Different pretreatment strategies to enhance fermentable sugar production from olive stone in the context of an olive-derived biomass biorefinery

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Introduction

The production of bioenergy and bioproducts from lignocellulosic biomass is a very promising way of reducing the dependence on oil and the effects of climate change in the transport and the chemical industry, as well as a real driving force for the development of lignocellulose based biorefineries. This approach is particularly relevant in rural areas with high density of residual biomass and by-product generation, as is the case of olive growing areas and the associated olive oil industry. The main residues and by-products produced in this industry are olive tree pruning (OTP), derived from the pruning operations in olive tree crop; extracted dry olive pomace (EOP), produced in olive oil pomace extraction industries; and olive leaves (OL) and stones (OS), produced at different steps of the olive oil extraction process in olive mills (Romero-García et al., 2014). Regarding OS, it is commonly used as a solid fuel due to its high heating power value; however, its consumption has suffered significant fluctuations in the last years: this has shown a possible scenario of a surplus stock resulting in difficulties in its commercialization. This situation has motivated the search for alternative upgrading processes, since OS is an abundant material in olive oil producing countries as for example, Spain, accounting for around 10% of the olive weight.

OS, as a lignocellulosic biomass material, requires an initial pretreatment step to break down the biomass structure and facilitate the recovery and use of main lignocellulose components, i.e., carbohydrates (cellulose and hemicellulose) and lignin, in the subsequent conversion process stages. Carbohydrates contained in OS biomass can be hydrolyzed by cellulolytic enzymes resulting in a stream of monosaccharides that could be used as sugar platform for several applications. In this work, different pretreatment techniques are tested for OS biomass, based in the use of steam explosion (SE) or extrusion technology in a two-step pretreatment strategy that includes a previous treatment with dilute acid. Although both types of pretreatment have shown to be effective as methods to alter lignocellulosic biomass and favor the release of sugars, they present differences in the operation conditions and in the main effects produced, which are necessary to evaluate prior to the selection of one or another procedure.

Materials and Methods

OS biomass (8% moisture and a particle size between 1 and 3.15 mm), supplied by a local company in Jaén (Andalusia, Spain), was analysed by triplicate according to Sluiter et al., (2010) to determine its chemical composition. The OS material was subjected to a two-step pretreatment process strategy. The first step was a treatment with sulphuric acid in autoclave at previously optimized conditions of 128ºC, 10.5 g acid/100 g OS and 33% (w/v) solids content, and the resulting slurry was next separated into a solid and a liquid fraction. The liquid fraction was analysed for sugar composition and the sugar recovery yield was calculated. Then, the solid fraction of acid-treated OS biomass was submitted to SE or extrusion pretreatment under different process conditions.

SE pretreatment was carried out in a small pilot unit equipped with a 2 L reaction vessel at 195, 210, and 225 ºC for 5 min, according to the results of previous works with olive stones (Ballesteros et al., 2001). As for extrusion pretreatment, it was carried out in a co-rotating twin-screw extruder with 6 barrels and length to diameter ratio (L/D) equal to 24. Different process conditions of temperature and were tested: 100, 125 and 150 ºC, at a catalyst (NaOH) to OS dry matter ratio (NaOH/DM ratio) of 15% (w/w), based on previous extrusion tests carried out in OS biomass (Doménech et al., 2020).

To evaluate the effectiveness of both pretreatments, the chemical composition of sequentially-pretreated OS materials was determined as indicated above for untreated OS and compared to raw OS biomass. Moreover, enzymatic hydrolysis (EH) tests in such materials were performed at laboratory scale using commercial enzymes [5% (w/v) solid fraction load, incubation with 15 FPU/ g solid of cellulase cocktail at 50 ºC for 72 h.], in order to calculate the sugar release yield and assess the enzymatic digestibility improvement after both pretreatment strategies. The SE-pretreated substrates needed a further milling step to promote enzymatic hydrolysis of carbohydrates. All sugar measurements were performed by High Performance Liquid Chromatography (HPLC) as described by Doménech et al., (2020).

Results and discussion

The results of OS biomass analysis showed the following average composition (in % dry weight basis): cellulose, 20.8 ± 0.2; hemicellulose, 25.9 ± 0.1(composed of xylan, 23.4 ± 0.1, galactan, 1.2± 0.0, arabinan, 1.2 ± 0.0 and mannan 0.1 ± 0.0), acetyl groups, 5.9 ± 0.1; acid insoluble lignin, 33.8 ± 0.6; acid soluble lignin, 1.7 ± 0.1; ash, 0.6 ± 0.0 and extractives, 6.4 ± 0.5. Total carbohydrates content accounting for close to 50% of OS dry weight indicates the substantial potential of this feedstock as a source of fermentable sugars, although the significant content in lignin may predict difficulties for cellulolytic enzymes accessibility.

The results of the first pretreatment step with sulphuric acid showed the liquor contained 62.1 g/L of xylose, which corresponds to a xylose recovery of 71% of the xylose content in raw OS. The separated solid fraction was submitted to extrusion and SE pretreatment runs under different conditions and the resulting materials were tested for sugar production in EH experiments. The Figure 1 below shows the main results of sugar production from those substrates, both in g/L of main sugars released (glucose and xylose) and in % of the sugar content in the pretreated material (sugar release yield for glucose, GRY and xylose, XRY). First of all, the data reveal a great improvement of enzymatic hydrolysis performance in pretreated OS substrates in comparison to raw OS, showing the effectiveness of both techniques. By comparing both pretreatments, significant differences are found in sugar concentration in EH media (glucose, up to 18 g/L in SE vs 7 g/L in extrusion; xylose, 1.7 g/L in SE vs 7 g/L in extrusion) and also, although to a minor extent, in the enzymatic digestibility of pretreatment materials (glucose SRY, up to 78% in SE vs 61% in extrusion; xylose SRY up to 54% in SE vs 61% in extrusion). These results can be in part explained by the different performance of the pretreatments tested, which result in important dissimilarities in carbohydrates composition of pretreated materials. Thus, SE leads to a substrate with a higher content of cellulose due to xylan solubilisation during pretreatment, while the output of the extrusion pretreatment was a single solid stream that retained almost all carbohydrates.

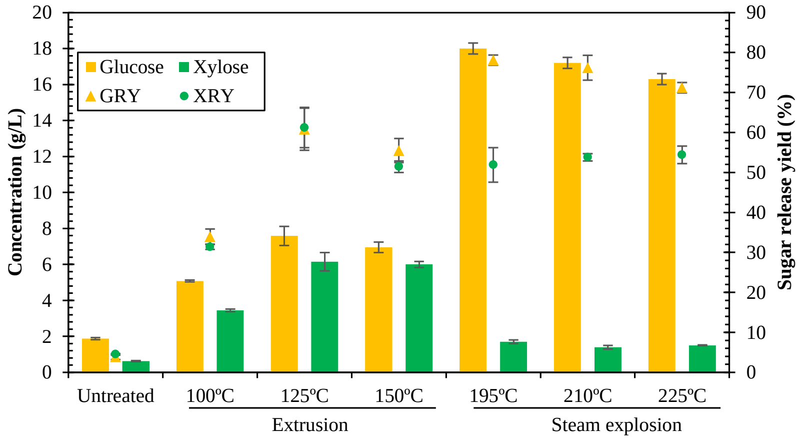

Figure 1. Sugar concentration (g/L) and release yield (% of sugar content in raw OS) for glucose and xylose produced by HE of sequentially-pretreated OS (acid treatment + steam explosion or extrusion).

Summarizing, from the results shown above it can be inferred that the most promising sequential fractionation scheme suggested for OS is an strategy to recover xylose in the first-acid step under the conditions tested herein, as well as cellulosic glucose after an SE step at 195 ºC for 5 min and EH, provided that a milling step is introduced before EH. However, it is necessary to consider the mass balance of the whole process under the two strategies, which will be also presented, to finally determine the most suitable way to process OS.

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