Effect of organosolv pretreatment on delignification and enzymatic hydrolysis of exhausted olive pomace

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1. Introduction

In the olive pomace industry, the exhausted olive pomace (EOP) is the main residual solid biomass generated after the extraction of the olive pomace oil with hexane. According to its composition, EOP could be used as feedstock for a lignocellulosic biorefinery (Contreras et al., 2020). It is an interesting alternative to its current application as biofuel since the former allows the production of bioenergy and value-added products such as bioactive compounds (Gómez-Cruz et al., 2020).
Therefore, the aims of this work are to valorize EOP through obtaining phenolic compounds applying two sequential extraction steps, and to evaluate the effect of organosolv pretreatment on the delignification and the enzymatic hydrolysis of the extracted EOP for recovering glucose from cellulose. For the former step, water and acetone:water were tested, while the organosolv pretreatment was carried out with ethanol:water at different proportions using sulfuric acid as catalyst . Ethanol is considered a good, effective and relatively inexpensive delignification solvent (Huijgen et al., 2010).

2. Materials and methods

The EOP used in this work was subjected to two sequential extractions steps. First, a previously optimized aqueous extraction (85ºC, 10% solids and 90 min) was applied in a thermostatic water bath according to Gómez-Cruz et al. (2020). As a result, two fractions were obtained: an aqueous extract and a solid fraction (extracted EOP). In order to recover more antioxidant compounds and also to eliminate more extractives that may interfere in the subsequent steps, the extracted EOP was subjected to a second extraction and two solvents were tested: water at 85ºC, 10% solids and 90 min in a thermostatic bath, as before, and 70% acetone at 30ºC, 10% solids and 30 min in an orbital shaker. Organosolv pretreatment with 50% or 60% ethanol and catalyzed with 1% H_2SO_4 were then evaluated at different temperatures and extraction times in a laboratory scale, 1-L stirred tank reactor. Table 1 shows the conditions of the different pretreatments.

Table 1.Conditions applied to recover antioxidant compounds and to delignify the exhausted olive pomace.

Second extraction[a]	Pretreatment	Pretreatment conditions	
		Ethanol (%)	T (ºC)
70% acetone (85ºC, 10% solids, 90 min)	P1	50	110
70% acetone	P2	60	110
70% acetone	P3	50	130
Water (30ºC, 10% solids, 30 min)	P4	50	130
Water	P5	50	140

[a]This extraction was applied to the extracted EOP obtained from the first extraction step with water at 85ºC, 10% solids and 90 min. All organosolv pretreatment experiments were catalysed with H_2SO_4 1% using a solid loading of 15% for 60 min.

According to the National Renewable Energy Laboratory (NREL) methodology, the content of extractives, cellulose, hemicellulose, lignin, moisture and ash was determined (Sluiter et al., 2012) in: the raw EOP, solids resultingsfrom the different extraction steps, and the pretreated solids.

The total phenolic compounds (TPC) and the antioxidant capacity were determined in the extracts and the pretreatment liquors. The TPC was measured following the Folin–Ciocalteu assay according to Gómez-Cruz et al. (2020). Gallic acid was used as standard and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extracted EOP. Antioxidant activity were determined through ABTS and FRAP at 734 nm and 593 nm, respectively (Gómez-Cruz et al., 2020). Trolox was used as standard, and the results were expressed as milligrams of Trolox equivalents (TE) per gram of extracted EOP. All the

measurements were carried out by triplicate and a Bio-Rad iMarkTM microplate absorbance reader was employed (Hercules, CA, USA) with 96-well polystyrene microplates. The phenolic profile of the extracts was also obtained by capillary zone electrophoresis (CZE).

To choose the best organosolv pretreatment, the percentage of delignification was estimated. It was also considered the efficiency of the enzymatic hydrolysis to convert cellulose from the pretreated solid into glucose according to Martínez-Patiño et al. (2015).

3. Results and discussions

After the first water treatment (85ºC for 90 min), a second extraction was carried out to recover more antioxidant compounds and valorize EOP. The use of water as solvent is classified as a safe, green and environmentally friendly solvent and an alternative to organic solvents (Hartonen et al., 2017). In general, the effect of the water and acetone 70% in the second extraction step was very similar in terms of TPC and antioxidant capacities (DPPH, ABTS and FRAP). In terms of the content of extractives in the extracted EOP, the two solvents led to solids with around 10%. Considering the two sequential extraction steps, a total reduction of 76.2% of the amount of extractives was obtained in the extracted EOP with respect to the raw material. Consequently, all extraction condiction yielded solid enriched in both cellulose and hemicellulose due to the removal of extractives.

The pretreatments P1 and P2 showed the lowest delignification values, 11.8% and 16.7%, respectively. Pretreatments P3 and P4 showed similar delignification values, 43.2% and 46.0%, respectively, regardless of the previous extraction steps, i.e. a second extraction with 70% acetone or with water (Table 1). In particular, the pretreatment P5 produced the highest delignification value, about 52.8%. Thus, it was observed that an increase in temperature leads to a higher delignification; i.e. the lowest values were obtained at 110°C (P1 and P2), while the highest values were at 140°C (P5).

The effect of the different pretreatments on the enzymatic digestibility of the pretreated solids (referred to the glucose content in the extracted EOP) was also evaluated. For the pretreatments P1 and P2, the enzymatic digestibility was 29% and the enzymatic hydrolysis yield (EHY) (referred to the total glucose content in the crude EOP) was 27%. The highest value of enzymatic digestibility was achieved applying the pretreatments P4 and P5 (about 80%) and the enzymatic hydrolysis yield was up to 57% in both cases. Finally, the pretreatment P5 was chosen since it produced the highest delignification and achieved the highest enzymatic digestibility, which will be applied for future EOP valorization steps.

4. References

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