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Ultrasounds pretreatment of olive pomace to improve xylanase and cellulase production by solid-state fermentation

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HIGHLIGHTS

- Ultrasounds as potential pretreatment for lignocellulosic residues.
- Improve cellulase and xylanase production by solid-state fermentation.
- Evaluation of wastes from new olive oil extraction system.
- Physicochemical comparison of olive mill wastes.
- Selection of best producers of cellulases and xylanases.

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ABSTRACT

Olive mills generate a large amount of waste that can be revaluated. This work aim to improve the production lignocellulolytic enzymes by solid-state fermentation using ultrasounds pretreated olive mill wastes. The composition of olive mill wastes (crude and exhausted olive pomace) was compared and several physicochemical characteristics were significantly different. The use of both wastes in SSF was evaluated and a screening of fungi for xylanase and cellulase production was carried out. After screening, the use of exhausted olive pomace and *Aspergillus niger* led to the highest enzyme activities, so that they were used in the study of ultrasounds pre-treatment. The results showed that the sonication led to a 3-fold increase of xylanase activity and a decrease of cellulase activity. Moreover, the liquid fraction obtained from ultrasounds treatment was used to adjust the moisture of solid and a positive effect on xylanase (3.6-fold increase) and cellulase (1.2-fold increase) production was obtained.

1. Introduction

Over the past decade, the olive mills have changed their traditional extraction method from the three phase system to the two-phase system. This change improves the olive oil quality and reduces wastewaters disposal costs because the two phase system barely generates wastewaters (Khdair et al., 2015). However, it generates a wet olive pomace or crude olive pomace (COP) whose handling is more difficult and cause more disposal problems than the pomace obtained by the three phase system (Cayuela et al., 2010). The COP needs new alternatives to reduce its environmental and economic impact. In the 2014/15 season, global olive oil production was around 2,287,000 t, according to the International Olive Council. Per ton of processed olives, 800–950 kg of COP are produced (Azbar et al., 2004), as a result 1,829,600 t of COP are generated each year. Currently, olive oil industries recover residual olive oil from COP. This waste has around 3.5% residual oil in wet basis. In spite of the doughy consistency hinder the drying of COP, the extraction of residual oil is still applied (Rincón et al., 2012). After olive oil extraction and a drying step, the exhausted olive pomace (EOP) can be used in combustion processes. However, combustion of EOP causes environmental problems due to the emission of toxic compounds in gas form (Niaounakis and Halvadakis, 2006) and it requires high energy consumption to reduce the moisture content. Thus, it is necessary to search new alternatives that could increase the value of these wastes and reduce their environmental impact. The investment in the revalorization of this waste is a current opportunity, and it may also contribute to the protection of the environment.

The use of EOP as solid substrate in solid-state fermentation (SSF) is an attractive solution, since due to its lignocellulosic nature can be an inductor of lignocellulolytic enzymes. However, its direct use in biological processes is not possible due to its high

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organic load, presence of antimicrobial compounds such phenols and lipids, low pH value and low nutrients concentration (Morillo et al., 2009). These barriers can be overcome in biotechnology processes by mixing with other wastes (Salgado et al., 2014a, 2015; Oliveira et al., 2016) or by pre-treatments (Gianico et al., 2013).

Pre-treatments enhance the biodigestibility and porosity of the residues and increase the accessibility of microorganisms to the materials improving the production of enzymes by SSF. To select the most appropriate pre-treatment, several criteria must be taken into account such as to minimize loss of hemicelluloses and cellulose, do not require the addition of toxic chemicals, minimize the use of energy, low capital equipment and chemicals demand, as well as the possibility of an easier scale-up (Holtzapple and Humphrey, 1984). Sonication largely meets these criteria, it is a physical treatment that can increase size of pores and accessible surface area, decrease degrees of polymerization of cellulose and crystallinity and improve the biodegradability or enzymatic hydrolysis of the residues (Taherzadeh and Karimi, 2008). Ultrasound pre-treatment (US) causes cavitation bubbles formation in the liquid phase, the bubbles grow and then violently collapse when they reach a critical size. US is influenced by four main factors: specific energy, ultrasonic frequency, application time, and the characteristics of the substrate (Rincón et al., 2014). The US is widely used as pre-treatment for anaerobic digestion processes with promising results (Rincón et al., 2014). However, the use of US pretreated solid as substrate in SSF was barely studied.

The SSF also appears as an attractive alternative to submerged fermentation (SmF). In SSF the microbial growth and product formation occurs at or near the surface of the solid substrate particle having low moisture contents. There are several potential advantages for bioprocessing and production of various value-added products compared to submerged fermentation, because products have higher yield, lower energy requirements and produces less wastewater with less risk of bacterial contamination (Pandey et al., 2007).

In recent years, it has been an increasing interest in the production of enzymes using low-cost processes as SSF. In this sense the agro-industrial substrates are considered suitable for enzyme production by SSF. Cellulases and xylanases are lignocellulolytic enzymes with applications in several industrial sectors as biofuels industries. Given the advantages of enzymatic route in bioethanol production from lignocellulosic residues, there is an increasing demand in the production of enzymatic cocktails containing cellulases and xylanases that allows an efficient saccharification of cellulose in lignocellulosic residues (Zimbardi et al., 2013). Several fungi are able to produce this enzyme cocktail; *Trichoderma reesei* and *Aspergillus niger* are the main industrial sources of cellulases and xylanases due to their ability to secrete high quantities of hydrolytic enzymes. Recently, the filamentous fungi *Aspergillus uvarum* and *Aspergillus ibericus* have been identified as producers of cellulases and xylanases (Salgado et al., 2014b).

Considering that the use of olive pomace as alone solid substrate in SSF is difficult and the growing interest in enzyme cocktails containing cellulase and xylanase, the aim of this paper was to examine the effect of US on the production of enzymatic cocktails containing these enzymes. In addition, a screening of filamentous fungi was performed to select the most suitable fungi to produce this enzymatic cocktail, and the composition of COP and EOP were compared.

2. Materials and methods

2.1. Raw material

The crude olive pomace (COP) and exhausted olive pomace (EOP) were collected from olive oil industry of northern Portugal in season 2013/2014. COP is a semi-solid waste of the two-phase system that was recovered after olive oil extraction. EOP is obtained after recovery of residual olive oil from COP and dried to use in combustion processes. The COP and EOP were stored at −20 °C and at room temperature and dry conditions, respectively.

2.2. Characterization of olive pomace

Both olive pomaces were analysed for the physical–chemical characterization. It was determined the moisture, total solids and ashes. Total nitrogen and organic carbon were determined by a Thermo Finningan Flash Element Analyzer 1112 series, San Jose, CA (USA) and metals, such as Ca, K, Mg, Na, Zn, Cu, Fe, Mn, Cr, Ni and Pb were analysed in ashes using Flame Atomic Absorption and Atomic Emission Spectrometry (FFAS/FAES) FAAS/FAES. The organic constituents of olive pomace such as cellulose, hemicellulose and Klason lignin were characterized by quantitative acid hydrolysis (QAH) in a two-stage acid treatment. The first stage with 72% wt H_2SO_4 at 30 °C for 1 h and the second stage after dilution to 4% wt H_2SO_4 at 121 °C for 1 h. The solution was filtered through a Gooch crucible to retain lignin and these were introduced in the hot air oven at 105 °C. The filtrate was analysed by High Performance Liquid Chromatography system using a Jasco830-IR intelligent refractive-index detector and a Varian MetaCarb 87H column. The column was eluted with 0.005 M H_2SO_4 and the flux was 0.7 mL/min at 60 °C. The total fat content was extracted with diethyl ether, in a Soxtec System HT2 1045 Extraction Unit. In order to analyse the free reducing sugar, proteins and total phenols in olive pomaces, an extraction with water (S:L ratio, 1:5 w/v) was performed. Free reducing sugars, total phenols and free protein were measured according to Salgado et al. (2014b). These determinations were performed in triplicate.

Table 1
Screening of filamentous fungi to verify ability to produce extracellular cellulases and xylanases.

Fungi	Code	Cellulases E:I	SD	Xylanases E:I	SD
Aspergillus niger	01UAs181	1.120^{cd}	0.000	1.064^{abc}	0.030
Aspergillus niger	01UAs183	1.064^{b}	0.008	1.034^{ab}	0.001
Aspergillus niger	CECT 2088	1.058^{b}	0.001	1.054^{abc}	0.000
Aspergillus niger	CECT 2700	1.067^{bc}	0.001	1.087^{bc}	0.028
Aspergillus niger	CECT 2915	1.140^{d}	0.000	1.258^{d}	0.029
Aspergillus ibericus	MUM 03.49	1.123^{d}	0.003	1.245^{d}	0.032
Aspergillus ibericus	MUM 2004	1.111^{bcd}	0.000	1.061^{abc}	0.000
Aspergillus ibericus	03UAs268	1.086^{bcd}	0.022	1.033^{ab}	0.077
Aspergillus uvarum	MUM 08.01	1.123^{d}	0.071	1.200^{d}	0.032
Aspergillus carbonarius	01UAs130	1.123^{d}	0.030	1.112^{c}	0.047
Trametes versicolor	MUM 04.100	0.985^{a}	0.003	1.000^{a}	0.020

Means were evaluated by least significant difference method of Fisher (LSD, $p = 0.05$). Values with the same letter within a column are not significantly different (PN0.05).