SIMULTANEOUS PRODUCTION OF B-GLUCIDASE AND EXTRACTION OF ANTIOXIDANT COMPOUNDS BY SOLID-STATE FERMENTATION OF WINERY AND OLIVE MILL WASTES

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Abstract

Wineries and olive mills wastes have a high content in phenolic compounds. This work aims to evaluate the production of β-glucosidase by solid-state fermentation (SSF) of these wastes to increase the extraction of phenolic antioxidant compounds. Filamentous fungi were growth on solid wastes under SSF. The β-glucosidase activity and antioxidant activity was measured in the extract obtained after 7 days of growth at 25 ºC.

The results showed that the use of exhausted olive pomace as substrate allowed to achieve the maximum production of β-glucosidase. Fungi that achieved the highest production of enzyme were *A. niger* CECT 2088 and *A. niger* MUM 03.49 with 28.31 ± 0.03 and 22.2 ± 0.2 U/g dry substrate. In addition, all studied fungi increased the antioxidant capacity of aqueous extract obtained after SSF of all wastes respect to unfermented wastes. The SSF showed to be a potential clean technology to extract antioxidant compounds.

INTRODUCTION

Global Food production must increase by 60% by 2050 due to the growth of world population (FAO). This increment will cause a higher production of agro-food wastes. On the other hand, the European commission has an ambitious strategy of circular economy. In this strategy, turning agro-food wastes into a resource is a priority for closing the loop, avoiding that the agro-food wastes to be burned or landfilled.

Olive oil and winery wastes are an excellent source of natural antioxidants as phenolic compounds. More than 30 biophenols and related compounds have been identified in olive mill wastes (Obied et al., 2005). Hydroxytyrosol is one of the major phenolic compounds in olive fruit which has pharmacological and antioxidant activity (Bendini et al., 2007). Grape pomace also has several phenolic compounds (phenolic acids, flavonoids, monomers and superior phenolic) with antioxidant activity, anti-inflammatory properties, anti-proliferation and cancer therapy (Tournour et al., 2015).

These compounds can be extracted by conventional solvent extraction (Rodríguez-Solana et al., 2014). However, the total recovery of them can be difficult because those compounds are present as insoluble bound form conjugates with sugars, fatty acids or amino acids (Dey et al., 2014). In addition, the public awareness of health, environment and safety hazards associated with the use of organic solvents in food processing and the possible solvent contamination of the final products together with the high cost of organic solvents, led to the development of a new and clean technology (Lafka et al., 2011). Extraction of natural phenolics by enzymes produced by solid-state fermentation (SSF) is a useful technique. Different carbohydrolases produced by the microorganisms can release the bound phenolics into soluble form (Bhanja et al., 2009). β-glucosidase are carbohydrate-hydrolysing enzymes that have important influence on the mobilization of soluble phenolics (Bei et al., 2018).

This study evaluated the production of β-glucosidase by solid-state fermentation (SSF) of olive mill and winery wastes and the extraction of phenolic antioxidant compounds after SSF.

METHODOLOGY

Raw material

Crude olive pomace (COP) and exhausted olive pomace (EOP) were collected from olive oil industry, exhausted grape marc (EGM) and vineshoots trimming (VTS) from the winery industry during the season 2016/2017. These residues were dried at 65 ˚C during 24 hours and stored at room temperature.

Microorganisms

Five fungi with potential for β-glucosidase production were selected in a previously stage. *Aspergillus ibericus* MUM 03.49, *Aspergillus ibericus* MUM 04.86 and *Aspergillus ibericus* 03UAs268 were obtained from MUM (Micoteca of University of Minho, Braga, Portugal) and *Aspergillus niger* CECT 2915 and *Aspergillus niger* CECT 2088 from CECT (Valencia, Spain) culture collection. They were revived on malt extract agar (MEA) plates. To obtain inoculum for SSF, the selected fungi were subcultured on MEA slants, and incubated at 25 ˚C for 6 days.

Solid-state fermentation

SSF process was carried out in glass petri dishes with 2 g of dry substrate sterilized at 121 ˚C for 15 minutes. The inoculation was performed following the method described by Salgado et al. (2014). SSF were incubated at 25 ˚C for 7 days. The extraction of enzymes was carried out by method described by Salgado et al. (2014). All SSF were performed in duplicate.

Analysis of antioxidant capacity and β-glucosidase

The antioxidant activity by DPPH scavenging method described by Oliveira et al. (2018) using trolox equivalent antioxidant capacity. The β-glucosidase was measured using the method described by Leite et al. (2016).

RESULTS

Olive mill and winery wastes were fermented by five *Aspergillus* strains. Figure 1 shows the production of β-glucosidase by SSF of these wastes using different fungi. In the case of VTS, all fungi growth and produced β-glucosidase*. A. niger* CECT 2088 achieved the maximum production using this substrate. In the same way, this fungus obtained the highest enzyme activity by fermentation of EOP. On the other hand, the use of EGM or COP as substrate led to lower β-glucosidase activity. For all fungi except for *A. niger* CECT 2915 and *A. ibericus* MUM 04.86, fermentation of EOP allowed to achieve the maximum β-glucosidase. Thus, this agro-food waste obtained after extraction residual olive oil, it has the potential to be used as substrate in SSF for enzymes production. Nowadays, the EOP is used as heating biofuel but its combustion generates important quantities of CO, CO_2 and polycyclic aromatic hydrocarbons (Colom-Diaz et al., 2017). In addition, the environmental objective of European Union gives preference to a recycle and reuse of agro-food wastes over their combustion. For these reasons, alternative solutions should be added for EOP.

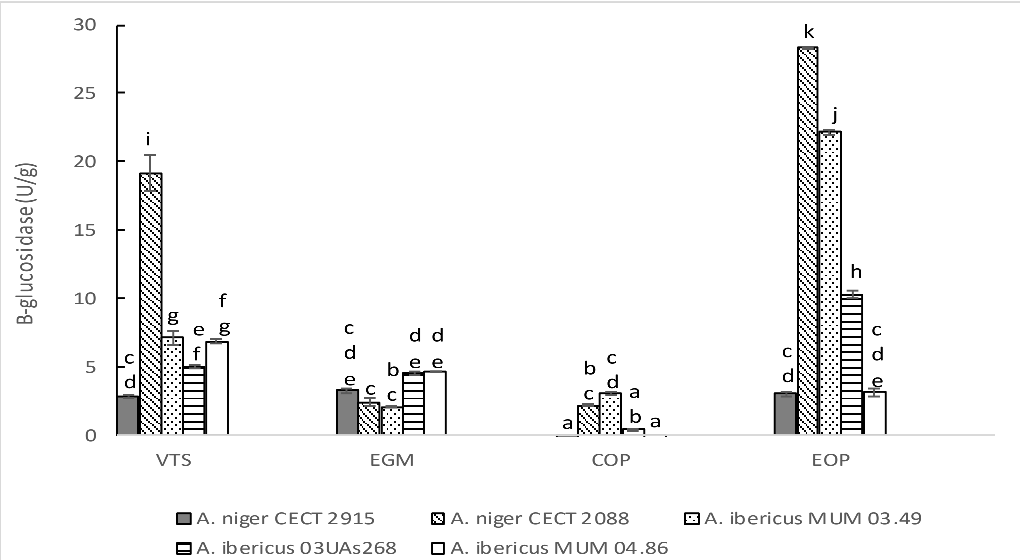

Figure 1. β-glucosidase activities obtained by SSF of olive mill and winery wastes

The extraction of antioxidant compounds by SSF of different olive mill and winery wastes was evaluated. Figure 2 displays the antioxidant activity of aqueous extract after SSF of the four wastes studied and the antioxidant activity of control of each unfermented waste. As can be observed, the extract with higher antioxidant activity was obtained from COP.

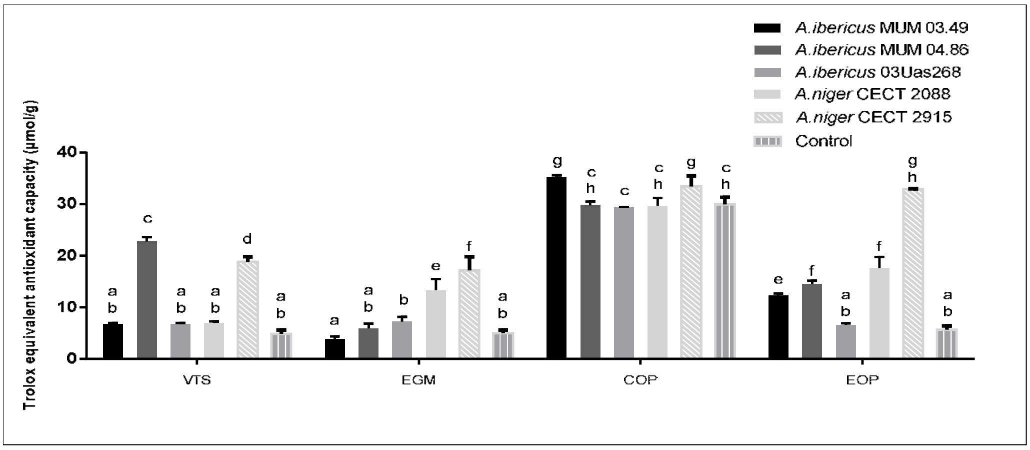

Figure 2. Antioxidant activity of aqueous extracts from fermented and unfermented wastes

However, the increase of extraction after SSF in comparison with unfermented COP was lower, only *A. ibericus* MUM 03.49 and *A. niger* CECT 2915 showed an increase significant. On the other hand, a higher increase of antioxidant activity was observed by SSF of EOP, VTS and EGM with *A. niger* CECT 2915 over unfermented wastes.

CONCLUSIONS

SSF showed to be a clean technology to improve the extraction of antioxidant compounds from olive mill and winery wastes and to produce β-glucosidase, which have many applications in several industrial sectors. The use of EOP as substrate allowed to achieve the maximum enzyme production and extraction of antioxidant compounds.

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