylose can be considered a valuable carbon source for transformation to xylitol or ethanol by pentose-fermenting yeasts [36, 37]. Sugar and inhibitory concentrations found at different process conditions are shown in Table 4 below.

The results of sugar concentration refer only to glucose and xylose, the main sugars detected. In relation to xylose in PH_{LHW} , an increase in the concentration is found when temperature raises from 170ºC to 190ºC up to 13 g/l, but higher temperature results in a sharp decline of xylose detected, indicating sugar degradation phenomenon. In DA experiments, results show a clear effect of acid concentration in hemicellulose breakdown and sugar release within the three temperatures tested, although in a different manner. At 170ºC, increased acid concentration in the media results in a rise in xylose recovery (in monomeric form) in prehydrolyzate up to close to 18 g/l, the highest value found, that correspond to roughly 50% of xylose content in EOP_{WE}. Nonetheless, at 190ºC the effect is the opposite and the presence of increasing acid in the pretreatment media provokes a decrease in sugar recovered (from 6.6 to 1.8 g/l), and at 210ºC almost no xylose is detected.

As a consequence of increasing temperature in LHW from 170 to 210ºC, the concentration of acetic acid, furfural and HMF significantly raises, in accordance with boosted hemicellulose breakdown and, in the case of furfural and HMF, a decrease in sugar recovery. When values of inhibitory compounds are compared in LHW and DA for the same temperature, higher values are always found in DA trials, due to more severe conditions leading to more xylose degradation. Acetic acid is produced as a consequence of deacetylation of hemicelluloses and its concentration in the PH varies with the severity of the pretreatment, from 0.99 g/l at 170ºC in

LHW to around 6.6 g/l at 210ºC, both in PH_{LHW} and PH_{DA}. Deacetylation of hemicellulose chains by hydrothermal pretreatments as LHW and DA has been reported to favor enzymatic digestibility based on alleviation of steric restrictions provoked by acetyl groups in cellulase accessibility to cellulose [38] and also because of the reduction of biomass recalcitrance by the formation of more easily hydrolyzed xylo-oligomers with fewer branches [39]. Contrarily, furfural and HMF derive from dehydration of pentonse and hexose sugars, respectively, and their presence should be minimized in any pretreatment, provided that the prehydrolyzate is used in some way as fermentation media [20, 36]. Regarding this, several techniques such as overliming or liquid-solid extraction have been proven to be very effective for detoxification of lignocellulose hydrolyzates [40].

The results presented above indicate that LHW and DA at the conditions tested mainly affect at xylan component of EOP_{WE}, producing a maximum release of close to 50% of the initial content in DA at 170ºC 2% acid. PH could be detoxified and used as a valuable liquid stream for microorganism propagation and growth, or for fermentation to ethanol or xylitol. This latter approach has been successfully tested in olive tree pruning (OTP) by Martinez-Patiño et al. [36], who submitted the prehydrolyzate from DA of OTP, detoxified by overliming, to fermentation with the ethanologenic bacteria *E.coli* for ethanol production.

3.2.2. Sugar recovery yields after LHW pretreatment

Based on the results shown in Tables 3 and 4, a series of yields were calculated, in order to estimate the fractionation of main sugars, glucose and xylose, into the solid and liquid streams generated after the pretreatment of EOP previously water-extracted. The analysis of these results provides an insight of the effect of LHW and DA in the main components of EOP_{WE}, being crucial to select those pretreatment conditions leading to a total maximum sugar recovery.

Results of SY for glucose indicate that after LHW and DA, a major part of the glucan contained in EOP_{WE} is retained in the solid fraction of pretreated material (in the figure SY(EOP)), with the

harshest conditions (DA at 210ºC, 2% acid) leading to severe glucan breakdown and solubilization. In our experiments, minor glucose solublization in PH (SY PH) may be partially related to the presence of glucose containing-water extractives in EOP_{WE}, which would be easily solubilized during LHW.

In relation to xylan component retained in EOP_{WE} after pretreatment (Figure 2, panel b), results indicate that both LHW and DA are effective to dissolve hemicellulose, being xylose increasingly solubilized as temperature and acid concentration in the media levels up. Consequently, SY (EOP) values drastically fall from the maximum value over 60% at LHW at 170ºC, to no recovery in the solid at the most severe conditions in DA at 210ºC. However, although at these conditions a great part or all xylan is solubized, no xylose is detected in PH, indicating sugar losses.

The effectiveness of water catalyzed-LHW to remove hemicellulose and generate pretreated substrates with increased enzymatic accessibility has been reported in different types of lignocellulosic biomasses [15, 17, 31]. In our LHW experiments, increasing solubilization of xylan occurs as temperature rises from 170 to 190ºC, although recovery values (32 and 38%) remain under the maximum value occurring at 170ºC and 2% acid (51%).

On the other hand, in LHW trials a great part of the sugars detected in PH_{LHW} are in oligomeric form (see Table 4), which is also a well-known feature of hydrothermal pretreatments such as LHW [31]. Contrarily, by acidifying the pretreatment media in DA, all xylose is recovered in monomeric form, which implies no need of subsequent hydrolysis of oligosaccharides if monomeric xylose is needed as carbon source.

Looking at the total sugar recovery yield values in LHW and DA shown in Figure 3, it is clear that LHW at 170ºC and 190ºC and DA at 170ºC (1 and 2% acid) result in the most favorable conditions aimed at maximizing xylan/xylose recovery after EOP processing, adding up the recoveries in the solids and prehydrolyzates generated. Increased severity conditions lead to extensive losses of xylan component. Regarding glucan/glucose recovery, in general good recovery values of 92-98% are obtained after LHW or DA pretreatment at temperatures of 170-

381 190ºC, although at 210ºC in LHW the recovery diminish to 85% and in DA at 210ºC and 2%
382 acid, to the lowest value of 43%.
383
384 3.2.3. Enzymatic hydrolysis of EOP after LHW/DA pretreatment
385 The effect of WE and LHW/DA pretreatment on the enzymatic digestibility of solids generated
386 after pretreatment ($EOP_{LHW/DA}$) was evaluated by performing EH tests under laboratory
387 conditions. Results of EH yields for glucan (EH_G) and xylan (EH_X) and sugar production yields
388 by EH in relation to raw EOP (SP_{EHG} and SP_{EHXY}), are shown in Table 5 below, together with
389 the results of control experiments.
390 Firstly, it can be observed that the value of the control EOP_{WE} exceeds by 1.6 fold the yield of
391 untreated EOP, supporting the hypothesis that water extraction favors the enzymatic digestibility
392 of EOP, as demonstrated in other related byproducts such as OTP [27]. Also, Franko et al., [28]
393 have recently reported a similar phenomenon occurring by the removal of water-soluble
394 extractives of steam pretreated softwood barks that result in increased glucose yield of 10-30%.,
395 depending on the softwood.
396 Regarding the enzymatic digestibility of $EOP_{LHW\ and\ DA}$ substrates, it is clearly increased in
397 comparison to control EOP_{WE} at all pretreatment conditions tested, with increments up to 3 and
398 18 fold for glucan and xylan, respectively. Only in LHW trials at170ºC, slight effect is found in
399 EH_G (33 vs 36%). The highest EH_G values (80-95% of theoretical) are found under the most
400 severe conditions, in DA trials at all temperatures with 2% acid, LHW at 210ºC and DA at 210ºC
401 with 1% acid.
402 The results obtained in this work are substantially higher than those obtained by Fernandes et
403 al. [10] in EOP submitted to dilute acid hydrolysis with 3.5% (w/w) in autoclave at 130ºC for 130
404 min, which were < 10% of theoretical. In another feedstock such as OTP; Negro et al. [20]
405 found high glucan conversion values of 63 and 88% of theoretical when pretreating OTP by
406 acid-catalyzed steam explosion at 175 and 195ºC, respectively, in laboratory EH experiments at
407 10% (w/v) consistency. Although our experiments have been carried out only at 5%, good

performances of EOP_{LHW} at higher solid loads are expected because of the similar nature of the residues. Further research will be planned to investigate this.

As shown in Table 3, $EOP_{LHW\ and\ DA}$ substrates at the conditions leading to higher EH_G values reveal low or negligible xylan content, which is consistent with the knowledge that hemicellulose removal improves the hidrolyzability of lignocellulosic materials [14, 41]. Several factors have been described to cause the positive effect of hemicellulose removal on cellulose accessibility that has been demonstrated in numerous lignocellulosic materials. For example, Adani, et al. [42] have reported that the removal of hemicelluloses influences the nano-porous structures distribution of cell-walls, while other researchers as Zhang et al. [41] claims that xylan shows high affinity to cellulose, thus absorbing on the surface and hindering cellulase access.

Xylan digestibility is also influenced by pretreatment severity, increasing as severity rises and reaching yields close to 100% in DA experiments at 190, 2% acid and LHW at 210ºC. However, the rather low xylan content of solid at these conditions (0.2 and 1.2%) reduces greatly the significance of the result.

When sugar production yields by EH are referred to raw EOP (SP_{EH} in Table 5), it is necessary to take into account the solid losses that occur in the different processing steps, which are reflected in the CSRY [see formula (1)]. The values of SP_{EH} shown in Table 5 for glucan component follow a similar pattern than that of EH_G, while xylose values are influenced by the significant effect of LHW or DA pretreatment step in the xylan content of pretreated solids. Thus, the extensive breakdown of xylan from raw EOP during pretreatment results in a low production by EH of the pretreated solids, in spite of showing very high digestibility yield. The calculation of SP_{EH} is very useful to determine the overall sugar yield shown below, that combines the production or release of sugars from solid and liquid streams generated throughout EOP processing.

3.2.4. Overall sugar yield

The values calculated for overall glucose and xylose production yield according to formula (7) are shown in Figure 4. The results show that by processing EOP in a strategy encompassing WE, followed by both LHW and DA pretreatment, reasonably good glucose production and recovery yields can be achieved at particular process conditions. So, LHW at 210ª and DA at 170 and 190ºC and 2% acid lead to maximum yields around 85% of glucose content in raw EOP. On the other hand, OSY values calculated for xylose show some limitation of the process strategy, specifically in the pretreatment step, to release and recover xylose from EOP, which is reflected in the maximum OSY_{XYL} of 62% in DA trials at 170ºC and 2% acid.

The above results of sugar release yield from EOP after a process consisting of pretreatment and EH are in the range of others reported in the literature in different biomass residues submitted to LHW, DA or steam-explosion pretreatments (Table 6). For example, similar high OSY values of 88% for glucose and even higher for xylose (85%) were obtained by Ballesteros et al. [27] in OTP biomass pretreated by steam explosion at 187ºC and 30 minutes., while Martinez Patiño et al. [36] reported somehow lower yield around 70% sugar yield for glucose by pretreating the same feedstock by DA. In general, hydrothermal pretreatments like LHW, DA or SE have been proven to be very effective to attain good overall sugar recoveries, provided that the most adequate process conditions are selected.

Summarizing, the best conditions to pretreat EOP_{WE} would be DA at 170ºC and 2% acid that result in maximum release and recovery of both glucose and xylose sugars. Figure 5 shows a mass balance of the different EOP components during overall processing of under the best conditions. According to these results, 1 ton of EOP could yield as a whole 167 kg of glucose and 68 kg of xylose, which accounts for 78% of the total amount of those sugars in EOP, and 65% of the total carbohydrates. Further studies on the possibility of converting the sugars contained in the different streams generated along the process into valuable compounds as bioethanol or xylitol are needed to establish the real potential of this material. Also, the revalorization of the compounds recovered in the water extract, i.e., mannitol and phenols, must be reinforced to improve the potentiality of EOP use within a biorefinery context.

4. CONLCLUSIONS

The strategy tested in this work to process EOP, which consists of a water extraction step followed by LHW or DA pretreatment and EH of the water-extracted and pretreated solids has been proven to be effective in fractionating EOP biomass and in reducing the recalcitrance of lignocellulose EOP structure to enzymatic attack. The water extraction step allows the recovery of an important amount of glucose that otherwise could be lost in the subsequent step together with other compounds of interest such as phenols and mannitol, which could be processed for further valorization as antixodants and sweeteners, respectively.

The pretreatments tested in water-extracted EOP, i.e., LHW and DA, under selected conditions have proven to be effective in improving the enzymatic digestibility of pretreated EOP in comparison to unpretreated material, although some differences between methods are found. While with both LHW and DA a similar result in terms of overall glucose yield can be achieved, the presence of acid in DA pretreatment leads to a more effective release and recovery of xylose in the prehydrolyzate resulting in the maximum overall xylose yield. Although in this work the fractionation approach of EOP biomass has been studied and proven to be feasible under the processing strategy tested, it represents only a first step in the assessment of the utilization of EOP as feedstock in a potential biorefinery.It must be continued with further studies on bioconversion of sugars and compounds released and techno-economic evaluations of the whole process.

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624 **Table 1**. Advantages and disadvantages of Liquid Hot Water (LHW) and Dilute acid (DA) methods for the pretreatment of lignocellulosic
625 biomass

Pretreatment method	Advantages	Disadvantages
LHW	• No addition of catalyst or chemicals • Low formation of degradation products • High hemicellulose-derived sugars recovery • Low-cost reactors due to low corrosion potential	• High energy requirement • High water demand • Not developed at commercial scale • Lignin can be partially solubilized and redeposited onto biomass as pretreated slurry cools down
DA	• High hemicellulose solubilization yield (in momomeric form) • Extensive development of reactors for DA • Possibility of using less corrosive acids than H_2SO_4 • Widely used at industrial scale	• Depending on the process conditions, degradation of hemicelluloses • Possible formation of pseudolignins • Need of neutralization step • High investment costs for reactor construction

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631 **Table 2.** Chemical composition of raw EOP (EOP_{RM}) and the sample after water extraction
632 (EOP_{WE}). Data represent average value of triplicates and standard deviation.

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Main component	EOP_{RM}	EOP_{WE}
	% (dry weight basis)	
Extractives, *thereof*	42.0 ± 1.18	14.9 ± 1.1
Water*	37.50 ± 1.5	9.05 ± 0.97
*Glucose in water extractives***	*8.32 ± 0.65*	*1.43 ± 0.03*
Ethanol	4.53 ± 0.41	5.80 ± 0.51
Glucan	10.4 ± 0.34	15.5 ± 0.61
Xylan	9.5 ± 0.28	15.2 ± 0.46
Arabinan	1.0 ± 0.01	1.3 ± 0.05
Galactan	1.0 ± 0.02	1.4 ± 0.05
Acetyl groups	2.1 ± 0.03	2.6 ± 0.15
Lignin, *thereof*	23.7 ± 0.09	37.6 ± 0.53
Acid-insoluble***	22.1 ± 1.41	35.1 ± 0.23
Acid-soluble	1.6 ± 0.03	2.5 ± 0.15
Ash	9.4 ± 0.05	6.1 ± 0.63
TOTAL	99.1	94.6

634

635 *Corrected for soluble ash
636 ** Included in water extractives value
637 ***Corrected for acid-insoluble ash
638

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Table 3. Chemical composition of the EOP_{WE} material after LHW or DA pretreatment ($EOP_{LHW\ or\ DA}$) at different conditions, and Solid Recovery Yield values (SRY_{LHW} and CSRY). Data represent average value of triplicates and standard deviation.

Component (% dry matter)	**LHW**			**DA**					
				1% acid			2% acid		
	170ºC	190ºC	210ºC	170ºC	190ºC	210ºC	170ºC	190ºC	210ºC
Glucan	16.6 ±0.5	18.8 ±0.2	20.9 ±0.3	20.4 ±0.4	21.5 ±0.7	20.9 ±0.4	24.3 ±1.0	22.1 ±0.9	11.1 ±0.2
Xylan	11.7±0.2	7.0±0.03	1.2±0.06	7.2±0.4	1.3±0.1	--	5.0±0.1	0.2±0.04	--
Arabinan	0.2±0.02	0.11±0.0	--	--	--	--	--	--	--
Galactan	0.6±0.03	0.4±0.04	--	0.3±0.03	--	--	--	--	--
Acetyl groups	2.7±0.14	1.5±0.05	0.4±0.02	1.6±0.20	0.5±0.05	0.1±0.02	--	--	--
Acid-insoluble lignin	52.4±0.7	65.6±0.9	68.5±0.1	61.7±1.7	68.9±0.9	73.7±0.8	67.4±0.9	68.6±1.8	82.5±0.8
Acid-soluble lignin	2.7±0.14	2.9±0.5	3.1±0.1	3.1±0.9	3.1±0.9	2.3±0.2	2.6±0.1	2.8±0.04	2.1±0.07
Total lignin	55.1	68.5	71.6	64.8	72	76	70.0	71.7	84.6
Ash	4.0±0.09	4.5±0.1	4.5±0.04	3.7±0.1	3.6±0.13	4.3±0.2	3.2±0.05	4.7±0.01	5.1±0.13
% $SRY_{LHW/DA}$	77.0	69.8	59.8	64.4	60.2	57.0	53.9	53.4	52.5
%CSRY	49.3	44.7	38.3	41.2	38.5	36.5	34.5	34.2	33.6

"--"= not found

Table 4. Concentration (g/l) of main sugars and soluble compounds detected in prehydrolyzates after LHW or DA pretreatment at different conditions. Values in brackets in sugars column show percentage of sugars in oligomeric form.

Pretreatment	Condition (Acid concentration in DA media; T)		Glucose	Xylose	Acetic acid	Furfural	HMF
LHW		170ºC	4.1 (43.3)	3.1 (95.2)	0.99	0.03	0.06
		190ºC	2.0 (63.0)	13.1 (95.4)	2.36	0.38	0.08
		210ºC	1.0 (40.4)	1.7 (51.3)	6.67	2.39	0.36
DA	1%	170ºC	4.5 (12.0)	15.3 (35.4)	2.16	0.9	0.4
		190 ºC	2.2 (2.2)	6.6 (13.0)	5.4	6.5	1.1
		210ºC	1.1 (21.6)	0.1 (19.0)	6.6	6.6	2.5
	2%	170ºC	5.3 (0)	17.5 (0)	3.9	2.6	0.6
		190 ºC	5.4 (0)	1.8 (0)	6.2	1.6	1.6
		210ºC	2.0 (0)	0	6.7	7.3	2.3

Table 5. Enzymatic hydrolysis yield of $EOP_{LHW\ or\ DA}$ for glucan and xylan and sugar production yield by EH in relation to raw EOP.

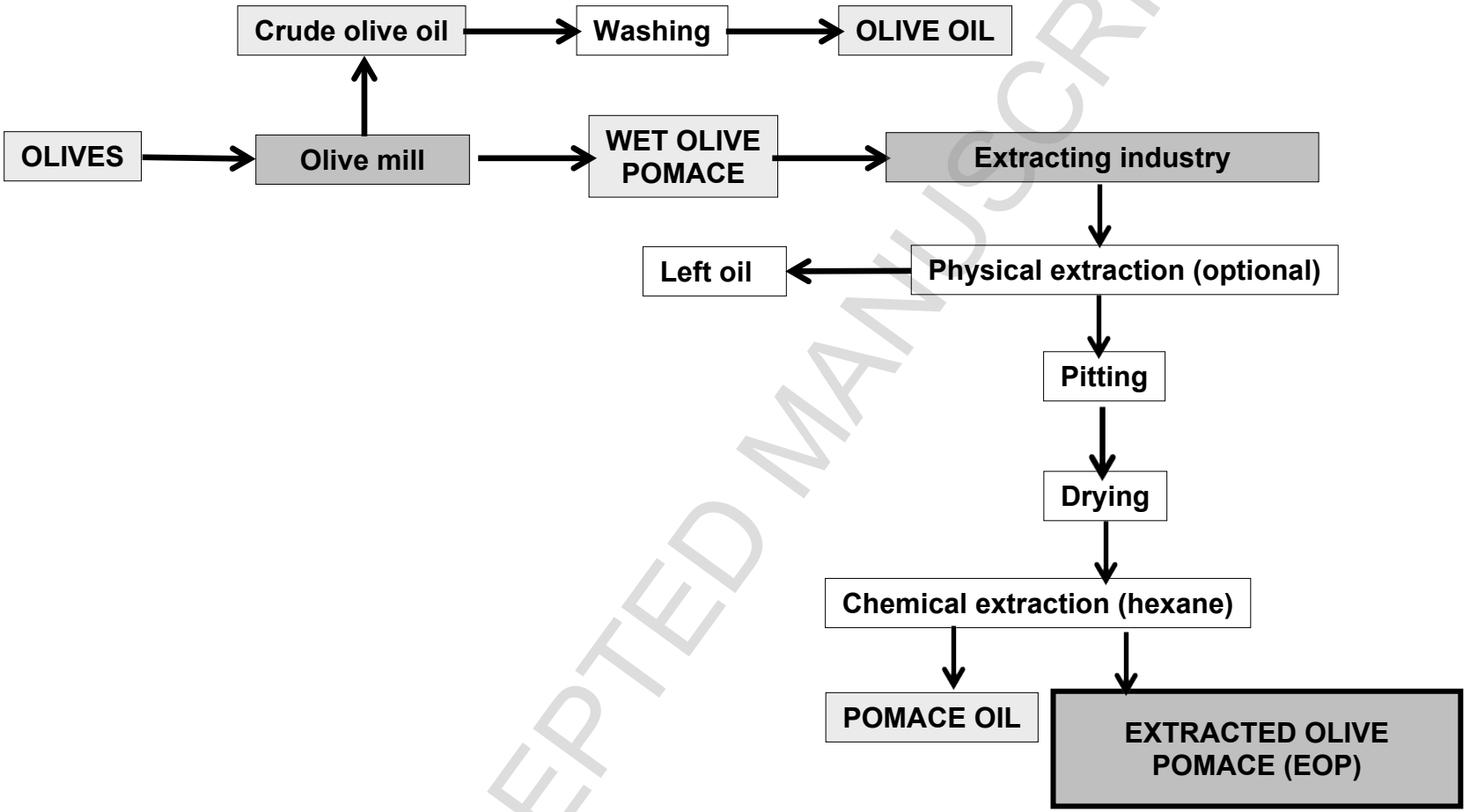
Pretreatment	**Condition (T, acid concentration in DA media)**	**EHY (% of theoretical)**		**SP_{EH} (% of sugar content in raw EOP)**	
		Glucan	Xylan	Glucose	Xylose
LHW	170ºC	35.9	16.0	16.2	9.4
	190ºC	60.1	36.0	28.6	11.6
	210ºC	94.4	99.8	42.1	4.7
DA	170ºC, 1%	58.6	14.7	25.3	4.3
	170ºC, 2%	76.4	57.0	36.5	9.0
	190ºC, 1%	65.9	59.5	31.0	3.1
	190ºC, 2%	84.0	93.0	35.5	0.7
	210ºC, 1%	91.5	n.a.	38.7	n.a.
	210ºC, 2%	81.6	n.a.	16.3	n.a.
	Control EOP_{WE}	*33.2*	*5.34*	n.a	n.a
	Control raw EOP	*20.8*	*2.50*	n.a	n.a

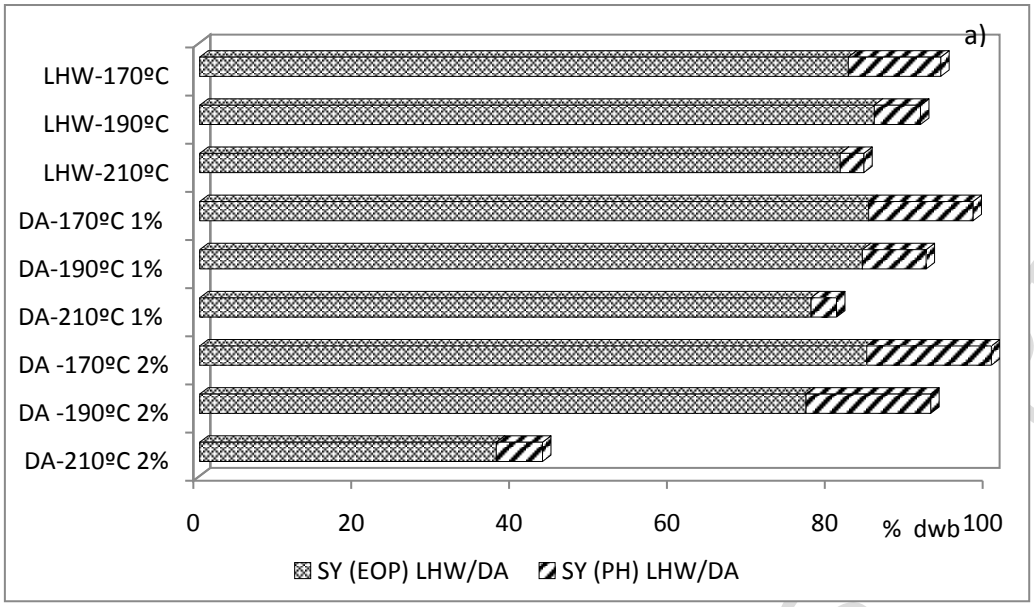
n.a. not applicable

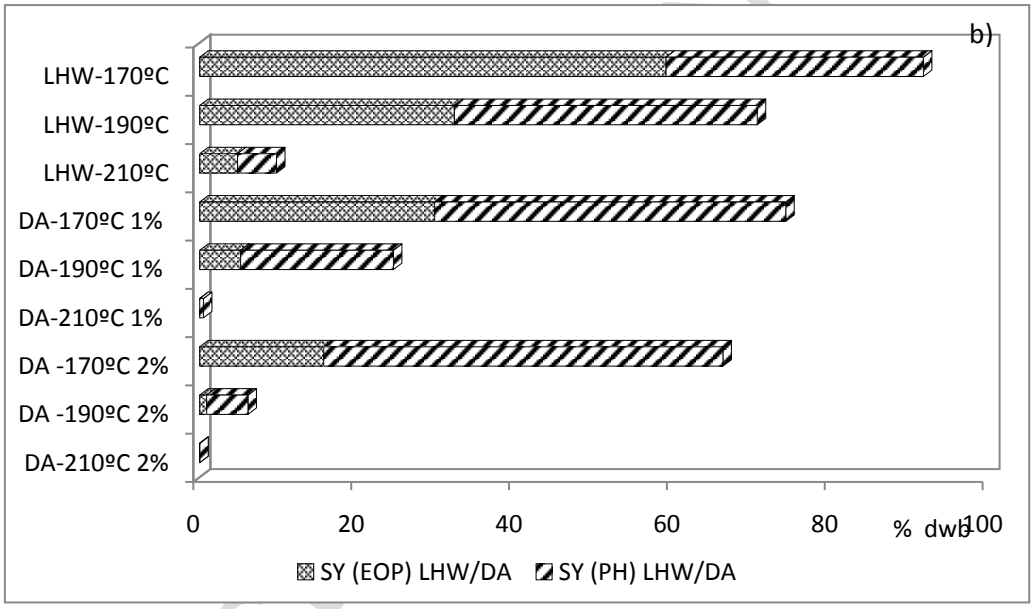
Table 6. Summary of feedstocks, process conditions and sugar yield from selected cases of study that use Liquid id Hot Water (LHW), Dilute acid (DA) or Steam Explosion (SE) for the pretreatment of different lignocellulosic biomass feedstocks

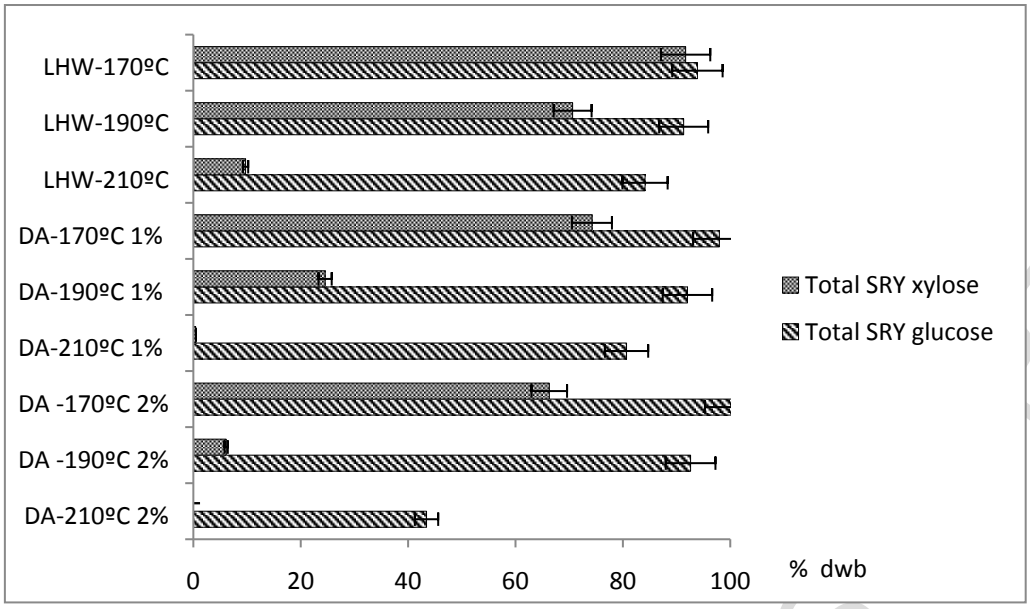
Pretreatment	Feedstock	Process conditions	Sugar yield (% of sugar content in raw material)		Reference
			Glucose	Xylose	
LHW	Corn cobs	160ºc, 10 min	82	71	[43]
	Wheat straw	188ºC, 40 min	76	38	[15]
	Olive pomace	210ºC, 4 min	74	n.d.	[16]
DA	Olive tree pruning	H_3PO_4 1.5% (w/v), 170ºC	68	78	[36]
	Olive tree pruning	H_2SO_4 1% (w/w), 190ºC	75	n.d.	[30]
	Cardoon biomass	H_2SO_4 0.2% (w/v), 200ºC for glucose H_2SO_4 0.1% (w/v), 180ºC for xylose	81	93	[44]
SE	Banana rachis residue	198ºC, 5 min (impregnated with H_2SO_4 1.5% (v/v)	87……	n.d.	[45]
	Olive tree pruning	187ºC, 30 min	88	85	[27]
	Rapessed straw	200ºc, 1.5 min	99……	n.d.	[46]
	Suagar cane bagasse	215ºC, 5 min	87……	n.d.	[47]

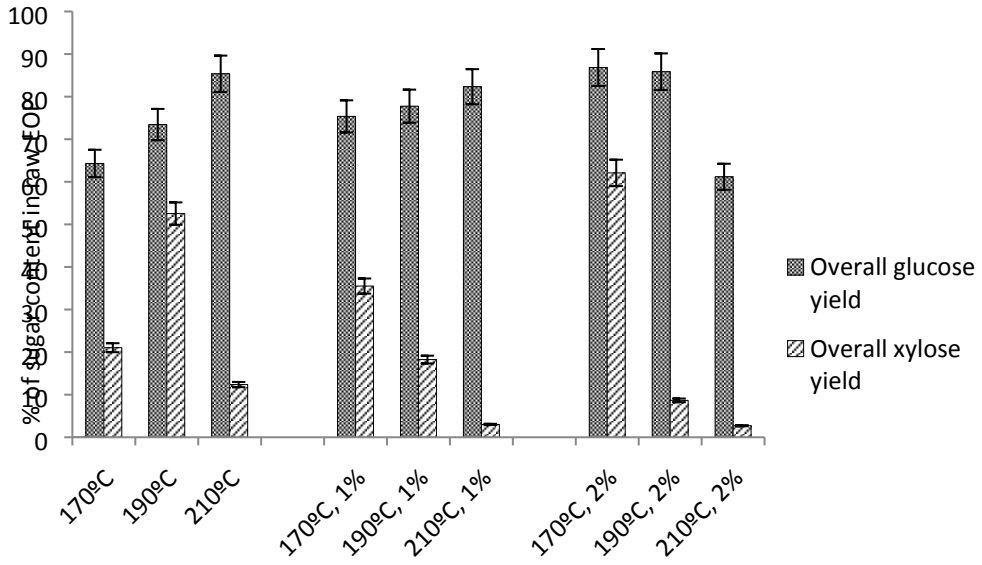
n.d: not disclosed




a)
LHW-170ºC
LHW-190ºC
LHW-210ºC
DA-170ºC 1%
DA-190ºC 1%
DA-210ºC 1%
DA -170ºC 2%
DA -190ºC 2%
DA-210ºC 2%
0
20
40
60
80
% dwb
100
SY (EOP) LHW/DA
SY (PH) LHW/DA


b)
LHW-170ºC
LHW-190ºC
LHW-210ºC
DA-170ºC 1%
DA-190ºC 1%
DA-210ºC 1%
DA -170ºC 2%
DA -190ºC 2%
DA-210ºC 2%
0
20
40
60
80
% dwb
100
SY (EOP) LHW/DA
SY (PH) LHW/DA





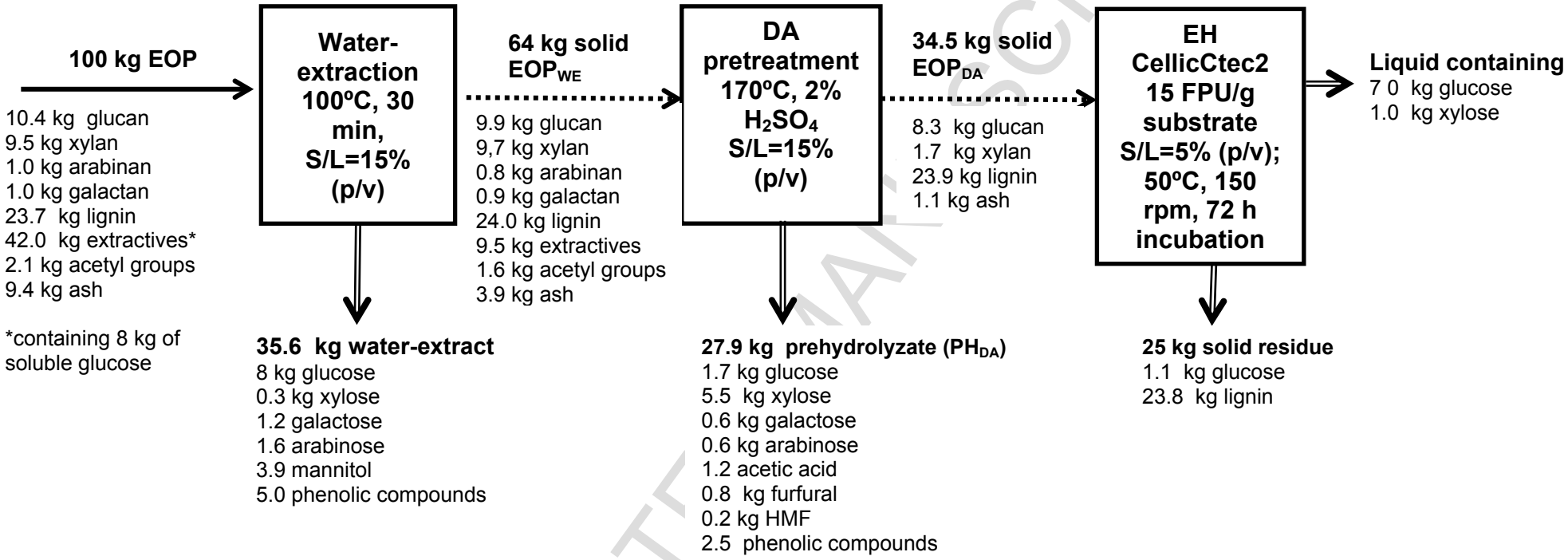


Figure 1. EOP generation scheme followed in traditional two-phases olive oil extraction system in Spain. Adapted from Manzanares, P. et al., 2017. Spanish Journal of Agricultural Research 15(3), 12 p.

Figure 2. Sugar recovery yields for glucose (a) and xylose (b) in solid fraction and prehydrolyzate of EOP pretreated by LHW or DA at different process conditions, in relation to EOP submitted to a water extraction step (EOP_{WE}). Values are shown in percentage of sugar content in EOP_{WE}

Figure 3. Total glucose and xylose recovery yields in LHW or DA pretreatment of EOP_{WE} at different process conditions, including the water extraction step. Values are shown in percentage of sugar content in EOP_{WE}.

Figure 4. Overall sugar yield (OSY) of glucose and xylose after WE and LHW or DA pretreatment of EOP at different process conditions. Values are shown in percentage of sugar content in raw EOP.

Figure 5 – Mass balance of 100 kg of EOP, submitted to water-extraction, pretreatment by dilute acid at 170ºC and 2% sulphuric acid and enzymatic hydrolysis.

- Extracted olive oil pomace (EOP) is studied as novel lignocellulosic feedstock
- LHW or Dilute acid pretreatments successfully fractionate EOP biomass
- A water extraction step allows recovering valuable compounds as mannitol and phenols