Evaluation of olive tree pruning biomass as feedstock to produce hydrolytic enzymes and antioxidant compounds through solid-state fermentation

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Introduction

Olive oil production is constantly increasing due to the good qualities associated with its consumption (Dahdouh et al., 2023). However, this industry produces also high number of different by-products or residues such as olive tree pruning (OTP), olive stones and pomace (Romero-García et al., 2014). From among these wastes, OTP biomass was selected as the raw material for this research work.

The pruning of the olive tree is performed every two years after the fruit harvesting with the aim of increasing the olive production and facilitating fruit collection (Romero-García et al., 2021). According to the study performed with data between 2004 and 2016, the average annual production of OTP biomass in Spain was 3.9 million (Marquina et al., 2021). The residue generated in this procedure is composed by approximately 25 % leaves, 50 % thin branches and 25 % thick branches (Romero-García et al., 2014). This biomass is usually burned, which implies the loss of biomass that can be reused to obtain bioproducts since its composition is mainly cellulose, hemicellulose and lignin.

The fungal solid-state fermentation (SSF) is the most used technic to produce hydrolytic enzymes such as cellulases and hemicellulases. These enzymes are essential to produce fermentable sugars from lignocellulosic biomass. However, some authors have observed the production of antioxidant compounds during fermentation in the solid state (Filipe et al., 2020). Thus, the purpose of this work is to evaluate the production of enzymes and antioxidant compounds through solid-state fermentation of OTP with *Aspergillus niger* and *Trichoderma reesei*. Moreover, the fermentation conditions will be optimized to produce the maximum enzyme activity based on statistical experimental design considering the moisture content, the inoculum concentration and the fermentation time as the main variable to optimize.

Material and methods

Olive tree pruning biomass (OTPB) was collected and crushed in a local olive grove in Jaen, Spain, after fruit harvesting. The OTPB was dried at room temperature in the laboratory and milled using a blade mill Retsch SM 100 (Haan, Germany), obtaining a particle size of 1 mm. Before the SSF, the solid was autoclaved at 121ºC and 20 min.

The SSF was performed with the two fungi: *Aspergillus niger* and *Trichoderma reesei*. Each fungus was grown in a petri dish with potato dextrose agar and incubated at 28ºC for 5 days. After that, spores were collected with NaCl 0.1 M, and the concentration of spores were counted in a Neubauer chamber. The fermentation was carried out in a petri dish with 8 grams of solid. A nutrient solution or distilled water was used to adjust the moisture content of the fermentation. Enzyme production was optimized by studying the moisture content between 60 - 80 % and the fermentation time between 0 - 76 h.

After the fermentation, the medium was extracted with NaCl 0.1 M by adding 10 mL per gram of dry solid. The mixture was mixed at 150 rpm for 30 min at room temperature. After that, the extract was centrifuged at 5500 rpm for 15 min, and filtered through 45 µm. The enzyme extract was frozen at -25 ºC until the analysis of enzyme and antioxidant activity were performed.

Results and discussion

Preliminary results obtained from the solid-state fermentation with *Aspergillus niger* showed higher growth when the fermentation was performed with the addition of a nutrient solution. Also, the cellulase activity produced after 5 days of fermentation, without the optimization of the process, was higher in that case when it was compared with the fermentation performed with water.

After optimizing the moisture content, the fermentation time and the inoculum concentration, it is expected to obtain a crude enzyme cocktail with a high content of cellulases and hemicellulases which can be used to hydrolyse OTPB. In this way, it can be obtained a broth rich in sugars that could be fermented to different by-products such as ethanol or lactic acid.

Additionally, it is expected to increase the production of antioxidant compounds after the solid-state fermentation of OTPB.

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