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Production of bioactive compounds by solid-state fermentation of oilseed cakes

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ABSTRACT: Vegetable oils are an important part of human diet. By-products from vegetable oil's industry are a reliable source of protein and fat, with variable amounts of fibre, and a source of phenolic compounds. However, their digestibility by some animals is difficult due to polysaccharides and lignin content. This work aims to assess the use of sunflower cake (SFC), rapeseed cake (RSC) and soybean cake (SBC) as substrate in solid-state fermentation (SSF) with filamentous fungi *Rhyzopus oryzae* and *Aspergillus ibericus*, for the production of animal feed additives and bioactive substances such as lignocellulolytic enzymes and antioxidant phenolic compounds. Results showed the highest cellulase and xylanase activities were achieved with *A. ibericus* using SFC and SBC as substrates. Highest β-glucosidase activity was observed in SSF with *R. oryzae* using RSC as substrate. *R. oryzae* release the maximum amount of total phenols and SSF improved antioxidant capacity of RSC and SBC extracts.

1 INTRODUCTION

Global population is increasing and it is expected to reach more than 9 billion people by 2050 (Food and Agriculture Organization, 2009). Food demand will increase over the next decades due to population growth and rising incomes by the families.

Agro-food wastes are a consequence of food industry production, which can be used in biotechnology industry. The use of agro-food wastes as raw materials for the production of added-value compounds using biotechnological process has economic and environmental impact (Lio and Wang, 2012; Horita *et al.*, 2015). Over the last decade, initiatives such as ZeroWaste Europe have been established to promote alternatives for a positive valorization of wastes instead of their incineration or landfill disposal. Biotechnological eco-friendly approaches for valorization of wastes, besides their environmental and economic benefits, also creates new business opportunities and workplaces (Zero Waste Europe, 2011). Production of value-added compounds from wastes is a key point to circular economy.

Nowadays, despite their industrial applications, vegetable oils play an important role in human diet. Major sources of vegetable oils are soybean, rapeseed and sunflower seeds. Oilseed cakes are the main by-products obtained after the extraction of these vegetable oils. These byproducts are mainly composed by proteins, fibers, carbohydrates, minerals and vitamins (Sunil *et al.*, 2016). These by-products are mainly used as animal feed in ruminant diets or to complement monogastric animal rations and also as fuel in thermal power stations (Matthäus, 2002; Lomascolo *et al.*, 2012;

Sunil *et al.*, 2016). These by-products, however, can contain antinutritional factors that can be harmful to animals and do not match their nutritional needs (Ajila *et al.*, 2012).

Soybean, sunflower and rapeseed are natural sources of antioxidants (Pandey *et al.*, 2000). During extraction of vegetable oils natural antioxidants present in the oilseeds are separated into liposoluble and hydrophilic fractions (Schmidt and Pokorný, 2005). Despite this fact, oilseed cakes still have large amounts of antioxidants that have not been damaged or removed during oil extraction process. Antioxidant activity of these meals is mainly due to the presence of phenolic compounds such as phenolic acids, flavonoids and lignans (Schmidt and Pokorný, 2005).

Oilseed cakes are lignocellulosic materials, suitable to be used as substrate in biotechnological process like solid-state fermentation (SSF). SSF is defined as a fermentation process that occurs in the absence or near-absence of free water to which is applied a natural or inert substrate used as solid support. The solid support must contain enough moisture to support the growth and metabolism of microorganisms. This fermentation reproduces the conditions close to the natural habitat of microorganisms; and filamentous fungi are the ones that better adapt to SSF (Pandey, 1992; Pandey *et al.*, 2000).

This study assessed the production of animal feed additives such as lignocellulolytic enzymes (cellulases, xylanases and β-glucosidase) and bioactive compounds such as antioxidant extracts, using sunflower, rapeseed and soybean cakes as substrate in SSF.

2 METHODOLOGY

2.1 *Raw material*

Sunflower cake (SFC), rapeseed cake (RSC) and soybean cake (SBC) were collected from companies related with the vegetable oils industry from Portugal. Residues were dried at 65°C for 24 hours and stored at room temperature.

2.2 *Microorganisms*

Rhyzopus oryzae 10.260 and *Aspergillus ibericus* 03.113 were obtained from Micoteca of University of Minho, Braga, Portugal (MUM). Fungi were cultivated in potato dextrose agar (PDA). In order to obtain inoculum for SSF, the selected fungi were subcultured in PDA slants and incubated at 25°C for 7 days.

2.3 *Solid-State Fermentation*

SFC, RSC and SBC were used as substrate in SSF assays to evaluate the two fungi. SSF process was carried out in 500 mL Erlenmeyer flasks with 10 g of dried residue with moisture level adjusted to 75% (w/w) in wet basis. Erlenmeyer flasks with the solid substrate were sterilized at 121°C for 15 minutes. Inoculation process was performed following the method described by Sousa et al. (2018). The extraction of enzymes was performed at the end of 7 days of fermentative process, with water, at 4°C in a solid/liquid ratio of 1:5, and 1 h of stirring, at 150 r.p.m. Following, extracts were filtered through a net. The liquid fraction was centrifuged (4 000 r.p.m.). All SSF were performed in duplicate.

2.4 *Enzymes activity, antioxidant capacity and total phenols of fermented extracts*

Cellulases and xylanases activity was quantified using 2% carboxymethylcellulose (CMC) and 1% xylan as substrate in 0.05 M citrate buffer (pH 4.8), respectively. The enzymatic reaction was carried at 50°C for 30 and 15 min respectively. The reducing sugars released during the enzymatic reaction were quantified by 3,5-dinitrosalicylic (DNS) method and measured at 540 nm. β-glucosidase activity was quantified using the method described by Leite *et al.*, (2016). Antioxidant capacity

of fermented extracts was quantified using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. 200 μL of fermented extracts were placed in a 96 well microplate and 100 μL of DPPH (0.3 mM, in methanol) was added. The mixture reacted in the dark for 30 min. Then, the variation of absorbance was measured at 520 nm. All samples were performed in duplicate. Methanol was used as blank solution and DPPH methanolic solution was used as control. Known amounts of Trolox were used to build a standard curve. Scavenging activity of fermented extracts was expressed as micromole of Trolox equivalents per g of dry solid substrate ($\mu mol \cdot g^{-1}$).

Total phenols were determined by the Folin – Ciocalteu method (Commission Regulation (EEC) No. 2676/90).

3 RESULTS

3.1 *Enzymes activity*

SFC, RSC and SBC were fermented with *R. oryzae* and *A. ibericus*. Both fungi were able to grow in the different residues. It is possible to observe the production of cellulase, particularly by *A. ibericus* (Figure 1). For this species, higher production of cellulase was found with SFC and SBC than with RSC. Contrary, it was for RSC that more cellulase was produced by *R. oryzae*, however at lower values than 20 U/g.

Figure 2 shows xylanase activity obtained after analysis of crude extracts. It is possible to observe that residues fermented with *A. ibericus* achieved higher values of xylanase activity when compared to residues fermented with *R.* oryzae.

In the case of SSF with *A. ibericus* there are no significant differences among obtained values of xylanase activity. Regarding the fermentation performed with *R. oryzae*, it was observed a maximum of xylanase activity using RSC as substrate, but not statistically different from the values obtained with SFC.

β-glucosidase activity was also detected in fermented (Figure 3) where the highest β-glucosidase activity was achieved in the fermentation with *R. oryzae*, using RSC as substrate. No statistically significant differences were found between β-glucosidase activity in fermentation with both fungi, using SFC and SBC as substrate.

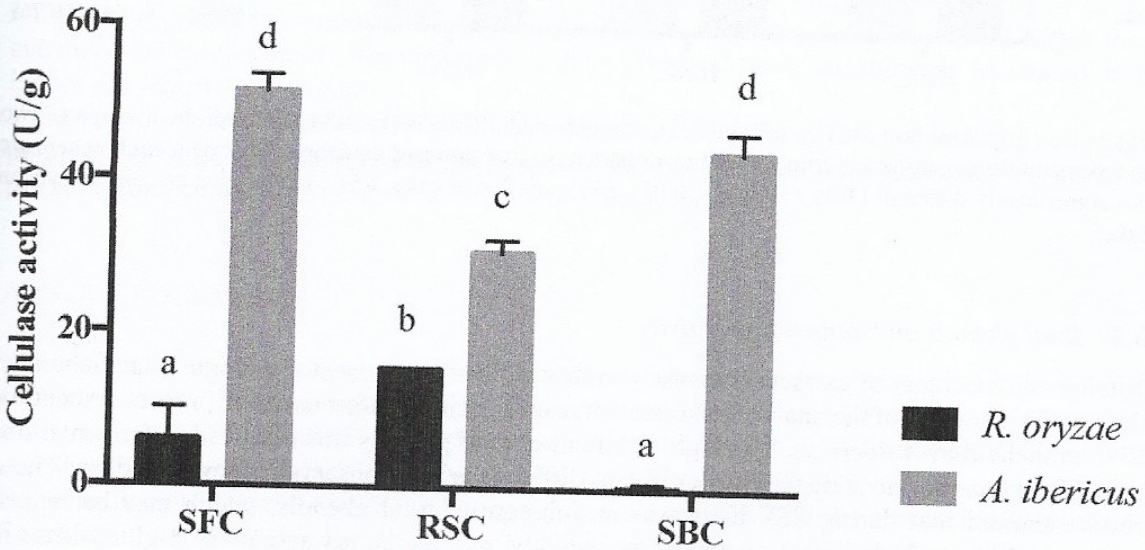

Figure 1. Cellulase activity of crude extracts obtained after SSF. Results represent the average of two independent fermentation experiments and error bars represent standard deviation. Bars with equal letters are not significantly different (Tukey test; $P < 0.05$). SFC, sunflower cake; RSC, rapeseed cake; SBC, soybean cake.

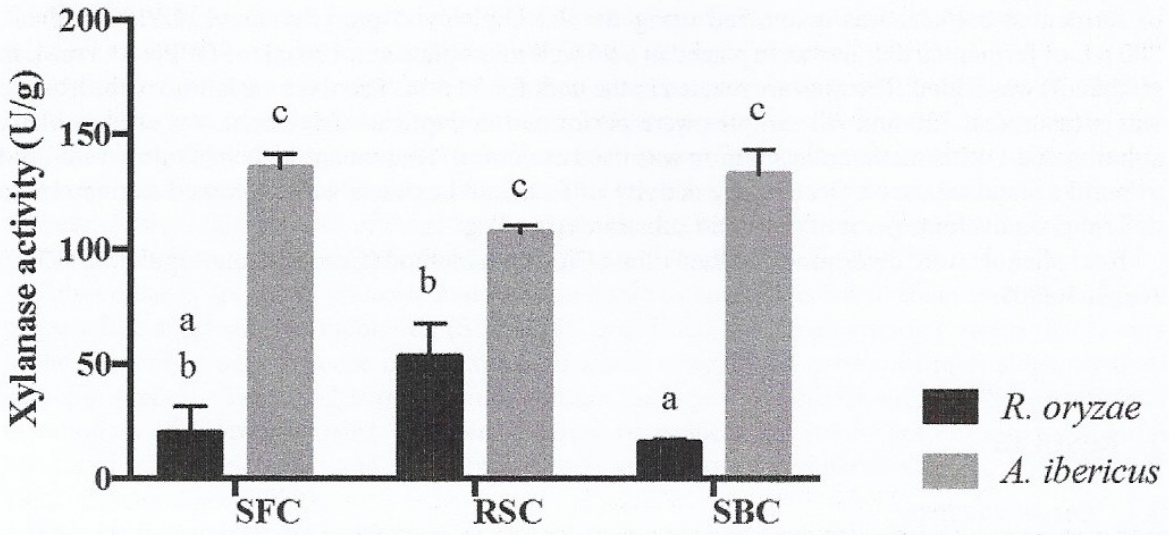

Figure 2. Xylanase activity of crude extracts obtained after SSF. Results represent the average of two independent fermentation experiments and error bars represent standard deviation. Bars with equal letters are not significantly different (Tukey test; $P < 0.05$). SFC, sunflower cake; RSC, rapeseed cake; SBC, soybean cake.

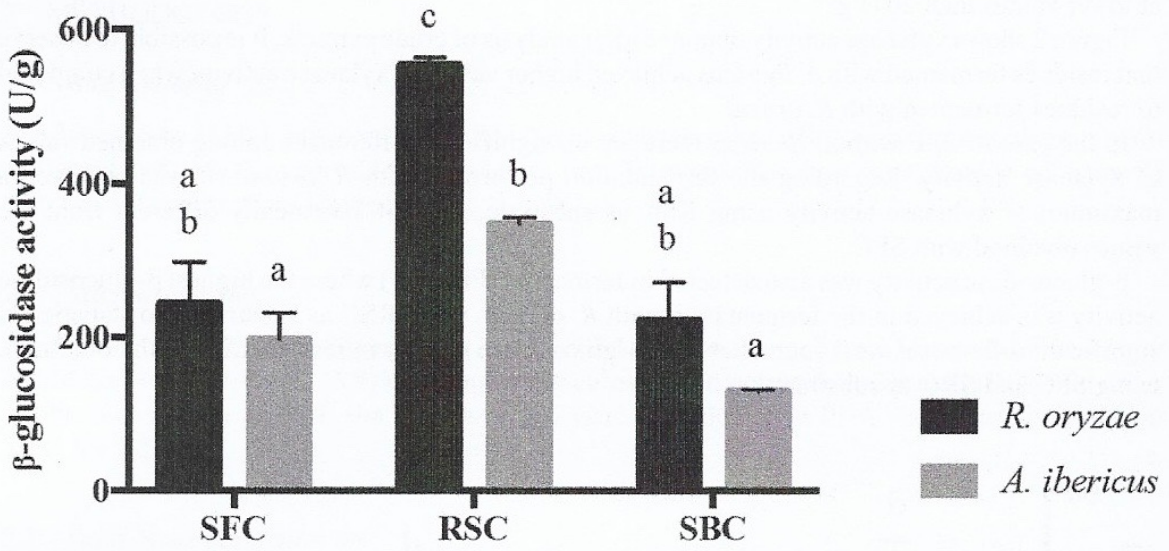

Figure 3. β-glucosidase activity of crude extracts obtained after SSF. Results represent the average of two independent fermentation experiments and error bars represent standard deviation. Bars with equal letters are not significantly different (Tukey test; $P < 0.05$). SFC, sunflower cake; RSC, rapeseed cake; SBC, soybean cake.

3.2 *Total phenols and antioxidant activity*

Total phenols content of extracts from the residues studied are presented in Figure 4a. Generally, the phenolic content of the analyzed extracts increased during the fermentative process, except for SFC fermented by *A. ibericus*. The higher content of total phenols was achieved in fermentations using *R. oryzae* but no statistically significant differences were observed among residues. These results showed that during SSF there was an increase of total phenols, which may be related with carbohydrate-hydrolyzing enzymes. As stated before, the higher activity of β-glucosidase in fermented extracts was achieved with *R. oryzae*, using RSC as substrate. These enzymes have been reported to be involved in mobilization of phenolic compounds during SSF (Vattem and Shetty, 2002; Bhanja *et al.*, 2009).

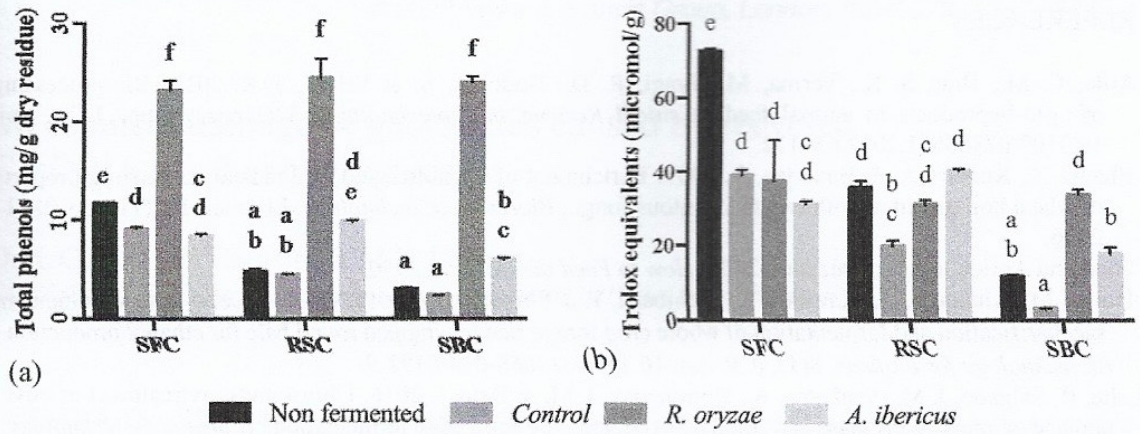

Figure 4. a) Total phenols content of initial substrate (non-fermented), control (solid after sterilization) and residues after SSF. b) Antioxidant capacity of initial substrate (non-fermented), control (solid after sterilization) and residues after SSF. Bars with equal letters are not significantly different (Tukey test; $P < 0.05$). SFC, sunflower cake; RSC, rapeseed cake; SBC, soybean cake.

Antioxidant capacity of non-fermented and fermented extracts is depicted in Figure 4b. The higher antioxidant capacity was obtained in non-fermented extract of SFC. In the case of RSC and SBC it was possible to observe an increase of antioxidant capacity after SSF when compared to control.

Extracts from *A. ibericus* contain smaller amounts of phenolic compounds rather than extracts from *R. oryzae*. Despite this fact, it was possible to observe that regarding to SFC and RSC, extracts from SSF by different fungi do not show statistically significant differences. In the case of SBC there was a clear difference between antioxidant capacity of fermented extracts.

4 CONCLUSIONS

SFC, RSC and SBC proved to be suitable substrates for SSF. During SSF lignocellulolytic enzymes were produced that can be used as feed additives. *A. ibericus* induced a higher activity of cellulases using SFC and SBC as substrate, and also the higher activity of xylanases was achieved with *A. ibericus*. β-glucosidase activity was highest when RSC was fermented with *R. oryzae*. These fungi enhanced the extraction of phenolic compounds in every substrate and this may be related to the higher activity of β-glucosidase.

SSF by *A. ibericus* improved the antioxidant capacity of RSC and SBC extracts. Phenolic compounds present in extracts from SSF by *R. oryzae* did not show the same antioxidant capacity as phenolic compounds present in extracts from SSF by *A. ibericus*.

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