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Improved lignocellulolytic enzyme production and antioxidant extraction using solid-state fermentation of olive pomace mixed with winery waste

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Abstract: Olive pomace is characterized by its low nutritional value and high phenolic content, which hinders its direct use as animal feed, fertilizer, or as a substrate in bioprocesses such as solid-state fermentation (SSF). A possible strategy for bioprocessing olive pomace by SSF is the mixture of olive mill wastes with other wastes produced in the same region, such as winery wastes. This may improve the production of bioactive compounds like enzymes and antioxidant phenolics. A simplex-centroid design was used to evaluate the use of olive mill and winery wastes alone or in combination as a substrate for SSF with *Aspergillus niger* and *Aspergillus ibericus*. Synergistic effects of combinations of crude olive pomace (COP), exhausted olive pomace (EOP), vine trimming shoots (VTS), and exhausted grape marc (EGM) were observed in the production of xylanases, cellulases, β-glucosidases, and in the variation in total phenolics and antioxidant activity of SFF extracts. A multiple response optimization was carried out, leading to the following optimal mixture of substrates: for *A. niger*, 23% (w/w) COP, 30% EGM, 33% VTS, 14% EOP; for *A. ibericus*, 30% EGM, 36% VTS, 34% EOP. The scale-up to tray bioreactor with optimal substrate made it possible to achieve the maximum xylanase, cellulase, and β-glucosidase production of 189.1 ± 26.7, 56.3 ± 2.1 and 10.9 ± 0.8 U/g, respectively. The antioxidant activity of fermented wastes was also improved 2.2-fold as compared with unfermented wastes. Thus, a combination of olive mill and winery wastes in SSF is a potential strategy to increase their value and to develop a circular strategy in these industries.

Key words: biorefinery; olive mill wastes; solid-state fermentation; lignocellulolytic enzymes

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Introduction

Olive oil is an important product in the Mediterranean agro-food sector. Mediterranean countries produced 94.5% of total world olive oil in season 2016/17 and Spain was the highest world producer with 1.3 million tons (International Olive Oil Council, 2018). It has been predicted that world olive oil production will increase by 22% in 2018/19 relative to 2016/17, achieving 3.13 million tons (International Olive Oil Council, 2018). As a result, there will be an increase in waste after olive oil extraction. The olive oil yield from processed olives is about 20%;[1] thus the world production of olive mill wastes (OMW) is estimated at 12.5 million tons in 2019. It was estimated by an international wine organization that, in 2018, 282 million of hectoliters of wine were produced (International Organization of Wine, 2018). The wine yield from grapes is about 70%, the remaining 30% is the grape marc. It can thus be estimated that 12.1 million of tones of grape marc were produced in 2018. The vine trimming shoots are normally burned in a field, causing greenhouse gases. The costs of treatment of this large quantity of agro-industrial waste affects the economic benefits of this industry. There is thus an urgent need to transform them into resources that can be used as feedstock in biorefineries. Agro-industrial waste constitutes a low-cost resource for large-scale commercial biorefineries.[2]

Recently, olive-oil mills changed the olive-oil extraction system to reduce water use, producing a wet solid waste called two-phase olive mill waste (TPOMW).[3] This change in the extraction process reduced water consumption and wastewater generation, but TPOMW continues to be a waste that is harmful to the environment because it has a negative effect on soil, aquifers, and air quality.[4] Two-phase olive mill waste is a mixture of olive pulp, skin, stone, and water and can be classified as crude olive pomace (COP) and exhausted olive pomace (EOP), depending on the percentage of residual oil in the final product.[5] Crude olive pomace still contains around 3.5% residual oil and EOP is obtained after the extraction of the residual oil from COP through centrifugation and drying processes.[6] It has a high moisture content (about 70%), low porosity, and a doughy texture that can reduce aeration.[3] It also contains toxic compounds such as polyphenols, poly-alcohols and volatile fatty acids, which have phytotoxic effects and may inhibit the growth of microorganisms.[7] On the other hand, TPOMW is a lignocellulosic material[6] with antioxidant compounds[8] that has high potential for use as biomass in the biorefinery industry.

Solid-state fermentation (SSF) is a technology for the production of microbial products that have the potential to be used in the feed, fuel, food, chemical, and pharmaceutical industries.[9] Solid-state fermentation is a fermentation process that occurs on solid substrates without free water.[10] The use of SSF in biorefinery processes is an alternative strategy to conventional physical and chemical treatments to produce value-added products from agro-industrial wastes.[11] Filamentous fungi are good degraders of lignocellulosic materials because they release lignocellulolytic enzymes and exhibit hyphal penetration into the substrate were they grow.[12] Fungal enzymes release phenolic antioxidant compounds[13] and break down the hemicellulose-cellulose matrix, thus releasing fermentable sugars[14,15] which favor fungal growth.[16] *Aspergillus ibericus* has demonstrated its capacity to produce these enzymes in previous work.[14] Solid-state fermentation is therefore a technology that allows the exploitation of all fractions obtained by fermentation, following the concept of a circular economy.

Under SSF, fungi have proven that they are able to produce a myriad of enzymes with different applications, including detergent manufacturing, food processing, textile and pharmaceutical industries, medical therapy, molecular biology, bioremediation, and biological control.[17] The enzymatic complexes known as cellulases are capable of degrading lignocellulosic residues, this ability making them highly desirable for industry use worldwide, in ethanol production, the treatment of waste papers, fruit-juice extraction, single-cell protein, cotton processing, pulp bleaching, and animal feed additives.[18] Using lignocellulosic wastes in SSF induces the production of fungal cellulases.[19] Endo-glucanases, cellobiohydrolases, and β-glucosidases enzymes all act upon different components of cellulose, hydrolyzing it.[18] Xylanases (endo-1,4-β-D-xylanases) hydrolyze hemicellulose and the internal bonds of xylan chains present in lignocellulosic materials, thus acting upon the cellulose and lignin present in plant cell walls.[20]

Antioxidants are important compounds, helping organisms to prevent oxidative stress caused by reactive oxygen species (ROS), which are harmful molecules, damaging and altering the carbohydrates, nucleic acids, lipids, DNA, and proteins. Oxidative stress can cause immunosuppression and slow growth.[21,22] The use of synthetic antioxidants has been raising concern due to possible human health hazards. Natural and cheap antioxidant compounds have been pointed out as a natural and cheap alternative that is more adequate for the feed industry.[21] Natural antioxidants like phenolic compounds are present within plant cell walls, although they are not very efficient when bound to the cellulose matrices of the walls.[23] Fungi are the most promising group of microorganisms that produce enzymes capable of degrading plant cell walls.[24] By employing these microorganisms, SSF offers a way to increase the phenolic compound content using agro-industrial by-products.[25]

The use of OMW as a single substrate can inhibit fungus growth and enzyme production due to the low porosity of substrate and high content of phenolic compounds.[26] Thus, a mixture of OMW with other agro-industrial wastes can promote fungal growth and improve enzyme production.[27] Statistical mixture designs are a suitable tool for studying different mixture compositions and for evaluating how they affect a specific response.[27]

The aim of this work was to study the use of SSF as a technology for obtaining value-added products from olive pomaces, following the concepts of biorefinery and the circular economy. In this study, mixtures of olive pomace with winery wastes were evaluated as substrates for improving the production of cellulases and xylanases, and the extraction of phenolic antioxidant compounds from olive pomace. To achieve the optimum mixture of these solid wastes, a simplex centroid mixture design was used. A kinetic study and scale-up in a tray bioreactor was then performed with the optimum mixture of wastes.

Materials and methods

Raw material and reagents

Four wastes were used in this work: COP, a semi-solid residue obtained after olive oil extraction; exhausted olive pomace (EOP), which results from the extraction of residual olive oil and drying of crude olive pomace; vine trimming shoots (VTS), produced with vine pruning; and exhausted grape marc (EGM), which originates from the winemaking and distillation processes. All wastes were obtained from industries in the north of Portugal in the 2017/18 season. These wastes originated from typical varieties of grapes and olives from this region. Vine trimming shoots and EGM were dried and milled (particle size < 1 mm). Wastes were stored at room temperature, except for COP, which was stored at 4 °C.

The following reagents were used in the study. Urea was added for SSF (VWR Chemicals ProLab; Radnor, Pennsylvania, United States). Peptone was used for inoculation (Chem Lab NV; Zedelgem, Bélgica) together with Tween-80 (Fisher Scientific; Hampton, New Hampshire, United States). The reagents for antioxidant activity were 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich; Sant Louis, Missouri, United States), methanol (Fisher Scientific), and Trolox (Sigma-Aldrich). Nitrophenyl β-D-glucopyranoside (Sigma-Aldrich), *p*-nitrophenol (Sigma-Aldrich), sodium carbonate (Fluka, Fisher Scientific), xylan (Sigma-Aldrich), citric acid monohydrate (VWR Chemicals), sodium citrate dihydrate (Inlab; Maharashtra, India), sodium carbonate (Fluka, Fisher Scientific), 3,5-dinitrosalicylic acid (Acros Organics, Fisher Scientific), xylose (Sigma-Aldrich), carboxymethylcellulose (Sigma-Aldrich), and glucose (Fisher Scientific) were used for β-glucosidase, xylanase, and cellulase activity. Folin–Ciocalteau reagent (AppliChem Panreac ITW Companies; Chicago, United States), sodium carbonate (Fluka, Fisher Scientific), caffeic acid (Fluka, Fisher Scientific), and ethanol (Fisher Scientific) were used to determine total phenol content.

Fungi

Two species of *Aspergillus* section *Nigri* were used in this work: *Aspergillus ibericus* MUM 03.49 was obtained from Micoteca da Universidade do Minho (Braga, Portugal), and *Aspergillus niger* CECT 2915 was obtained from a Spanish type Culture Collection (Valencia, Spain). The fungi were preserved in glycerol at −80 °C and then revived on malt extract agar (MEA) plates (20 g L^{-1} malt extract, 1 g L peptone, 20 g L^{-1} glucose, and 20 g L^{-1} agar). Cultures were made and renewed every month on potato dextrose agar (PDA) plates (15 g L^{-1} agar, 20 g L^{-1} dextrose, 4 g L^{-1} potato extract), and subcultured in PDA slants, being incubated at 25 °C for 7 days. These cultures were stored at 4 °C and the slants were used as inoculums for SSF.

Solid-state fermentation

Several substrate mixtures were designed according to a simplex-centroid mixture design (Table 1). Solid-state fermentation of each mixture was carried out in 500 mL plugged Erlenmeyer flasks. Each flask contained 10 g of substrate with humidity adjusted at 75% (w/w wet basis) and C/N ratio normalized at 15 with urea to ensure the growth of fungi. The flasks were then sterilized at 121 °C for 15 minutes. All runs were carried out with *A. ibericus* and *A. niger*. For the inoculation of the fungi into the substrate mixture, an autoclaved (121 °C for 15 min) peptone solution composed of 0.1% peptone and 0.01% Tween-80 was added into the PDA slants. The fungal spore concentration was then adjusted to 10^6 spores mL^{-1} using a Neubauer counting chamber, and 2 mL of the spore solution was inoculated into each flask. All flasks were then maintained at 25 °C for 7 days for the fungi to grow.

Bioactive compounds extraction

Enzymes and antioxidant compounds were recovered using distilled water at a solid/liquid ratio of 1:5 (w/v) and stirring at 150 rpm for 30 min in an incubator at room temperature. All solid residue was then filtered through a fine-mesh net and centrifuged at 9.7 g for 10 minutes at 4 °C. The resulting supernatant was filtered by vacuum through filter paper (Whatman grade 1) and the extract was then stored at −20 °C until analysis.

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Table 1. Characterization of the solid substrate of each experiment of simplex centroid design.

Runs	Solid mixture				Final composition in dry solid						
	COP (g)	EGM (g)	VTS (g)	EOP (g)	Cell. (%)	Hemice (%)	Lignin (%)	Ashes (%)	CP (%)	TP (%)	RS (%)
1	10	0	0	0	12.5 ± 0,9	22.3 ± 0.8	43.2 ± 0.5	6.6 ± 0.5	3,75 ± 0,6	0.84 ± 0.03	9.6 ± 0.6
2	0	10	0	0	14.4 ± 0.2	10.2 ± 0.4	66.6 ± 0.5	9.1 ± 0.6	13.5 ± 0.4	0.17 ± 0.01	0.4 ± 0.01
3	0	0	10	0	42.4 ± 0.5	23.8 ± 0.4	34.1 ± 0.6	3.6 ± 0.4	3.75 ± 0.1	0.17 ± 0.01	0.45 ± 0.01
4	0	0	0	10	12.9 ± 0.2	28.9 ± 0.05	55 ± 2	3.4 ± 0.2	5.75 ± 0.4	0.71 ± 0.01	3.3 ± 0.2
5	5	5	0	0	13.5 ± 0.6	16.3 ± 0.6	54.9 ± 0.5	7.9 ± 0.6	8.7 ± 0.5	0.51 ± 0.02	5 ± 0.3
6	5	0	5	0	27.5 ± 0.7	23 ± 0.6	38.7 ± 0.6	5.1 ± 0.5	3.8 ± 0.4	0.51 ± 0.02	5 ± 0.3
7	5	0	0	5	12.7 ± 0.6	25.6 ± 0.4	49.1 ± 1.3	5 ± 0.4	4.8 ± 0.5	0.77 ± 0.02	6.5 ± 0.4
8	0	5	5	0	28.4 ± 0.35	17 ± 0.4	50.3 ± 0.6	6.4 ± 0.5	8.6 ± 0.3	0.17 ± 0.01	0.4 ± 0.01
9	0	5	0	5	13.7 ± 0.2	19.6 ± 0.2	60.8 ± 1.3	6.3 ± 0.4	9.6 ± 0.4	0.44 ± 0.01	1.9 ± 0.1
10	0	0	5	5	27.7 ± 0.4	26.4 ± 0.2	44.6 ± 1.3	3.5 ± 0.3	4.8 ± 0.3	0.44 ± 0.01	1.9 ± 0.1
11	3.33	3.33	3.33	0	22.9 ± 0.5	18.6 ± 0.5	47.5 ± 0.5	6.4 ± 0.5	6.9 ± 0.3	0.39 ± 0.02	3.5 ± 0.2
12	3.33	3.33	0	3.33	13.1 ± 0.4	20.3 ± 0.4	54.4 ± 1	6.3 ± 0.4	7.6 ± 0.5	0.57 ± 0.02	4.4 ± 0.3
13	3.33	0	3.33	3.33	22.3 ± 0.5	24.8 ± 0.4	43.7 ± 1	4.5 ± 0.4	4.4 ± 0.3	0.57 ± 0.02	4.4 ± 0.3
14	0	3.33	3.33	3.33	23 ± 0.3	20.8 ± 0.3	51.4 ± 1	5.3 ± 0.4	7.6 ± 0.3	0.34 ± 0.01	1.4 ± 0.1
15	2.50	2.50	2.50	2.50	20.5 ± 0.5	21.3 ± 0.4	49.7 ± 0.9	5.7 ± 0.4	6.7 ± 0.4	0.47 ± 0.02	3.4 ± 0.2

COP, crude olive pomace; EGM, exhausted grape marc; VTS, vine trimming shoots; EOP, exhausted olive pomace; Cell, cellulose; Hemicel, hemicellulose; CP, crude protein; TP, total phenols; RS, reducing sugars.

Enzyme activities

For xylanase determination, beechwood xylan (1% in citrate buffer 0.05 N at a pH of 4.8) was used as a substrate, and carboxymethylcellulose (CMC) (2% in citrate buffer 0.05 N at a pH of 4.8) was used as a substrate for cellulase. The release of reducing sugars after enzymatic hydrolysis was determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller *et al*., 1959): 250 µL of beechwood xylan solution and 250 µL of diluted sample with buffer were added to the test tubes (for the blank, 250 µL of citrate buffer 0.05 N at pH 4.8 was used instead of the diluted sample), which were then heated in a bath at 50 °C for 15 min. Thereafter, 500 µL of DNS was added to the mixture and the tubes were placed in a bath at 100 °C for 5 minutes; after cooling to room temperature, 5 ml of distilled water was added and the absorbance was read at 540 nm by an microplate reader (Bio-Tek; Winooski, Vermont, United States). For cellulase determination, the process was the same as that described above except for the substrate, which was CMC, and the incubation time, which was 30 min at 50 °C. The calibration curves were made with concentrations ranging from 0 to 2 gL^{-1} of glucose for cellulase determination and xylose for xylanase determination, both in citrate buffer. One unit of enzyme activity was defined as the amount of enzyme needed to release 1 µmol of glucose or xylose per minute under standard assay conditions for cellulase and xylanase, respectively. Results were expressed as units per gram ($U\,g^{-1}$) of dry substrate used.

β-Glucosidase was analyzed with *p*-nitrophenyl-*β*-D-glucopyranoside (PNG) as substrate. Then 100 µL of PNG and 100 µL of sample diluted in citrate buffer 0.05 N at pH of 4.8 were added to the test tubes. The tubes were then heated in a bath at 50 °C for 15 minutes. Then, 600 µL of Na_2CO_3 at 1 $mol\,L^{-1}$ and 1.7 mL of distilled water were added to the tubes and the absorbance was read at 400 nm with an ELISA reader. The calibration curve was made with *p*-nitrophenol with concentrations ranging from 0 to 100 $\mu g\,mL^{-1}$. One unit of enzyme activity was defined as the amount of enzyme needed to release 1 µmol of *p*-nitrophenol per minute under the assay conditions. Results were expressed in units per gram of dry substrate (U/g^{-1}).

Total phenols and antioxidant analysis

Total phenols (TP) were determined using the Folin–Ciocalteu method (Commission Regulation (EEC) No. 2676/90) with some modifications. The following were added to test tubes: 100 µL of a diluted sample (1:2) (or 100 µL of distilled water for the blank); 2 mL of Na_2CO_3 at 15%, 500 µL of Folin–Ciocalteu reagent, and 7.4 mL of distilled water. The tubes were then placed in a bath at 50 °C for 5 min, vortexed after cooling, and absorbance was read at 700 nm.

A calibration curve was performed with caffeic acid (CA) with concentrations ranging from 0 to 2 g L^{-1}. Thess total phenols were expressed as mg CA per g of dry solid. The total phenol variation (TPV) was calculated by subtracting the TP obtained in the control (without inoculation) from the TP of the fermented extract.

Antioxidant activity (AA) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. In a microplate, 200 µL of sample was added to each pit, with several dilutions in methanol, and a blank was made using only methanol and 100 µL of DPPH was added to these solutions. The microplate was placed in a dark space for 30 min and afterward, its absorbance was read at 517 nm. The calibration curve was prepared using the known quantities of 6-hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid (Trolox). Results were expressed in µmol of Trolox equivalent (TE) per gram of dry solid substrate (µmol TE g^{-1}). The antioxidant activity variation (AAV) was calculated by subtracting the AA of the control (without inoculation) from that of the fermented extract.

Experimental design and statistical analysis

A simplex centroid mixture design was used due to its ability to evaluate the presence of the synergistic or antagonistic effects of the mixtures of solid wastes. This design consisted of 15 runs with each independent variable (COP, EOP, EGM, and VTS) at five levels: 1 (100%), 1/2 (50%), 1/3 (33%), 1/4 (25%), and 0 (0%), the final amount of substrate was fixed (10 g). Each experiment was performed in duplicate and a control of each run was performed without inoculation with fungus. The dependent variables studied were cellulase, xylanase, β-glucosidase, total phenols, and antioxidant activity. The mixture proportions used in experiments with each fungus are presented in Table 1.

By applying multiple regression analysis on the experimental results, it was obtained the following equation that represents this model:

$$\begin{aligned} Y = {} & b_1 \cdot x_1 + b_2 \cdot x_2 b_3 \cdot x_3 + b_4 \cdot x_4 + b_{12} \cdot x_1 \cdot x_2 \\ & + b_{13} \cdot x_1 \cdot x_3 + b_{14} \cdot x_1 \cdot x_4 + b_{23} \cdot x_2 \cdot x_3 + b_{24} \cdot x_2 \cdot x_4 \\ & + b_{34} \cdot x_3 \cdot x_4 + b_{123} \cdot x_1 \cdot x_2 \cdot x_3 + b_{124} \cdot x_1 \cdot x_2 \cdot x_4 \\ & + b_{134} \cdot x_1 \cdot x_3 \cdot x_4 + b_{234} \cdot x_2 \cdot x_3 \cdot x_4 \end{aligned}$$

Where Y is the response variable, b are the coefficients of regression, and x are the independent variables. The experimental data were evaluated using the Statistica 10 software package. To calculate a substrate mixture that optimizes several dependent variables together, multiple response optimization was carried out with the Statgraphics Centurion XVI software.

Results and discussion

Chemical composition of the olive mill and winery wastes and their mixtures

The substrate composition is an important factor for the growth of fungi during SSF. The use of a single agro-industrial waste as a substrate does not provide the nutrients necessary for the growth of fungi and for the production of enzymes.[28] Thus, a mixture of wastes could be a suitable strategy to improve the production of enzymes by SSF.

As can be observed in Table 1, the use of a statistical mixture design allowed different fermentation medium composition to be studied by using different waste mixtures. This way, the composition of polysaccharides, lignin, crude protein, ash, and phenols can be related to the enzyme production and antioxidant compound extraction. It is known that the content of polysaccharides and lignin affects the growth of fungi and the production of cellulases and xylanases.[29] In this study, the agroindustrial waste that had higher cellulose content was VTS, which was about threefold higher than the other three wastes. The hemicellulose content was higher in EOP followed by VTS and COP, with EGM having the lowest content – about threefold lower than in EOP. The hemicellulose content was higher than the cellulose content in olive mill wastes, which was in agreement with results observed by other authors.[1] On the other hand, cellulose content was higher than hemicellulose in winery wastes. The waste that had the highest lignin content was EGM, and VTS was the waste with lower lignin content – twofold lower than EGM. Lignin in EGM is linked mainly to seed and skins of grapes,[30] while in olive pomace it is linked with the olive flesh and stones.[31] The high lignin content may make it difficult for microorganisms to gain access to the cellulose and hemicellulose.[29]

Exhausted grape marc showed the highest ash content, possibly due to the high level of crystallization and precipitation of tartrates during winemaking process.[32] The ash content was about twice as high in COP than in EOP, and this may be caused by the washing effect of minerals during the second extraction of residual olive oil. The lower ash content was observed in VTS, and this is in agreement with results obtained with several vine shoots from different grape varieties.[33] The ash content can be positively correlated with the biological efficiency of fungi in SSF.[34]

Crude protein content was low in all wastes except for EGM. The protein content of EGM can range between 6% and 15% depending on grape variety and the dead yeast that remains in grape marc after wine fermentation.[35] High protein content improves fungal growth during SSF.[34] The mixture of wastes increased the protein content of COP, EOP, and VTS when compared with the single substrates. The water-soluble total phenols content was higher in olive mill wastes than in winery wastes. Crude olive pomace has a higher total phenols content than EOP, and this may be due to the washing effect of removing the residual oil from COP. The TP content of olive pomace ranges between 0.6 and 2.4% depending on the extraction method, region, and crop variety.[1] Many phenolic compounds of olive and winery wastes are conjugated with components of the plant cell wall; thus, other treatments should be used to extract these compounds, namely the enzymes produced by fungi.[29]

Evaluation of olive mill and winery wastes as substrates using a simplex centroid mixture design

The simplex-centroid design is an effective tool to optimize the culture media for SSF.[27] The design allowed a comparison to be made between the use of olive mill wastes and winery wastes separately and mixed as substrates for the production of xylanase, cellulase, and β-glucosidase, and for extraction of TP and antioxidant compounds. Table 2 shows the results of the dependent variables studied in each run for both fungi tested.

Effect of the substrates on enzyme production by SSF

The experimental design allowed the optimum substrate composition for maximum enzyme production to be identified and allowed the evaluation of potential interaction effects between mixtures of wastes. Table 3 shows the regression effects and the statistical parameters, showing a good fit of the models, as highlighted by the coefficient of determination (R^2) and the F-value. The coefficient of determination was higher than 0.92 for all enzymes production by both fungi, except for cellulase production by *A. niger* (R^2 of 0.77). This indicates a good agreement between experimental values and the values predicted by the model. The F-value for the dependent variables xylanase, TPV, and AAV produced by *A. niger*, and for xylanase produced by *A. ibericus* indicates that the models were statistically significant ($P < 0.05$). Regarding the use of solid wastes separately, EGM and VTS showed higher cellulase production for both fungi.

Table 2. Results of dependent variables studied in simplex centroid design for the two fungi.

Runs	*A. niger*					*A. ibericus*				
	Xyl ($U\,g^{-1}$)	Cel ($U\,g^{-1}$)	BGS ($U\,g^{-1}$)	TPV (mg $CA\,g^{-1}$)	AAV (µmol $TE\,g^{-1}$)	Xyl ($U\,g^{-1}$)	Cel ($U\,g^{-1}$)	BGS ($U\,g^{-1}$)	TPV (mg $CA\,g^{-1}$)	AAV (µmol $TE\,g^{-1}$)
1	26.8 ± 0.1	30.5 ± 0.1	13.8 ± 0.4	–0.6	–37.1	0.8 ± 0.7	6.1 ± 3	0.1 ± 0	–3.0	–47,9
2	66.6 ± 1.6	56.2 ± 0.6	9.7 ± 0.1	0.0	–23.0	28.1 ± 0.3	18.1 ± 0.9	0.1 ± 0	0.2	–25,3
3	43.2 ± 4.8	30.3 ± 5.8	10.3 ± 1.2	–0.3	–22.3	38.0 ± 1.9	26.9 ± 1.3	0 ± 0	–0.4	–12,4
4	23.8 ± 0.2	21.8 ± 0.7	9.8 ± 0.2	2.8	–11.6	36.5 ± 5.6	15.2 ± 2.6	0.5 ± 0.1	1.4	–25,4
5	16.2 ± 1.1	20.6 ± 4.3	5.7 ± 0.3	–0.6	–18.8	81.2 ± 2.6	24.0 ± 0.5	0.1 ± 0	0.0	–21,7
6	44.4 ± 0.4	32.8 ± 8.7	5.5 ± 0.8	–0.3	–11.2	17.7 ± 0.7	16.6 ± 2.1	11.6 ± 0.6	0.8	–17,2
7	22.8 ± 1.4	15.5 ± 0.4	5.6 ± 1	0.3	–26.6	18.5 ± 0.1	11.2 ± 0.3	7.7 ± 0.3	3.5	–35,8
8	79.6 ± 6.7	28.9 ± 9.1	8.7 ± 2.1	0.1	–2.9	36.5 ± 1.5	29.5 ± 4.8	1.2 ± 1.7	0.6	–14,7
9	62.7 ± 6.6	24.6 ± 1.2	4.9 ± 0.4	–0.6	14.5	66.4 ± 3.2	16.9 ± 0.1	12.4 ± 0.5	1.3	–26,8
10	75.2 ± 1.8	15.2 ± 1.8	9.0 ± 0.1	–0.8	38.2	26.8 ± 2.3	26.4 ± 5.8	25.5 ± 0.6	1.4	5,7
11	129.4 ± 15.8	20.2 ± 1	13.3 ± 1.4	–0.2	4.5	96.4 ± 2.9	27.3 ± 8.9	16.5 ± 0.6	0.0	–4,5
12	60.2 ± 2.1	13.6 ± 0.2	6.3 ± 1.1	0.6	–5.7	51.0 ± 1.5	16.2 ± 1.5	8.5 ± 0.9	–0.1	–29,3
13	45.7 ± 9.7	23.5 ± 0.9	6.6 ± 1.3	0.3	0.4	53.1 ± 3.9	26.5 ± 1.2	15.2 ± 0.8	–0.1	–15,9
14	85.5 ± 23	14.6 ± 0.5	14.1 ± 1.2	0.2	–13.3	85.0 ± 0.1	84.2 ± 7.4	22.2 ± 0.8	0.2	–19,2
15a	72.2 ± 0.9	37.7 ± 1.1	16.2 ± 2.5	0.8	3.0	63.2 ± 4.9	36.3 ± 0.5	18.0 ± 1.5	0.2	–9,3
15b	75.8 ± 0.9	38.8 ± 2.1	17.9 ± 0.6	0.9	–2.8	59.8 ± 2.4	22.6 ± 0.5	19.0 ± 0.9	1.2	–11,2
15c	74.2 ± 6.3	38.2 ± 2.2	15.5 ± 0.7	1.1	1.3	67.1 ± 1	30.6 ± 0.9	21.8 ± 1.2	0.5	–11,6

Results are presented as the mean (n = 2) ± SD; Xyl, xylanase; Cel, cellulase; BGS, β-glucosidase; TPV, variation in total phenols expressed as equivalents of caffeic acid; AAV, antioxidant activity variation expressed as equivalents of Trolox; TE, Trolox equivalents.

Table 3. Regression coefficients and ANOVA of the special cubic models.

	A. niger					*A. ibericus*				
RC	Xyl	Cel	BGS	TPV	AAV	Xyl	Cel	BGS	TPI	AAI
x_1	26.47	30.81*	13.84***	–0.64**	–36.96***	0.49	6.09	0.10	–3.03*	–44.48**
x_2	66.27**	56.53**	9.71**	0.03	–22.91**	27.81	18.03*	0.21	0.20	–20.04*
x_3	42.81*	30.63*	10.34**	–0.32	–22.21**	37.71*	26.85**	–0.05	–0.38	0.33
x_4	23.46	22.08	9.89**	2.80***	–11.49*	36.17*	15.16*	0.60	1.38	–15.05
x_1x_2	–109.26	–102.19	–26.12*	–1.21	41.51	281.37**	49.11	–1.34	4.93	48.74
x_1x_3	50.55	–1.54	–27.82*	0.39	70.29**	7.34	2.03	46.67**	9.25	61.97
x_1x_4	3.01	–53.73	–26.64*	–3.07**	–12.41	13.88	3.72	28.35	16.53**	18.38
x_2x_3	111.79	–68.88	–7.05	0.93	75.61**	28.21	29.60	62.15**	2.05	–17.57
x_2x_4	82.70	–68.92	–21.32	–8.12***	123.73**	150.57	2.69	48.62**	1.42	4.91
x_3x_4	179.59*	–54.61	–6.14	–8.43***	217.16***	–27.60	23.00	102.9***	2.84	60.18
$x_1x_2x_3$	1854.42**	231.68	273.93**	5.65	367.92*	808.68	15.19	131.79	–2.33	372.96
$x_1x_2x_4$	387.77	287.37	129.82	36.11***	99.43	–830.93	–110.09	–2.32	–40.90	–138.29
$x_1x_3x_4$	–562.47	441.35	91,96	27.25**	–107.13	505.27	177.15	–79.56	–55.23	82.54
$x_2x_3x_4$	–268.21	217.40	252,26**	33.10**	–1027.4***	666.05	382.87	–46.09	–8.43	–186.48
Statistical parameters										
Model (SS)	12557.2	1566.4	269.05	12.2	4828.4	10347.8	1178.9	1196.7	23.8	2490.4
Total error (SS)	606.703	461.67	15.14	0.11	61.17	798.88	102.37	21.13	2.76	181.89
R^2	0.9539	0.7723	0.9467	0.9908	0.9874	0.9283	0.9201	0.9827	0.896	0.932
R^2 adjusted	0.7542	0	0.716	0.951	0.9333	0.6177	0.5739	0.9075	0.4453	0.637
F-value	4.78	0.68	4.1	24.93	18.22	2.99	2.66	13.07	1.99	3.16

RC, regression coefficients; R^2, coefficient of determination; Xyl, xylanase; Cel, cellulase; BGS, β-glucosidase; TPV, total phenols variation; AAV, antioxidant activity variation.
$^{***}P<0.01$; $^{**}P<0.05$; $^{*}P<0.01$.

The use of EOP and VTS separately showed higher xylanase production by *A. ibericus*, and this can be linked to the high hemicellulose content in both wastes. The production of β-glucosidase by *A. niger* was similar for the four wastes used separately; however, there was no production of this enzyme by *A. ibericus* under these conditions.

The production of cellulases were not improved by mixture of wastes. On the other hand, the mixture of wastes significantly improved xylanase and β-glucosidase production. Mixture contour plots (Fig. 1) display the enzyme production variation using different amounts of olive mill and winery wastes. Figure 1 represents the three factors with a higher effect in each corner of the triangle, while the fourth factor was fixed with level 0. The internal points of the triangle show the enzyme production predicted by the model with different proportions of mixtures of wastes. The darker zone shows the optimum mixture, which allowed maximum enzyme production. As can be observed in Fig. 1(a) and 1(f), the optimum mixture to maximize xylanase production was similar for both fungi, with a mixture of COP, EGM, and VTS. The xylanase production using the ternary mixture (run 11) was improved about fivefold by *A. niger* and 120-fold by *A. ibericus* in comparison with the use of COP as the unique substrate (run 1). Thus, the mixture of olive mill wastes with winery wastes was a suitable strategy for enhancing xylanase production.

A similar effect was observed in the production of β-glucosidase (Fig. 1(c) and 1(h)). However, in this case, the optimum mixture for each fungus was slightly different. The optimum mixture for β-glucosidase production by *A. niger* was obtained with equal proportions of EOP, EGM, and VTS, whereas for production by *A. ibericus* the optimum mixture was obtained with EOP and VTS in equal proportions.

The model predicted the maximum cellulase production by *A. niger* using only EGM as a substrate; however, the model fit was not satisfactory (Fig. 1(b) and 1(g)). Using *A. ibericus*, the optimum cellulase production was obtained with a mixture of similar proportions of EOP, EGM, and VTS. In this case, the mixture of EOP with winery wastes also improved cellulase production 14-fold. Other studies evaluated grape skins and olive pomace as substrates for xylanase, cellulase, and β-glucosidase production by other fungi. Romo-Sanchez *et al.*[36] studied the production

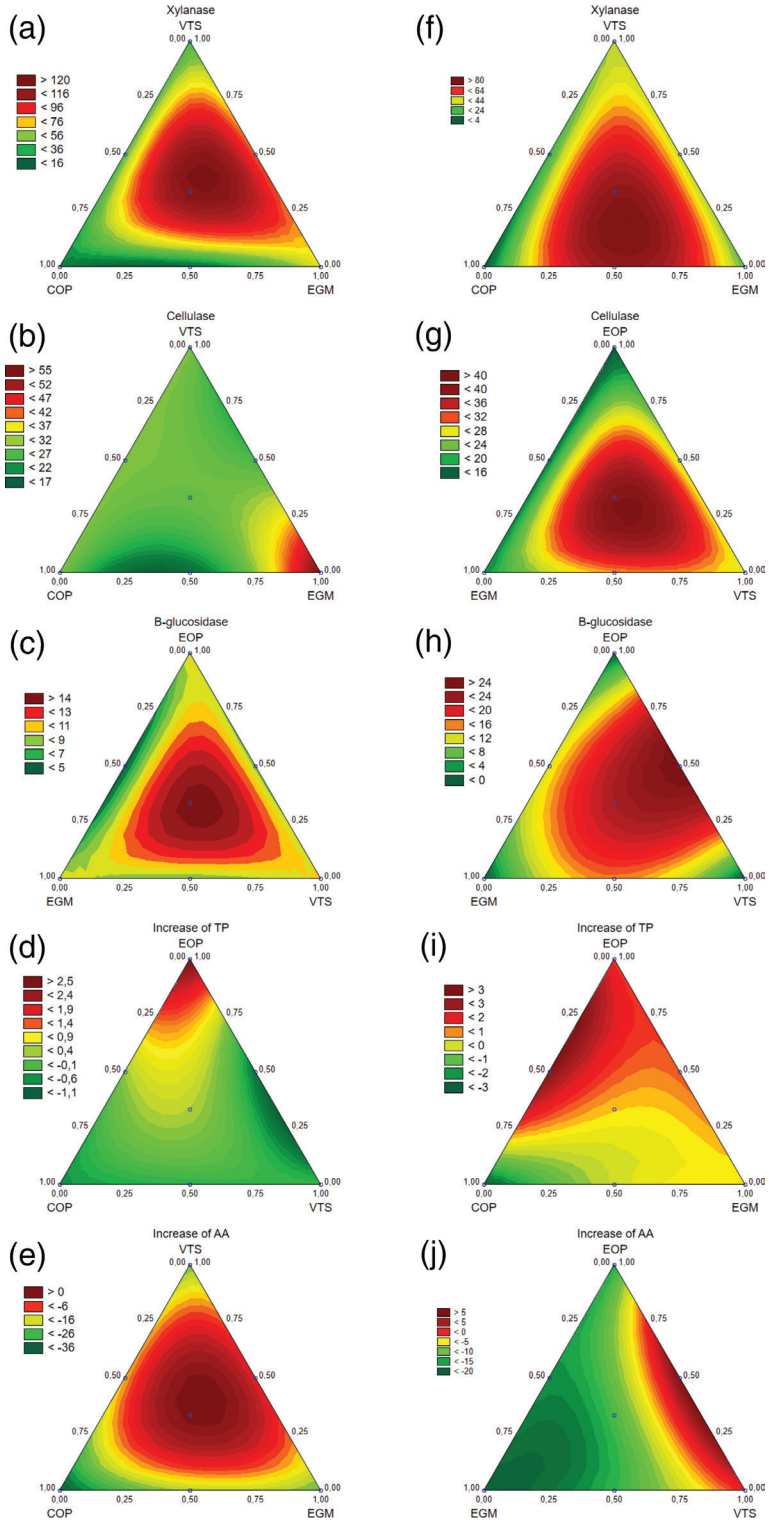

Figure 1. Contour plots for dependent variables of simplex centroid design: (a) xylanase by *A. niger*; (b) cellulase by *A. niger*; (c) β-glucosidase by *A. niger*; (d) variation of TP by *A. niger*; (e) variation of antioxidant activity by *A. niger*; (f) xylanase by *A. ibericus*; (g) cellulase by *A. ibericus*; (h) β-glucosidase by *A. ibericus*; (i) variation of TP by *A. niger*; (j) variation of antioxidant activity by *A. ibericus*.

of these enzymes by *A. niger* and *A. fumigatus*, using grape skin and olive pomace supplemented with wheat bran, and the maximum xylanase activity (47 U g^{-1}) was achieved by *A. niger* using grape skin as a substrate and a 15-day fermentation period. The production of enzymes by *A. fumigatus* using only olive pomace or olive pomace supplemented with wheat bran as substrates led to lower production of xylanase (8 U g^{-1}), cellulase (4 U g^{-1}), and β-glucosidase (1 U g^{-1}). These values were lower than the ones obtained in this work by *A. ibericus* (96.4 U xylanase g^{-1}, 84.2 U cellulose g^{-1} and 25.5 U β-glucosidase g^{-1}) and by *A. niger* (129.4 U xylanase g^{-1}, 38.8 U cellulose/g and 17.9 U β-glucosidase g^{-1}) using the optimal mixtures of olive mill and winery wastes. The use of olive pomace as the sole substrate in SSF is difficult; thus, wheat bran is frequently used as a supplement to improve enzyme production.[36–38] However, the cost of wheat bran is high relative to other agro-industrial wastes, such as winery wastes.

Effect of substrate on the release of total phenols by SSF

The release of water-soluble total phenols by the SSF of mixtures of olive mill and winery wastes was also studied. As can be seen in Table 2, TP increased or decreased depending on the substrate used in SSF. The SSF of COP (run 1) led to a large reduction in TP after 7 days; however, SSF of EOP (run 4) led to a TP increase of 1.4 mg CA g^{-1} during the same period. The reduction in total phenols can be due to the polymerization of free phenolics by oxidative enzymes.[39] The capacity for the reduction of total phenols was also observed in the fermentation of olive mill wastewater by *A. ibericus*.[40] The degradation of ferulic, coumaric, and syringic acids by *A. niger* was also observed.[41] The increase in TP in the SSF of EOP may be due to the action of β-glucosidase and xylanases that can hydrolyze phenolic compounds linked to polysaccharides of agro-industrial waste.[39]

The coefficients of determination were 0.99 for SSF by *A. niger* and 0.93 for SSF by *A. ibericus*, showing a good fit between experimental and predicted values. The F-value for SSF by *A. niger* was 18.22, indicating that the model was significant ($P < 0.05$). As can be observed in Table 3, almost all factors significantly affected ($P < 0.05$) the increase in total phenols. All ternary interaction terms, except for ($x_1x_2x_3$), showed a high positive effect ($P < 0.05$) for SSF by *A. niger*. On the other hand, for SSF by *A. ibericus* the highest significative ($P < 0.05$) effect was the binary term (x_1x_4).

The maximum extraction of total phenols (3.5 mg CA/g) was achieved in SSF of the two olive mill wastes studied (COP and EOP). These wastes had higher total phenol content than winery wastes (Table 1); thus they have greater potential to extract total phenols. It is estimated that 98% of the phenolic compounds of olive fruit remain in olive pomace.[42] Fig. 1(d) and 1(i) shows the contour plots for the variation of TP depending on the mixture of EOP, COP, and VTS used for SSF by *A. niger*, and the mixture of EOP, COP, and EGM used for *A. ibericus*. As can be observed, the optimum region for SSF by *A. niger* was near to the EOP corner, and for SSF by *A. ibericus* was in the center of the side between EOP and COP. This indicates that the technique of SSF for the extraction of TP from olive pomace was more suitable for wastes from olive mills. Other techniques for the extraction of phenolic compounds from olive pomace using organic solvents, such as ethanol mixed with water, showed lower values of TP than those obtained by SSF. De Bruno *et al.*[42] studied different solvents and times of extraction, and the maximum extraction of TP obtained was 1.7 mg of CA g^{-1} dry pomace. This value was twofold lower than the maximum achieved in the present study by SSF of a mixture of COP and EOP. Rubio-Senent *et al.*[8] evaluated phenolic extraction from olive pomace using hydrothermal treatments at 160 °C, and obtained a maximum extraction of 3.4 mg of CA g^{-1} fresh pomace, which expressed in dry weight should be 0.9 mg CA g^{-1} dry pomace.

Effect of substrates on antioxidant activity by SSF

The antioxidant activity of aqueous extracts obtained from the SSF of wastes was compared with that of extracts from autoclaved and non-inoculated wastes. Solid-state fermentation by *A. niger* showed a maximum antioxidant activity of 38 µmol TE/g when VTS and EOP were used as substrates. For SSF by *A. ibericus*, a decrease of antioxidant activity after 7 days of SSF it was observed in all experiments, except for run 1, where a mixture of COP, EGM, and VTS was used as substrate. The fermentation time can be a factor that affects the degradation of antioxidant compounds. Dulf *et al.*[39] observed an increase in antioxidant activity in the first days of SSF and a reduction in antioxidant activity after sixth day of SSF.

The coefficient of determination for SSF by *A. niger* was close to 1, showing a good fit and F- value, indicating that the model had a significant effect ($P < 0.05$). The coefficient of determination for SSF by *A. ibericus* was high (0.93) but the F-value was low, indicating that the model did not have a significant effect. Almost all coefficients of regression for SSF by *A. niger* had statistically significant effects ($P < 0.05$), and the interaction between wastes exerted a positive effect on the increase of antioxidant activity after SSF by *A. niger*. Both the binary (x_1x_3, x_2x_3, x_2x_4, x_3x_4) and ternary ($x_1x_3x_1$)

terms showed a significant ($P < 0.01$) positive effect on the increase of antioxidant activity. The ternary term explains that the optimal region in the contour plot (Fig. 1(e)) was obtained with a mixture in equal proportions of COP, EGM, and VTS. In the case of SSF by *A. ibericus*, the contour plot (Fig. 1(j)) indicates that the maximum antioxidant activity was obtained with a mixture of EOP and VTS. The increase in antioxidant after SSF is linked to the action of enzymes produced by fungi that transformed the phenolic compounds of the substrate into aglycone phenolic molecules with high antioxidant activity.[43] The increase in antioxidant activity by the action of enzymatic cocktail on agro-industrial wastes, as citrus residues, was previously evaluated and led to an increase of antioxidant activity (DPPH method) of 17%.[43] In the present work, the maximum antioxidant activity (run 10) was fivefold higher with the SSF than with the unfermented mixture of EOP and VTS. Previously, Teles *et al.*[29] studied the increase of antioxidant activity by SSF of grape marc mixture with wheat bran, and only observed an increase of antioxidant activity (ABTS method) of 1.5-fold after 96 h compared with the control.

Optimization of substrate composition by multiple response

To optimize all dependent variables jointly, multiple response optimization was applied. Table 4 shows the optimal mixture of wastes that allow high production of enzymes and high extraction of total phenols to be achieved, and an increase of antioxidant activity in SSF by *A. niger* and *A. ibericus*. As can be observed in Table 2, the optimum mixture of wastes that maximizes enzyme production does not coincide with the optimum waste mixture that allows a high increase in total phenols and antioxidant activity to be obtained. The maximum value of all dependent variables is thus lower than the optimal conditions for each variable evaluated separately. The validation of the model was performed using the optimal mixture of wastes as substrates for both fungi. The production of xylanases, cellulases, and β-glucosidases by *A. niger* under optimal conditions was 84.3 ± 2.4, 47 ± 1.6, and 9.6 ± 0.2 U g^{-1}, respectively. The production of xylanases and β-glucosidases was lower than the predicted value, while the production of cellulases was higher. The variation of total phenols and antioxidant activity of the extract from fermented solid was 2.3 and 31.4, lower than extract from unfermented solid. In SSF by *A. ibericus*, the production of xylanases, cellulases, and β-glucosidases was similar to the values predicted by the model, which were 78.1 ± 0.4, 39 ± 1.2, and 21.2 ± 3 U g^{-1}, respectively.

Kinetic of SSF by *A. ibericus* under optimum substrate composition

The production of enzymes and the variation in total phenols and antioxidant activity with fermentation by *A. ibericus* was evaluated using the optimal substrate conditions. The SSF was performed in a tray-type bioreactor with 50 g of dry substrate. Figure 2a shows the production of enzymes and the increase in ergosterol, which was used as an indirect indicator of fungal growth. This is an effective method to determine fungi biomass mainly in the first two phases of mycelium growth.[44]

On the first day of SSF no increase in the ergosterol content was observed. Thereafter, the ergosterol content increased from day 2 to day 11 of fermentation. The production of enzymes started after 2 days of fermentation. Xylanase was the enzyme that showed the highest increase during the first days of SSF, with the maximum production being achieved on the seventh day. Cellulase production achieved the maximum activity on the fourth day, and β-glucosidase achieved the maximum production on the second day. Figure 2b presents the evolution of antioxidant activity and the release of total phenols soluble in water. There was a higher increase (twofold) in antioxidant activity on the third day of SSF, and then the activity decreased. This effect was also observed in the SSF of berry pomaces by *A. niger*,[45] where the antioxidant activity decreased after the fourth day of SSF to the initial value before SSF. This could be linked to the polymerization of free phenolic compounds released in the first days of SSF.[39] Teles *et al.*[29] also observed a negative correlation between

Table 4. Optimization of multiple responses.

Optimum value	Mixture of wastes				Response variables (predicted value)				
	COP (%)	EGM (%)	VTS (%)	EOP (%)	Xyl (U g^{-1})	Cel (U g^{-1})	BGS (U g^{-1})	TPI (mg CA g^{-1})	AAI (μmol TE g^{-1})
SSF by *A. niger*	23	30	33	14	99.15	31.86	16.19	0.58	0.22
SSF by *A. ibericus*		30	36	34	73.83	40.61	22.77	0.15	–7.9

SSF, solid-state fermentation; COP, crude olive pomace; EGM, exhausted grape marc; VTS, vine trimming shoots; EOP, exhausted olive pomace; Xyl, xylanase; Cel, cellulase; BGS, β-glucosidase; TPV, variation in total phenols expressed as equivalents of caffeic acid; AAV, antioxidant activity variation.

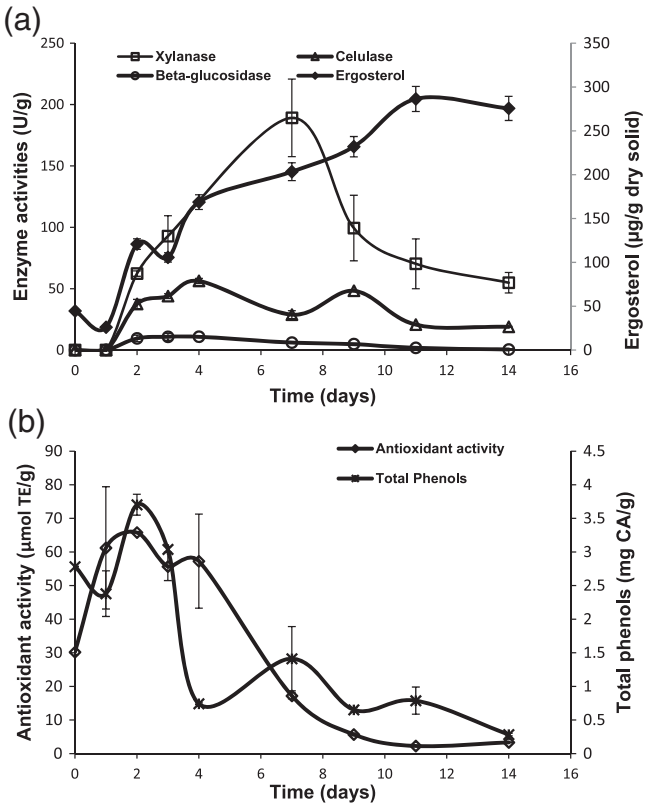

Figure 2. Evolution of xylanase, cellulase, β-glucosidase, and ergsterol (a) and the antioxidant activity and phenols (b) during SSF by *A. ibericus* with optimal substrate.

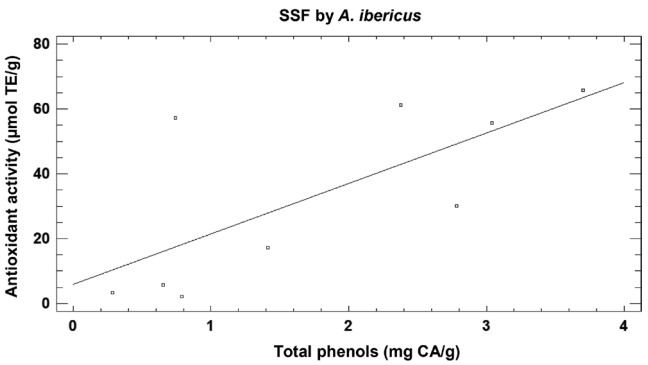

Figure 3. The relationship between antioxidant activity (DPPH method) and total phenolic compounds of extract from SSF by *A. ibericus* with optimal substrate.

cellulase and β-glucosidase production with anthocyanins, which can be mainly responsible for the antioxidant activity.

Figure 3 shows the linear regression of antioxidant activity and total phenols. The relationship between antioxidant activity and total phenols showed a significant correlation ($P = 0.0295$) and the R^2 statistic indicated that the model explained 51.48% of the antioxidant activity variability.

The correlation between total phenols and antioxidant activity was studied by several authors. Dulf *et al.*[45] did not find significant correlation between total phenols and antioxidant activity in extracts from fermented berry pomace by *A. niger*, since the antioxidant activity can be linked to only specific types of phenolic compounds as flavonols and anthocyanins, which could not be extracted. Likewise, no correlation between total phenols and antioxidant activity was found in the extracts from olive mill wastes.[46]

Conclusions

The present study showed that olive pomace can be bioprocessed to obtain bioactive compounds. The study of mixtures of olive mill wastes with winery wastes as the substrate for SSF using a simplex-centroid design made it possible to select the optimal substrate for the production of enzymes and to increase the extraction of total phenols and antioxidant activity by SSF. The mixture of wastes caused an increase in xylanase, cellulose, and β-glucosidase production by *A. niger* and *A. ibericus* compared with the use of olive pomace as the only substrate. A short period of SSF increased the extraction of antioxidant compounds and allowed maximum production of cellulase and β-glucosidase to be achieved. However, maximum xylanase production was achieved after 7 days of SSF. The extract from the fermented mixture of olive mill and winery wastes by *A. ibericus* showed a significant correlation between antioxidant activity and total phenols. Based on these results, it can be concluded that SSF can be a suitable tool to obtain bioactive compounds from olive pomace.

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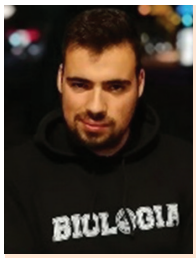


Diogo Filipe

Diogo Filipe has an MSc in aquatic biological resources from the Faculty of Sciences at the University of Porto (FCUP). He studied biological engineering during his stay at Center of Biological Engineering at the University of Minho and wishes to apply his knowledge to current issues in aquaculture and capture fisheries. Outside of this work he has a background in fish immunology, using molecular biology and fish parasitology techniques. He is also interested in preserving endangered fish populations and the ocean's ecosystems.

Helena Fernandes

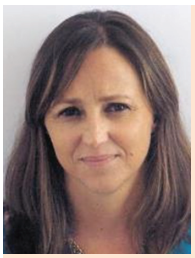
Helena Fernandes has a master's degree in aquatic biological resources from the University of Porto and started her PhD in 2017, developing her research at the Faculty of Sciences of University of Porto (FCUP, Porto), at the Interdisciplinary Center for Marine and Environmental Research (CIIMAR, Porto), and at the Center of Biological Engineering (CEB, Braga). She focuses on the reutilization of agro-industrial by-products through solid-state fermentation, with the aim of obtaining value-added compounds and novel feed ingredients for inclusion in the diets of aquaculture fish species.

Carolina Castro

Carolina Castro obtained her PhD in 2016 in biology at Faculty of Sciences of Porto University. She is currently a postdoctoral researcher at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Porto University. Her main research interest focuses on the nutrition and welfare of marine fish of interest for aquaculture – more specifically, on the nutritional effects of macronutrients and ingredients on metabolic and digestive capacity and oxidative stress. In recent years she has been collaborator in numerous national and industry funded projects, authoring and co-authoring several international conferences and research papers in the fields of fish nutrition and aquaculture.

Helena Peres

Helena Peres (female, PhD) has been a research nutritionist at CIIMAR – the Interdisciplinary Center of Marine and Environmental Research, University of Porto, Portugal since 2003 and Invited Associate Professor at FCUP since 2010. She has been the director of the Fish Nutrition and Immunobiology group at CIIMAR since 2010, and the director of the animal sciences laboratory – Aquatic Species Course (CAL-AQUA), since 2016. She has been involved in R&D projects and market-driven projects with national and international industries. Her domain of specialization is fish nutrition, with a particular interest in nutritional strategies for reducing the environmental impact of aquaculture and modulating wellbeing, stress, and infection resistance in fish.



Isabel Belo

Isabel Belo graduated in chemical engineering at the University of Porto, Portugal, and gained her PhD in 2000 in chemical and biological engineering at the University of Minho, Braga, Portugal. She is assistant professor in the Biological Engineering Department of the Engineering School of University of Minho, and a staff researcher of the Center of Biological Engineering (CEB). Isabel Belo is the director of the bioprocess and biosystems laboratory of CEB and is the director of the doctoral program in chemical and biological engineering at the University of Minho. Her main research activities are related to bioprocess engineering, particularly bioprocess development and optimization and fermentation technologies.

Aires Oliva-Teles

Aires Oliva-Teles is a full professor in the Faculty of Sciences, Porto University, and a coordinator of the Fish Nutrition and Immunobiology group at CIIMAR. He has been working as a fish nutritionist for more than 30 years, being involved mainly in the study of nutrition and feeding of Mediterranean marine fish species of interest to aquaculture.

José Manuel Salgado

José Manuel Salgado obtained his PhD in food science and technology at the University of Vigo (Spain) in 2010. He is a faculty researcher in the biosystems group of the Center of Biological Engineering at the University of Minho (Portugal). He has contributed significantly to the development of a circular economy in the agro-food industry, particularly regarding the revalorization of agro-food wastes by biotechnology processes. He has studied the fractionation of lignocellulosic materials and different fermentation techniques for the reuse of agro-industrial wastes. His current research is focused on solid-state fermentation to be applied in biorefineries. He has developed the valorization of wastes from agro-food industries in order to obtain high value-added products such as enzymes, antioxidant compounds, and animal feed with high nutritional value using solid-state fermentation processes.

