Biorefinery of olive pomace waste: optimization of Organosolv pretreatment process

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The olive-oil sector holds an important role in the economy of some Mediterranean countries such as Greece, Italy and Spain. However, there are differences in terms of the extraction system that each country uses. Greece production is based on 3-phase systems (3P), while 90% of Spanish olive mills are using 2-phase system (2P). The use of one system or another leads to significant differences in the generated wastes. 3P olive mills produce two types of wastes, namely olive pomace (OP) and olive mill wastewater, whereas 2P systems produce a mixture of wastewater and OP denoted as TPOMW (two-phase olive mill waste), that has been recently used to obtain another type of oil called "orujo" olive oil. The waste derived from this process is known as olive mill waste (OMW) (del Pozo et al., 2018; Dourou et al., 2016). The whole amount of waste is generated during the 2-month period of harvesting and pressing season, which causes significant problems regarding its handling and processing (Dourou et al., 2016). The traditional uses of these residues involve the combustion or their use as a fertilizer. However, the direct use of these waste streams on soils is not favorable due to the presence of, among others, high content of salt, organic acids, phenolic compounds and oils. Several biotechnological solutions that have been proposed, suggest bioremediation processes and are focused on the elimination and/or recovery of phenolic compounds (Galanakis et al., 2018; Hamza et al., 2012; Khoufi et al., 2006). In this work we propose the use of OP, TPOMW and OMW as a source of sugars towards the formulation of fermentable culture media with a great potential in the biorefinery sector. An integral key step for the exploitation and valorization of lignocellulosic biomass, is the application of an initial pretreatment that will convert raw materials to a form amenable to enzymatic degradation (Yang et al., 2008). For this purpose, organosolv technology was chosen as the pretreatment method, in which organic solvents or their aqueous solutions are used as the pretreatment medium. Organosolv pretreatment enables the efficient fractionation of biomass components, the separation of cellulose and lignin, generating less amounts of inhibitor compounds (Zhang et al., 2016). The aim of this study is to use organosolv technology to efficiently carry out the delignification of the material which will be subsequently saccharified by commercially available enzymes. In that way, the carbohydrates from cellulosic and hemicellulosic fraction will be hydrolyzed to yield fermentable sugars.

For the optimization of the conditions of the organosolv pretreatment, an experimental design was set up using a Central Composite Rotatable Design (CCRD) and performed by employing the software STATISTICA 13.0. In order to select the upper and lower limits for the variables of the factorial design, preliminary tests studying the effect of the solvent (acetone, ethanol/EtOH and tetrahydrofuran/THF), the temperature (100 or 174 ºC) and the presence or absence of 0.5% *w/w* sulfuric acid as a catalyst were assayed. The remaining solid fraction after pretreatment was washed and analyzed by quantitative acid hydrolysis (QAH) in a two-stage process to determine the cellulose, hemicellulose and lignin content.

The results indicate that the untreated material exhibits a percentage of carbohydrates susceptible to be separated from lignin by organosolv technology, but also high amounts non-structural water-soluble extractives (18.44 ± 0.42 %) and ethanol-soluble extractives (24.91 ± 0.45 %). Compositional analysis after pretreatments also shown that those factors that had the greatest effect over the carbohydrate content were the temperature, and the presence of the catalyst. Regarding the solvent, the acetone was the most suitable solution for the pretreatment, however the experiment conducted with EtOH: dH_2O in presence of 0.5% *w/w* H_2SO_4 enhanced up to 2.35-fold times the cellulose recovery in the solid fraction and resulted in reduction of the lignin content (around 10%). For that reason, EtOH and acid catalyst were chosen for further experiments in order to optimize the fractionation of OMW.

The following step of this study was the selection of the independent variables of CCRD (**Table 1**). These involved the time of pretreatment (X_1), the temperature (X_2), and the ratio organic solvent: water (X_3), while the response variables were the percentage of cellulose/hemicellulose recovery, and lignin removal. A total of 23 experiments were carried out and the results of the compositional analyses were used to generate a second-order polynomial equation. Three-dimensional surface plots were also generated to illustrate the relationship and interaction between the coded variables and the responses. The results demonstrated that organosolv pretreatment is an efficient method to obtain a carbohydrate-rich solid fraction that can be subsequently used for the production of fermentable sugars.

Table 1. Definition of independent variables and levels used for CCRD matrix

Independent variables		**Variable limits**				
Definition (Units)	Variable in model	**- α**	**-1**	**0**	**1**	**+ α**
Time (min)	X_1	6.36	20	40	60	73.65
Temperature (ºC)	X_2	55.91	90	140	190	224.09
Ratio EtOH: dH_2O	X_3	7.95	25	50	75	92.05

The objective of the organosolv pretreatment is to enhance the accessibility of the enzymes and to facilitate the release the carbohydrates from the cellulose and hemicellulose fraction. For that reason, the enzymatic hydrolysis of the solid fraction to obtain monomeric sugars was performed. These carbohydrates can be subsequently used as carbon sources by microorganisms and metabolized into high value-added bioproducts or biomolecules. With this last point, the present work will target not only to reduce the environmental pollution, but also to drive the production of "natural" biomolecules obtained through microbial metabolism. The biotechnological production of compounds and molecules allows for the reduction and/or elimination of synthetic processes and, at the same time, it receives a positive acceptance by the consumers and the industry. Although more experiments need to be performed regarding the load and the combinations of enzymes, the high percentage of cellulose recovered after the organosolv pretreatment renders the process promising for further optimization.

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References

del Pozo, C., Bartrolí, J., Puy, N., Fàbregas, E., 2018. Separation of value-added chemical groups from bio-oil of olive mill waste. Ind. Crops Prod. 125, 160–167.

Dourou, M., Kancelista, A., Juszczyk, P., Sarris, D., Bellou, S., Triantaphyllidou, I.E., Rywinska, A., Papanikolaou, S., Aggelis, G., 2016. Bioconversion of olive mill wastewater into high-added value products. J. Clean. Prod. 139, 957–969.

Galanakis, C.M., Tsatalas, P., Galanakis, I.M., 2018. Implementation of phenols recovered from olive mill wastewater as UV booster in cosmetics. Ind. Crops Prod. 111, 30–37.

Hamza, M., Khoufi, S., Sayadi, S., 2012. Fungal enzymes as a powerful tool to release antioxidants from olive mill wastewater. Food Chem. 131, 1430–1436.

Khoufi, S., Aloui, F., Sayadi, S., 2006. Treatment of olive oil mill wastewater by combined process electro-Fenton reaction and anaerobic digestion. Water Res. 40, 2007–2016.

Yang, B.; Wyman, C. 2008. Pretreatment: The key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod. Biorefin. 2, 26–40.

Zhang, K., Pei, Z., Wang, D., 2016. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. Bioresour. Technol. 199, 21–33.