Bioprocessing of oilseed cakes by fungi consortia: impact of enzymes produced on antioxidants release

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Abstract

Oilseed cakes (OC) present high potential as feedstock for the biobased industry. Biotechnological processes allow OC valorization by the production of diverse value-added products and simultaneously altering OC structure, improving their nutritional value, and boosting OC utilization in animal feed. This work explored the use of fungi consortium of two different species as a bioprocessing approach to improve the nutritional quality of OC, obtain enzymes and antioxidants by solid-state fermentation (SSF) of sunflower cake (SFC) and rapeseed cake (RSC). *Rhyzopus oryzae* and *Aspergillus ibericus* consortium led to the highest production of cellulase (135 U/g) and β-glucosidase (265 U/g) while maximum protease (228 U/g) was obtained with *A. niger* and *R. oryzae* consortium. Maximum xylanase production (886 U/g) was observed in SSF of RSC resulting in high hemicellulose reduction. The synergistic action of lignocellulosic enzymes resulted in fermented extracts with increased antioxidant potential with possible application as food additives against oxidative stress.

Keywords: co-cultures, filamentous fungi, antioxidants, enzymes, crude protein, fiber

1. Introduction

Lignocellulosic biomass is a widely bioavailable, natural, and promising material that has been exploited in many biotechnological processes. Biomass is obtained from atmospheric carbon dioxide (CO_2) and water, using sunlight as the energy source for the photosynthesis process (Yousuf et al., 2020). Agricultural wastes, industrial by-products, woody materials, energy crops, and human waste are some of the potential sources of lignocellulosic biomass. Global lignocellulosic biomass production accounts for approximately 181.5 billion tons annually (Paul & Dutta, 2018). Although the composition of this resource may change according to its origin, they are mainly composed of carbohydrates such as cellulose (35 – 50 %) and hemicellulose (25 – 30 %), lignin (15 – 20 %), lipids, ashes, and soluble components including proteins, sugars and phenolic compounds (Kumla et al., 2020; Menon & Rao, 2012).

The rapid and accelerated rate of global climate change implies a quick transition to a sustainable society that can reuse and valorize natural bioresources, such as lignocellulosic biomass. Biomass is generally complex and thus pre-treatments are essential to overcome their compound structure and obtain maximum profit from their components.

The vegetable oils industry is one of the main sources of lignocellulosic biomass. The impact of vegetable oils on human health is invaluable. They are rich in essential unsaturated fatty acids and various valuable bioactive compounds that contribute to the absorption of fat-soluble vitamins and ensure the normal function of the endocrine system (Kotecka-Majchrzak et al., 2020). Additionally, the exploitation of vegetable oils for biodiesel production as a replacement for fossil fuels largely contributes to the increased

50 production of oilseeds. It is expected that by 2029, 87% of world oilseeds production,
51 excluding soybean, will be crushed (OECD et al., 2020). Solid by-products obtained after
52 mechanical extraction of oil are referred to as cakes and are of lignocellulosic nature. The
53 global production of these commodities has increased over the past years. Edible oil cakes
54 are nutritionally valuable and can be used as a supplement in human diets, as an additive
55 in bakery, infant food, or as multipurpose supplements (Ancuța & Sonia, 2020). Non
56 edible oil cakes are used as green manure due to the presence of toxic compounds (Gupta,
57 Sharma, Sharma, et al., 2018). Application as animal feed, landfilling, or biofuel
58 conversion (heat or electricity) are among the most conventional practices to capitalize
59 oilseed cakes (Otles et al., 2015). However, the presence of toxic compounds and
60 antinutritional factors can affect animals' and humans' well-being. Sustainable
61 improvement of food chains is essential to overcome these issues resulting in value-added
62 products.

63 Sunflower cake (SFC) and rapeseed cake (RSC) are two of the most generated by-
64 products obtained from the vegetable oils industry. These by-products are characterized
65 for their high protein content, lipids, carbohydrates, and minerals. SFC has a protein
66 content ranging from 23-53% while crude fiber may vary from 17-33% (Ancuța & Sonia,
67 2020; Sousa et al., 2021). RSC has a high protein content between 14-40% and 5-15% of
68 crude fiber (Ancuța & Sonia, 2020). Additionally, these OC contain phenolic compounds
69 that act as antioxidants against oxidative stress (Ancuța & Sonia, 2020; Sousa et al.,
70 2021). Total carbon accounts for approximately 50% (w/w) of these products ensuring
71 they can be used as substrate in solid-state fermentation (SSF) as the carbon source for
72 microorganism's growth and metabolites production.

73 SSF is defined as a fermentation process that occurs in the absence or near absence
74 of free water to which is applied a natural or inert support used as substrate for the growth

of microorganisms (Pandey et al., 2000). However, the solid substrate must contain enough moisture to support the growth and metabolism of microorganisms. In this type of fermentation, microorganisms grow in conditions close to their natural *habitat* and filamentous fungi are the ones that better adapt to this environment.

The use of SFC and RSC as substrate for SSF, as a biological approach of OC treatment, has the potential to increase the nutritive value of these products (Sousa et al., 2021). Deconstruction of the lignocellulosic matrix through extracellular hydrolytic enzymes produced by fungi may favor the release of unextractable conjugated phenolics with potential antioxidant capacity. Phenolic acids, flavonoids, and lignans can be found in OC as free, esterified, or in condensed form. The action of these compounds can help reduce oxidative stress preventing the appearance of various types of cancer (Ancuța & Sonia, 2020). Phenolic compounds are the main type of antioxidants found in SFC and RSC. Amongst them, catechin, epicatechin, p-coumaric, and chlorogenic acid are the main antioxidants found in SFC while gallic acid, caffeic acid, ferulic acid, quercetin, luteolin, and sinapic acid are the major antioxidants present in RSC (Şahin & Elhussein, 2018; Senanayake et al., 2019; Teh & Bekhit, 2015). Phenolic compounds have redox properties which are related to the extinction of singlet and triplet oxygen, to decompose peroxides and absorb and neutralize free radicals.

Despite the commercial value of OC and its applications, through clean and low-cost bioprocess it is possible to increase their value and, in the same process, obtain value-added bioactive components. Through SSF it is possible to obtain commercially interesting enzymes with possible applications in industries such as textile, paper, or as a supplement in animal feed (Singh et al., 2021). Furthermore, valuable phenolic compounds with possible applications in the pharmaceutical, cosmetic, or food area as antioxidants, anti-inflammatory, chemoprotective or anti-carcinogenic agents

(Żymańczyk-Duda et al., 2018) can be obtained. Finally, SSF can contribute to obtaining an improved residue with increased nutritional properties such as reduced fiber content to be used in animal feed.

Understanding the behavior of isolated fungal strains in the biotechnological process is a step further for process optimization. SSF of OC using single filamentous fungi species has been previously described by Sousa et al. (2021). Those single cultures, under the same conditions, showed different profiles of enzymatic activity and antioxidant potential. While *Rhyzopus oryzae* proved to be the best producer of β-glucosidase leading to a higher concentration of total phenolic compounds (TPC) in fermented extracts, *Aspergillus niger* showed to be a good producer of cellulases and xylanases while having an important role in the obtainment of antioxidant-rich extracts and maintenance of crude protein. Microbial consortia can contribute to increasing process yields because of the high demand from industries, to obtain a more complete cocktail (enzymatic or antioxidant) or even to overcome individual limitations of monocultures. Fungi consortia occur ubiquitously in nature and the symbiotic relations between species allow them to suppress monoculture limitations. Synergistic interactions between fungal species may increase substrate use resulting in higher productivity, increased fungal species adaptability to the fermentative environment, and increased resistance to contamination by unwanted microorganisms (Alam et al., 2003). Additionally, nutritional limitations may be overcome by the interaction between compatible species. The use of fungal consortia can be a viable and interesting alternative to increase the catalytic potential of enzymatic cocktails. The successful obtainment of enzymatic rich extracts with increased activity over monocultures was reported by some authors using lignocellulosic residues such as cottonseed cake, sugarcane bagasse, and wheat bran (Oliveira Rodrigues et al., 2020; dos Santos et al., 2019; Grewal et al., 2019;

Maehara et al., 2018). However, less exploited is the use of fungal consortia to enhance the extraction of phenolics with relevant antioxidant potential.

This study evaluates the use of fungi consortium for fermentation of OC under SSF conditions. The work aimed to evaluate the potential use of fungi co-cultures on SSF of SFC and RSC to produce lignocellulolytic enzymes and antioxidant compounds, as well as to biomodify the nutritional properties of OC to improve their applications in animal feed.

2. Materials and methods

2.1. Oilseed Cakes

Two OC from vegetable oils production industries were used: SFC and RSC. OC were obtained from industries operating in Portugal. SFC was provided by Sorgal, S.A. and RSC was provided by IBEROL – *Sociedade Ibérica de Oleaginosas*, SARL. OC were dried at 65ºC for 24 hours and kept in closed containers in the dark at room temperature. These OC were previously characterized (Sousa et al., 2021), having both around 40% (w/w) of protein content, 8% of lignin, with slight differences in cellulose (14% to 16%), and hemicellulose of 11% and 14% for SFC and RSC, respectively.

2.2. Microorganisms

SFC and RSC were used as substrates in SSF experiments to assess the effect of fungi consortium using three fungi species*, R. oryzae*, *Aspergillus ibericus,* and *A. niger*. *R. oryzae* MUM 10.260 and *A. ibericus* MUM 03.113 were obtained from *Micoteca* of the University of Minho, Braga, Portugal. *A. niger* CECT 2915 was obtained from CECT (Colección Española de Cultivos Tipo, Valencia, Spain). Fungi were cultured in potato dextrose agar (PDA) plates and preserved at 4ºC.

150

151 *2.3. Solid-state fermentation*

152 SSF experiments were carried out using 500 mL Erlenmeyer flasks, containing 10 g of
153 dry substrate with moisture adjusted to 75% (w/w, wet basis) with distilled water. Flasks
154 were sterilized at 121ºC for 15 minutes. For inoculation, a sterile peptone solution (1 g L^{-1}
155 peptone and 0.1 g L^{-1} Tween 80) was added into PDA slants to collect spores and the
156 suspension concentration was adjusted to allow an initial fermentation inoculum of $2x10^5$
157 spores g^{-1} (per mass of dry solid). Flasks were kept at 25ºC for 7 days. Each experiment
158 was performed in duplicate.

159

160 *2.4. Extraction of bioactive compounds*

161 Antioxidant phenolic compounds and enzymes were recovered after SSF through
162 liquid/solid extraction using 50 mL of distilled water. Mixtures were stirred for 30 min in
163 an orbital incubator at 20ºC and afterward, were filtered through a fine-mesh net. The
164 resulting liquid fraction was centrifuged at 2264 *g* for 10 min at 4 ºC. The enzymatic
165 extract was recovered and stored at -20 ºC until analysis.

166

167 *2.5. Total phenolic compounds and antioxidant activity*

168 Total phenolic compounds (TPC) were determined using the Folin-Ciocalteau
169 method (Commission Regulation (ECC) No. 2676/90) with some modifications (Sousa
170 et al., 2021). TPC was expressed as mg GA per g of dry matter.

171 Antioxidant activity accessed by the DPPH method was performed as described
172 by Dulf et al. (2015) with some modifications (Sousa et al., 2021). The free radical
173 scavenging potential of extracts was expressed as micromoles of Trolox equivalents per
174 gram of dry matter (μmol g^{-1}).

175 Antioxidant potential quantified by the iron chelating activity (ICA) was
176 performed as described by Sousa et al. (2021). Extract's capacity to chelate ferrous ion
177 was expressed as EDTA equivalents (nmol/g).
178 Extracts capacity to scavenge the superoxide radical was determined using the
179 PMS-NADH non-enzymatic assay as described by Gangwar et al. (2014) with some
180 modifications (Sousa et al., 2021). The scavenging potential of fermented extracts was
181 expressed as micromoles of ascorbic acid equivalents per gram of dry matter (μmol g^{-1}).
182 The Ferric Reducing Antioxidant Power Assay (FRAP) was performed according
183 to Benzie & Strain (1996) with some modifications to adapt the assay for microplate
184 (Sousa et al., 2021). The reduction potential of fermented extracts was expressed as
185 ferrous sulfate equivalents (μmol/g). All assays were performed in duplicate.
186
187 *2.6. Enzymatic activities*
188 Cellulase and xylanase quantification was determined using
189 carboxymethylcellulose (CMC) (20 g L^{-1} in citrate buffer 0.05 N at a pH 4.8) and xylan
190 (10 g L^{-1} in citrate buffer 0.05 N at a pH 4.8) as substrate, respectively. Release of
191 reducing sugars after enzymatic hydrolyses was quantified using the 3,5-dinitrosalicylic
192 acid (DNS) method as previously described (Sousa et al., 2021).
193 *β*-glucosidase quantification was assessed using p-nitrophenyl-β-D-
194 glucopyranoside (PNG) as substrate (Sousa et al., 2021). One unit of enzyme activity was
195 defined as the amount of enzyme required to release 1 μmol of p-nitrophenol per 1 minute
196 at 50 ºC and pH 4.8. Values of *β*-glucosidase activity were expressed in units per gram of
197 dry solid (U g^{-1}).
198 Protease activity was measured using azo casein (5 g L^{-1} in sodium acetate buffer
199 50 mM, pH 5.0) as substrate (Sousa et al., 2021). One unit of enzyme activity was defined

as the amount of enzyme required to release 1 μmol of azopeptides in 1 minute at 37 ºC and pH 5.0. Values of protease activity were expressed in units per gram of dry substrate (U g-1).

2.7. Nutritional parameters

Crude protein of solid residues was estimated using a defined factor of 6.25, after analysis of nitrogen content by the Kjeldahl method (Mariotti et al., 2008).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated after quantification of the organic constituents such as cellulose, hemicellulose, and Klason lignin by quantitative acid hydrolysis (QAH) in a two-stage acid treatment (Leite et al., 2016).

2.8. Statistical analysis

Differences in experiments among the same OC were analyzed using a one-way analysis of variance (ANOVA) at a significance level of 5%. All dependent variables were compared by a *post hoc* Tukey's Honestly Significant Difference (HSD) test or Least Significant Difference (LSD) *post hoc* test, using Statgraphics Plus 5.1 (Manusgistics, Inc., Rockville, MD).

3. Results and discussion

3.1. Enzymes produced by SSF

Compared to single cultures, the use of fungal consortia is an interesting approach for OC bioprocessing due to the production of different enzymes by different species with concomitant impacts on the obtention of other bioproducts and on the final composition of the bioprocessed OC. In this work, three filamentous fungi, *R. oryzae*, *A. ibericus,* and

A. niger, were selected to explore the effect of using different fungal consortia of two species in SSF of two different OC, SFC and RSC.

The enzyme activities produced by each fungal consortia using SFC and RSC as substrate are presented in **Figure 1**. A significantly improved performance of enzyme production by the consortium *R. oryzae* + *A. ibericus* was observed for almost all enzymes monitored except for xylanases in SFC. As regards xylanase, slight differences were observed between co-cultures but the consortia *A. niger* + *R. oryzae* produced the highest enzymatic activity (**Fig. 1B**). Maximum cellulase (**Fig. 1A**) produced by the consortium *R. oryzae* + *A. ibericus* was 1.32 and 1.90-fold higher than the obtained by *A. ibericus* + *A. niger* and *A. niger* + *R. oryzae* consortia, respectively. It is worth stressing that cellulase activities obtained by all fungi co-cultures were higher than previously reported with single species in SFC using identical SSF conditions (Sousa et al., 2021). *R. oryzae* + *A. ibericus* consortium stood out regarding *β*-glucosidase activity (**Fig. 1C**), producing nearly 265 U/g that is 3.49-fold and 3.39-fold higher than the one obtained using *A. ibericus* + *A. niger* and *A. niger* + *R. oryzae* consortia, respectively. These values are also higher than those previously reported for monocultures (Sousa et al., 2021). Similarly, protease production (**Fig. 1D**) followed the same pattern with a maximum activity obtained by the consortium *R. oryzae* + *A. ibericus*, corresponding to a 2-fold higher enzymatic production compared with the other co-cultures. These results contrast with reported findings for monocultures, where *A. niger* was globally the best producer of enzymes by SSF using SFC (Sousa et al., 2021), revealing that a microbial consortium of weaker enzyme producers in a specific substrate may allow obtaining similar results than the attained with monocultures.

The produced enzyme's activities by SSF using RSC as substrate were quite similar for all consortia. Maximum cellulase production (**Fig. 1A**), was nearly 140 U/g in

SSF of RSC by *R. oryzae* + *A. ibericus* consortium that is 1.32-fold higher ($p < 0.05$) than the produced by *A. ibericus* + *A. niger*. Also in RSC, it is worth noticing that cellulase activities obtained in SSF by all consortia were above the previously reported values obtained with pure cultures of each species in SFC and RSC (Sousa et al., 2021). Also, xylanase activities (**Fig. 1B**) obtained in the herein reported work were higher than the values previously reported, where *A. niger* was the best producer (Sousa et al., 2021). The fungi consortium, *A. ibericus* + *A. niger,* produced the maximum xylanase activity of 886 U/g but the other consortia were able to achieve comparable values. The activity of β-glucosidase (**Fig. 1C**) follows the same pattern as cellulase with *R. oryzae* + *A. ibericus* alongside *A. niger* + *R. oryzae* consortia being the highest producers ($p < 0.05$). *R. oryzae* was previously reported as the best β-glucosidase producer in SSF of RSC (Sousa et al., 2021). All co-cultures produced similar values of protease activity (**Fig. 1D**), which were about 4-fold higher than the reported values for monocultures of these species (Sousa et al., 2021).

Overall, the consortium *R. oryzae* + *A. ibericus* showed the highest carbohydrases and proteases production efficiency, using SFC or RSC as substrate. According to previous studies in the literature. These data highlight that in SSF, the behavior of fungal consortia, regarding enzyme production, cannot be predicted according to the behavior of single cultures.

The benefits of microbial consortia for the production of enzymes were reported by other authors that obtained higher enzymatic production by SSF using microbial co-cultures when compared to monocultures. (Oliveira Rodrigues et al., 2020) tested five fungal strains in SSF experiments using raw sugarcane bagasse and wheat bran (1:1). The authors reported maximum production of β-glucosidase of 171 U/g using a consortium of *A. niger* SCBM01, *Trametes versicolor* 561, and *Pleurotus ostreatus* PL06, which

corresponds to a 2-fold increase compared to the maximum obtained using monocultures. Cellulase production (CMCase), presented similar values of enzyme activity between microbial consortium (73.32 U/g) and monocultures (82.70 U/g). Maehara et al. (2018) also tested the effect of monocultures over co-cultures using steam-exploded sugarcane bagasse and wheat bran (1:1) as substrate for SSF. The co-cultivation of *Trichoderma reesei* with *A. niger* produced the highest amounts of *β*-glucosidase (64.5 U/g) and xylanase (539.7 U/g). The use of cottonseed as substrate for SSF was exploited by Grewal et al. (2019), using mono and co-cultures. The highest production of cellulase (CMCase) and *β*-glucosidase was 155.41 U/g and 29.73 U/g, respectively. Maximum values were achieved using a consortium of *A. niger* (NRRL 3122), *T. reesei* (NRRL 6156), and *Phanerochaete chrysosporium* (MTCC 787). Santos et al. (2019) studied the consortium of *A. niger* SCBM1 and *A. fumigatus* SCBM6 using sugarcane bagasse and wheat bran as substrate for SSF. The authors reported maximum xylanase and *β*-glucosidase activity of 375 U/g and 80 U/g, respectively.

Protease production by SSF using fungi consortia has been less studied and available data with monocultures showed lower activity values than the ones herein reported. Oberoi et al. (2012) reported a maximum protease activity of around 40 U/g using a consortium of *A. niger* and *T. reesei*, using kinnow waste and wheat bran in a ratio of 4:1. Castro and Sato (2013) reported maximum production of protease of 59.87 U/g by *A. oryzae* LBA01, using wheat bran as substrate for SSF; and Gupta et al. (2018) reported maximum protease production of 52.5 U/g by *A. niger*, using mahuca cake as substrate in SSF.

Experimental data regarding the production of lignocellulolytic and proteolytic enzymes obtained with this work are clearly above the average reported values in literature **Table 1**. The use of microbial consortia is a viable and clean alternative for the exploitation of lignocellulosic materials to produce enzymatic cocktails, without the

addition of external supplements. Microbial consortia exist within nature establishing symbiotic relations and, in this case, the results of the positive interactions are evident, resulting in an increased enzymatic production over monocultures.

3.2. Antioxidant potential of fermented extracts of oilseed cakes

Extracts of fermented SFC and RSC were analyzed to assess TPC and antioxidant properties (**Fig. 2**). Maximum extraction of TPC from SFC was observed in fermented extracts obtained from the consortium *R. oryzae* + *A. ibericus* (**Fig. 2A**), where TPC was 4.84 and 1.83-fold higher ($p < 0.05$) than the obtained with *A. ibericus* + *A. niger* and *A. niger* + *R. oryzae* consortia, respectively. Additionally, only *R. oryzae* + *A. ibericus* consortium was able to outpace the concentration of TPC obtained by the control. Previous work by Sousa et al. (2021) reported higher extraction of TPC from fermented OC by *R. oryzae* monoculture in comparison to *A. ibericus* and *A. niger*. This fact may explain the higher values of TPC as a result of SSF with *R. oryzae* + *A. ibericus* and *A. niger* + *R. oryzae* consortia compared to *A. ibericus* + *A. niger* consortium. Moreover, the action of lignocellulolytic enzymes is well described as a key factor influencing the extraction of phenolics from lignocellulosic fractions (Macedo et al., 2021; Martillanes et al., 2021; Sharma & Arora, 2015). The action of lignocellulolytic enzymes, namely β-glucosidase, on OC matrix allowed the release of water-soluble phenolic compounds. The highest production of this enzyme observed in *R. oryzae* + *A. ibericus* consortium led to a higher concentration of TPC. In fact, the enzymatic profile observed for β-glucosidase presents similarities to the TPC profile. Variations in these profiles may be attributed to the action of other enzymes. Extracts of fermented RSC using fungi consortia had similar concentrations of water-soluble phenolics. *A. niger* + *R. oryzae* consortium stood out,

showing the maximum concentration of TPC with statistically significant differences ($p < 0.05$) over control.

Figure 2B-E depicts the results of four different antioxidant assays used to assess the antioxidant potential of fermented SFC and RSC. Fermented extracts of SFC obtained by consortia *A. ibericus* + *A. niger* and *A. niger* + *R. oryzae* exhibited a highest scavenging potential of free radicals (**Fig. 2B**) over *R. oryzae* + *A. ibericus* consortium. However, these consortia were not able to outpace the scavenging potential of free radicals observed in the control. On the other hand, the scavenging potential of free radicals was successfully increased throughout SSF using RSC as substrate. An increased scavenging potential of 2.79-fold over control ($p < 0.05$) was observed for the three consortia.

Iron chelators may act as antioxidants by their action in scavenging reactive oxygen species (ROS) but also by reducing the quantity of available iron that undergo Fenton reactions, resulting in a decreased amount of generated hydroxyl radical (•OH) (Adjimani and Asare 2015). The highest ability to chelate iron of extracts of fermented SFC (**Fig. 2C**) was obtained by *R. oryzae* + *A. ibericus* consortium. This extract presented a chelation potential 2.78-fold higher than *A. ibericus* + *A. niger* consortium and 1.79-fold compared to *A. niger* + *R. oryzae* culture. Despite an increase of the chelation potential of nearly 36 % of *A. niger* + *R. oryzae* extracts over control, no statistically significant differences were observed. *A. ibericus* + *A. niger* consortium fermented extracts of RSC achieved maximum chelation ability, using this substrate. However, the chelation potential obtained by the consortia was between 2 and 3.2-fold lower than control. This fact may be explained by fungal interactions and consequent inhibitions. Previously, Sousa et al. (2021) reported the increase of chelation power of fermented extracts of RSC using monocultures. Additionally, extracts antioxidant potential may be related to antioxidants produced by the fungi itself. Consortia where *A. niger* was present

led to extracts with similar or higher chelation potential. The work of Sousa et al. (2021) reported a maximum chelation potential obtained by *A. niger* when compared to *R. oryzae* and *A. ibericus* monocultures.

Superoxide anion is one of the main ROS and it is very difficult to maintain this anion stable for a long period. It is related to several harmful biological processes such as protein denaturation and lipid peroxidation (Araki & Kitaoka, 2001). The scavenging of superoxide anion (**Fig. 2D**), exhibited by fermented extracts of SFC from *A. ibericus* + *A. niger* consortium achieved similar values as the control. Still, this consortium obtained a scavenging potential of 2.33 and 70-fold higher than *R. oryzae* + *A. ibericus* and *A. niger* + *R. oryzae* consortia, respectively. Extracts from *A. niger* + *R. oryzae* consortium had scavenging potential almost null. *A. ibericus* may be the key factor regarding this antioxidant activity maintaining a symbiotic relationship with the other fungi and explaining the low values obtained by *A. niger* + *R. oryzae* consortium. In previous work, Sousa et al. (2021) reported a higher scavenging potential of SFC extracts fermented by monocultures of *A. niger* and *A. ibericus* when compared to *R. oryzae* monocultures using the same substrate. The presence of the fungi *A. ibericus* in co-cultures using RSC as substrate led to a higher scavenging potential over *A. niger* + *R. oryzae* consortium. No statistically significant differences ($p < 0.05$) were observed between *R. oryzae* + *A. ibericus* and *A. ibericus* + *A. niger* consortia. These consortia were, respectively, responsible for an increase of nearly 27 and 21-fold over control. Although with a lower increase, *A. niger* + *R. oryzae* consortium was able to significantly increase the scavenging potential of superoxide anion by about 11-fold over control.

The reduction of oxidants is a path to minimizing their harmful effects related to oxidative stress. Additionally, the iron reduction from Fe^{3+} to a more stable form, Fe^{2+}, interrupts the oxidation chain. **Figure 2E** depicts the reduction power of SFC and RSC

fermented extracts. SSF using the consortium *R. oryzae* + *A. ibericus* resulted in a 17% increase of reduction potential over control and corresponds to 1.6 and 1.86-fold higher values than the obtained with *A. ibericus* + *A. niger* and *A. niger* + *R. oryzae* consortia. On the other hand, all extracts of fermented RSC by all consortia had similar reduction potential. SSF had a clear positive effect on this antioxidant activity using RSC as substrate, resulting in around 21, 24 and 27-fold enhancement over the control, for *R. oryzae* + *A. ibericus*, A. *ibericus* + *A. niger,* and *A. niger* + *R. oryzae* consortia, respectively.

Generally, it was proven the potential of SFC and RSC as substrate of SSF to produce bioactive compounds with antioxidant potential, using fungal consortia. Few studies have been found regarding the use of fungal consortia to produce extracts with proven *in vitro* antioxidant potential. Abd Razak et al. (2015) studied the interaction of *R. oligosporus* and *Monascus purpureus* to enhance the phenolic content and antioxidant potential of rice bran. The authors reported nearly a 5-fold increase of TPC and the reduction potential of fermented extracts. On the other hand, the authors did not observe statistically significant differences among results of scavenging potential of free radicals. Within the analysis of antioxidant potential assays, differences between each assay represent the specificity of each method. DPPH method measures the scavenging potential of free radicals while superoxide assay measures the extracts' capacity to scavenge the superoxide anion. ICA method is based on the chelation potential of extracts while FRAP measures their reducing ability. Different patterns between antioxidant assays were observed in this work and were depicted in **Figure 2**. Also, the TPC of extracts is not correlated with their antioxidant potential. It is known that fungal hydrolytic enzymes such as β-glucosidase and xylanase, may increase TPC and antioxidant potential (Bhanja et al., 2009). These enzymes act directly on the substrate

400 leading to an increase in the availability of free hydroxyl groups on phenolic structures.
401 This action results in increased content of free phenolic groups and consequently an
402 increase of the antioxidant potential of the substrate. Additionally, hydrolysis of phenolic
403 conjugates may release free phenolic compounds and low molecular weight molecules
404 with higher antioxidant potential (Sharma & Arora, 2015).

405 Chen et al. (2020) tested a microbial consortium of the fungus *Monascus anka* and
406 the gram-positive bacteria *Bacillus subtilis* for the enhancement of phenolic fractions
407 liberation from oats. The author reported a 23.7-fold increase of TPC after 12 days of
408 fermentation and a reduction of more than 50% of the IC_{50} value resulting in an increment
409 of antioxidant potential when compared to unfermented oats.

410 SSF with specifically fungal consortia is worth exploration due to the symbiotic
411 relations established by microorganisms. However, in some cases may happen some type
412 of inhibition between species. Sousa et al. (2021) reported a higher antioxidant potential
413 of monocultures fermented extracts using the same species as the ones present in these
414 consortia. *A. niger* monocultures led to extracts with a better overall response regarding
415 the different antioxidant assays while *R. oryzae* to the highest reported values of TPC.
416 Also, a higher chelation potential of fermented RSC with *A. niger* monoculture was
417 reported compared to the herein obtained values, but extracts obtained with fungal
418 consortia showed a reducing potential 2.2 and 1.3-fold higher than the maximum values
419 reported for monocultures.

420

421 *3.3. Nutritional parameters of fermented oilseed cakes*

422 Crude protein and fiber fractions including cellulose, hemicellulose, and lignin,
423 are important nutritional parameters in animal feed. **Table 2** shows crude protein and
424 fiber fractions of fermented SFC and RSC.

SFC and RSC are protein-rich substrates exhibiting a content of around 400 g/kg of protein per dry mass of solid. During SSF of SFC, fungal consortia did not significantly affect the amount of crude protein. On the other hand, RSC crude protein was significantly increased during SSF by the *Aspergillus* species consortia. The work of Sousa et al. (2021) reported a decrease of crude protein after SSF of SFC and RSC, using monocultures of the same species as in this work. The ability of fungi consortia to maintain or increase the crude protein of OC's shows that the interaction between species overcomes monoculture limitations. According to INRAE et al. (2020) feed tables, fermented SFC and RSC present crude protein values within the range recommended for animal feed, even exceeding some common SFC and RSC values. The fiber fractions of OC's were significantly affected during SSF. Unlike ruminants, in monogastric nutrition, dietary fiber is represented by carbohydrates present in plant cell walls that are indigestible by endogenous enzymes (McDonald et al., 2011). Particularly, cellulose and lignin fractions decrease feed digestibility as they are the least usable fractions of crude fiber and due to the production of lignocellulosic enzymes in SSF, a decrease in these fractions was expected.

Hemicellulose is a polysaccharide of the plant cell wall. It surrounds cellulose microfibrils and therefore, it comprises the exterior part of the lignocellulosic materials, which is more accessible to microorganisms. Compared to controls, there was a reduction in hemicellulose fractions of 23% in SSF (statistically significant by LSD Post Hoc test with $p < 0.05$) by *R. oryzae* and *A. ibericus* consortium, using SFC as substrate. The consortia *A. ibericus* and *A. niger* and *A. niger* and *R. oryzae* were able to respectively reduce hemicellulose by 21% and 17%, however without statistically significant differences. The effect of microbial fermentation in the decrease of hemicellulose was even higher when RSC was used as substrate. Maximum reduction was obtained by *A.*

niger and *R. oryzae* consortium achieving 56% of reduction, followed by *A. ibericus* and *A. niger* consortium with 49% reduction and, *R. oryzae* and *A. ibericus* with 42% (all reductions are statistically significant by LSD Post Hoc test with $p < 0.05$).

As regards cellulose content, microbial degradation through SSF unaltered or increased cellulose concentration. Consequently, the cellulose content of fermented OC was equal or slightly higher than the values on the control on a dry matter basis. These results are in consonance with enzymes activity where was observed higher enzymatic activity of xylanase in fermented extracts, compared to cellulase. Xylanase can break down hemicellulose and this enzyme is produced in larger quantities.

Lignin, the most recalcitrant component of lignocellulosic biomass, was not degraded throughout SSF. Therefore, the final concentration of this component significantly increased in the final fermented biomass on a dry matter basis in both SFC and RSC, due to the degradation of the other fibrous fractions by fungal enzymes during SSF. The presence of high lignified cell wall components can result in a decrease in the nutritive value of the fermented feed. However, a proportion of insoluble fiber is necessary for animal feeding to enhance gut health and intestinal passage rate. Overall, lignin concentrations in fermented OC were within the values observed in other feed ingredients such as cereal by-products according to INRAE et al. (2020).

All species used in this work are generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA), thus their use for the degradation of lignocellulosic materials does not entail any obstacle for the use of the fermented OC as animal feed.

4. Conclusions

Synergistic interactions between fungal species in SSF were demonstrated, resulting in an increased performance to produce enzyme rich extracts with an additional property that is their high antioxidant potential, with great interest for many applications such as feed, food, cosmetics, and pharmaceutical industries. Additionally, despite the action of lignocellulosic enzymes produced by fungal consortia was mainly reflected on hemicellulose reduction, fermented OC fibrous fractions are within the parameters used in the feed industry.

Conflict of Interest

The authors confirm that they have no conflicts of interest concerning the work described in this manuscript.

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REFERENCES

Abd Razak, D. L., Abd Rashid, N. Y., Jamaluddin, A., Sharifudin, S. A., & Long, K. (2015). Enhancement of phenolic acid content and antioxidant activity of rice bran fermented with Rhizopus oligosporus and Monascus purpureus. *Biocatalysis and Agricultural Biotechnology*, *4*(1), 33–38. https://doi.org/10.1016/j.bcab.2014.11.003

Adjimani, J. P., & Asare, P. (2015). Antioxidant and free radical scavenging activity of iron chelators. *Toxicology Reports*, *2*, 721–728. https://doi.org/10.1016/j.toxrep.2015.04.005

Alam, M. Z., Fakhru'l-Razi, A., Abd-Aziz, S., & Molla, A. H. (2003). Optimization of compatible mixed cultures for liquid state bioconversion of municipal wastewater sludge. *Water, Air, and Soil Pollution*, *149*(1–4), 113–126.

Ancuța, P., & Sonia, A. (2020). Oil Press-Cakes and Meals Valorization through Circular Economy Approaches: A Review. *Applied Sciences*, *10*(21), 7432. https://doi.org/10.3390/app10217432

Araki, T., & Kitaoka, H. (2001). The mechanism of reaction of ebselen with superoxide in aprotic solvents as examined by cyclic voltammetry and ESR. *Chemical and Pharmaceutical Bulletin*, *49*(5), 541–545. https://doi.org/10.1248/cpb.49.541

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bhanja, T., Kumari, A., & Banerjee, R. (2009). Enrichment of phenolics and free radical scavenging property of wheat koji prepared with two filamentous fungi. *Bioresource Technology*, *100*(11), 2861–2866. https://doi.org/10.1016/j.biortech.2008.12.055

Chen, G., Liu, Y., Zeng, J., Tian, X., Bei, Q., & Wu, Z. (2020). Enhancing three phenolic fractions of oats (Avena sativa L.) and their antioxidant activities by solid-state fermentation with Monascus anka and Bacillus subtilis. *Journal of Cereal Science*, *93*, 102940. https://doi.org/10.1016/j.jcs.2020.102940

Castro, R. J. S., & Sato, H. H. (2013). Synergistic effects of agroindustrial wastes on simultaneous production of protease and α-amylase under solid state fermentation using a simplex centroid mixture design. *Industrial Crops and Products*, *49*, 813–821. https://doi.org/10.1016/j.indcrop.2013.07.002

Oliveira Rodrigues, P., Gurgel, L. V. A., Pasquini, D., Badotti, F., Góes-Neto, A., & Baffi, M. A. (2020). Lignocellulose-degrading enzymes production by solid-state fermentation through fungal consortium among Ascomycetes and Basidiomycetes. *Renewable Energy*, *145*, 2683–2693. https://doi.org/10.1016/j.renene.2019.08.041

Santos, B. V., Rodrigues, P. O., Albuquerque, C. J. B., Pasquini, D., & Baffi, M. A. (2019). Use of an (hemi) cellulolytic enzymatic extract produced by Aspergilli species consortium in the saccharification of biomass sorghum. *Applied Biochemistry and Biotechnology*, *189*(1), 37–48. https://doi.org/10.1007/s12010-019-02991-6

Dulf, F. V., Vodnar, D. C., Dulf, E.-H., & Toşa, M. I. (2015). Total Phenolic Contents, Antioxidant Activities, and Lipid Fractions from Berry Pomaces Obtained by Solid-State Fermentation of Two Sambucus Species with Aspergillus niger. *Journal of Agricultural and Food Chemistry*, *63*(13), 3489–3500. https://doi.org/10.1021/acs.jafc.5b00520

Gangwar, M., Gautam, M. K., Sharma, A. K., Tripathi, Y. B., Goel, R. K., & Nath, G. (2014). Antioxidant capacity and radical scavenging effect of polyphenol rich

546 Mallotus philippenensis fruit extract on human erythrocytes: an in vitro study. *The*
547 *Scientific World Journal*, *2014*. https://doi.org/10.1155/2014/279451
548 Grewal, J., Tiwari, R., & Khare, S. K. (2019). Secretome Analysis and Bioprospecting
549 of Lignocellulolytic Fungal Consortium for Valorization of Waste Cottonseed
550 Cake by Hydrolase Production and Simultaneous Gossypol Degradation. *Waste*
551 *and Biomass Valorization*, 1–16. https://doi.org/10.1007/s12649-019-00620-1
552 Gupta, A., Sharma, A., Pathak, R., Kumar, A., & Sharma, S. (2018). Solid State
553 Fermentation of Non-Edible Oil Seed Cakes for Production of Proteases and
554 Cellulases and Degradation of Anti-Nutritional Factors. *J Food Biotechnol Res*,
555 *2*(1), 4.
556 Gupta, A., Sharma, R., Sharma, S., & Singh, B. (2018). Oilseed as potential functional
557 food Ingredient. *Trends & Prospects in Food Technology, Processing and*
558 *Preservation, 1st Ed.; Prodyut Kumar, P., Mahawar, MK, Abobatta, W., Panja, P.,*
559 *Eds*, 25–58.
560 INRAE, CIRAD, & AFZ. (2020). *Composition and nutritive values of feeds for cattle,*
561 *sheep, goats, pigs, poultry, rabbits, horses and salmonids*.
562 https://www.feedtables.com
563 Kotecka-Majchrzak, K., Sumara, A., Fornal, E., & Montowska, M. (2020). Oilseed
564 proteins–properties and application as a food ingredient. *Trends in Food Science &*
565 *Technology*. https://doi.org/10.1016/j.tifs.2020.10.004
566 Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K.,
567 Vadthanarat, S., & Lumyong, S. (2020). Cultivation of mushrooms and their
568 lignocellulolytic enzyme production through the utilization of agro-industrial
569 waste. *Molecules*, *25*(12), 2811. https://doi.org/10.3390/molecules25122811
570 Leite, P., Salgado, J. M., Venâncio, A., Domínguez, J. M., & Belo, I. (2016).
571 Ultrasounds pretreatment of olive pomace to improve xylanase and cellulase
572 production by solid-state fermentation. *Bioresource Technology*, *214*, 737–746.
573 https://doi.org/10.1016/j.biortech.2016.05.028
574 Macedo, G. A., Santana, Á. L., Crawford, L. M., Wang, S. C., Dias, F. F. G., & de
575 Moura Bell, J. M. L. N. (2021). Integrated microwave-and enzyme-assisted
576 extraction of phenolic compounds from olive pomace. *LWT*, *138*, 110621.
577 https://doi.org/10.1016/j.lwt.2020.110621
578 Maehara, L., Pereira, S. C., Silva, A. J., & Farinas, C. S. (2018). One-pot strategy for
579 on-site enzyme production, biomass hydrolysis, and ethanol production using the
580 whole solid-state fermentation medium of mixed filamentous fungi. *Biotechnology*
581 *Progress*, *34*(3), 671–680. https://doi.org/10.1002/btpr.2619
582 Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting nitrogen into protein—
583 beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition*,
584 *48*(2), 177–184. https://doi.org/10.1080/10408390701279749
585 Martillanes, S., Ayuso-Yuste, M. C., Bernalte, M. J., Gil, M. V., & Delgado-Adámez, J.
586 (2021). Cellulase-assisted extraction of phenolic compounds from rice bran (Oryza
587 sativa L.): process optimization and characterization. *Journal of Food*
588 *Measurement and Characterization*, 1–8.
589 McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A., &
590 Wilkinson, R. G. (2011). *Animal Nutrition. Essex*. Prentice Hall, Pearson.
591 Menon, V., & Rao, M. (2012). Trends in bioconversion of lignocellulose: biofuels,
592 platform chemicals & biorefinery concept. *Progress in Energy and Combustion*
593 *Science*, *38*(4), 522–550. https://doi.org/10.1016/j.pecs.2012.02.002
594 Oberoi, H. S., Babbar, N., Dhaliwal, S. S., Kaur, S., Vadlani, P. V, Bhargav, V. K., &
595 Patil, R. T. (2012). Enhanced oil recovery by pre-treatment of mustard seeds using

crude enzyme extract obtained from mixed-culture solid-state fermentation of kinnow (Citrus reticulata) waste and wheat bran. *Food and Bioprocess Technology*, *5*(2), 759–767.

OECD, Food, & of the United Nations, A. O. (2020). *OECD-FAO Agricultural Outlook 2020-2029*. https://doi.org/10.1787/1112c23b-en

Otles, S., Despoudi, S., Bucatariu, C., & Kartal, C. (2015). Food waste management, valorization, and sustainability in the food industry. In *Food waste recovery* (pp. 3–23). Elsevier. https://doi.org/10.1016/B978-0-12-800351-0.00001-8

Pandey, A., Soccol, C. R., & Mitchell, D. (2000). New developments in solid state fermentation: I-bioprocesses and products. *Process Biochemistry*, *35*(10), 1153–1169. https://doi.org/10.1016/S0032-9592(00)00152-7

Paul, S., & Dutta, A. (2018). Challenges and opportunities of lignocellulosic biomass for anaerobic digestion. *Resources, Conservation and Recycling*, *130*, 164–174. https://doi.org/10.1016/j.resconrec.2017.12.005

Şahin, S., & Elhussein, E. A. A. (2018). Assessment of sesame (Sesamum indicum L.) cake as a source of high-added value substances: from waste to health. *Phytochemistry Reviews*, *17*(4), 691–700. https://doi.org/10.1007/s11101-018-9554-4

Senanayake, C. M., Algama, C. H., Wimalasekara, R. L., Weerakoon, W., Jayathilaka, N., & Seneviratne, K. N. (2019). Improvement of oxidative stability and microbial shelf life of vanilla cake by coconut oil meal and sesame oil meal phenolic extracts. *Journal of Food Quality*, *2019*. https://doi.org/10.1155/2019/1263629

Sharma, R. K., & Arora, D. S. (2015). Fungal degradation of lignocellulosic residues: An aspect of improved nutritive quality. *Critical Reviews in Microbiology*, *41*(1), 52–60. https://doi.org/10.3109/1040841X.2013.791247

Singh, A., Bajar, S., Devi, A., & Pant, D. (2021). An overview on the recent developments in fungal cellulase production and their industrial applications. *Bioresource Technology Reports*, 100652. https://doi.org/10.1016/j.biteb.2021.100652

Sousa, D., Salgado, J. M., Cambra-López, M., Dias, A. C. P., & Belo, I. (2021). Degradation of lignocellulosic matrix of oilseed cakes by solid state fermentation: fungi screening for enzymes production and antioxidants release. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.11490

Teh, S.-S., & Bekhit, A. E.-D. A. (2015). Utilization of oilseed cakes for human nutrition and health benefits. In *Agricultural biomass based potential materials* (pp. 191–229). Springer. https://doi.org/10.1007/978-3-319-13847-3_10

Yousuf, A., Pirozzi, D., & Sannino, F. (2020). Fundamentals of lignocellulosic biomass. In *Lignocellulosic biomass to liquid biofuels* (pp. 1–15). Elsevier. https://doi.org/10.1016/B978-0-12-815936-1.00001-0

Żymańczyk-Duda, E., Szmigiel-Merena, B., & Brzezińska-Rodak, M. (2018). Natural antioxidants–properties and possible applications. *J Appl Biotechnol Bioeng*, *5*(4), 251–258. https://doi.org/10.15406/jabb.2018.05.00146

FIGURE CAPTIONS

Figure 1. Activity of extracellular enzymes, cellulases (**A**), xylanases (**B**), β-glucosidases (**C**), and proteases **(D)** during SSF of SFC ■ and RSC ■ using fungal consortia. Results represent the average of two independent experiments and error bars represent standard deviation. Bars with equal letters for each substrate are not statistically different (Tukey test; $P < 0.05$).

Figure 2. Total phenolic content (TPC) and antioxidant potential of aqueous extracts of controls (oilseed cake sterilized) and fermented SFC ■ and RSC ■ using fungal consortia. **(A)** TPC; (**B**) DPPH radical scavenging activity; (**C**) iron chelation ability; **(D)** superoxide radical scavenging activity; **(E)** reducing ability. Results represent the average of two independent experiments and error bars represent standard deviation. Bars with equal letters for each substrate are not statistically different (Tukey test; $P < 0.05$).

654 **TABLES**

Table 1. Maximum enzymatic activities obtained by SSF using fungal consortia

Consortia	Solid substrates	Enzyme	Activity (U g^{-1})	Reference
Aspergillus niger SCBM1 + Trametes versicolor 561 + Pleurotus ostreatus PL06	Raw sugarcane bagasse + raw wheat bran (1:1)	Carboxymethylcellulase (CMCase)	73.3	Oliveira Rodrigues et al. (2020)
		β-glucosidase	171.1	
Trichoderma reesei + A. niger	Steam-exploded sugarcane bagasse + wheat bran (1:1)	β-glucosidase	64.5	Maehara et al. (2018)
		Xylanase	539.7	
Aspergillus niger + Trichoderma reesei +Phanerochaete chrysosporium	Cottonseed cake	CMCase	155.4	Grewal et al. (2019)
		β-glucosidase	29.7	
Aspergillus niger SCBM1 + Aspergillus fumigatus SCBM6	Sugarcane bagasse + wheat bran (1:1)	Xylanase	375	Santos et al. (2019)
		β-glucosidase	80	
Aspergillus niger + T. reesei	Kinnow waste + Wheat bran (4:1)	Protease	40	Oberoi et al. (2012)
Maximum enzymatic activity values reported in this work				
R. oryzae + A. ibericus	SFC and RSC	Cellulase	135	This work
A. ibericus + A. niger	RSC	Xylanase	886	
R. oryzae + A. ibericus	SFC	β-glucosidase	265	
A. niger + R. oryzae	RSC	Protease	228	

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Table 2. Nutritional composition of sunflower cake and rapeseed cake. The mean difference of values with different letters is significantly different, following a One-way ANOVA with LSD Post Hoc Test, $p < 0.05$.

Sunflower cake				
Composition (g Kg $^{-1}$)	Control	*R. oryzae* + *A. ibericus*	*A. ibericus* + *A. niger*	*A. niger* + *R. oryzae*
Crude Protein	413 ± 4	387 ± 36	371 ± 14	414 ± 8
Cellulose	112 ± 8^{a}	114 ± 1^{a}	119 ± 9^{ab}	137 ± 10^{b}
Hemicellulose	90 ± 7^{b}	69 ± 8^{a}	71 ± 9^{ab}	75 ± 6^{ab}
Lignin	116 ± 5^{a}	172 ± 0^{c}	175 ± 17^{c}	147 ± 23^{b}
Rapeseed cake				
Composition (g Kg $^{-1}$)	Control	*R. oryzae* + *A. ibericus*	*A. ibericus* + *A. niger*	*A. niger* + *R. oryzae*
Crude Protein	420 ± 7^{a}	441 ± 14^{ab}	453 ± 10^{b}	444 ± 6^{ab}
Cellulose	104 ± 3^{a}	125 ± 3^{b}	118 ± 6^{b}	96 ± 5^{a}
Hemicellulose	93 ± 1^{c}	54 ± 3^{b}	47 ± 3^{ab}	41 ± 4^{a}
Lignin	149 ± 5^{a}	199 ± 0^{b}	213 ± 17^{b}	227 ± 23^{b}

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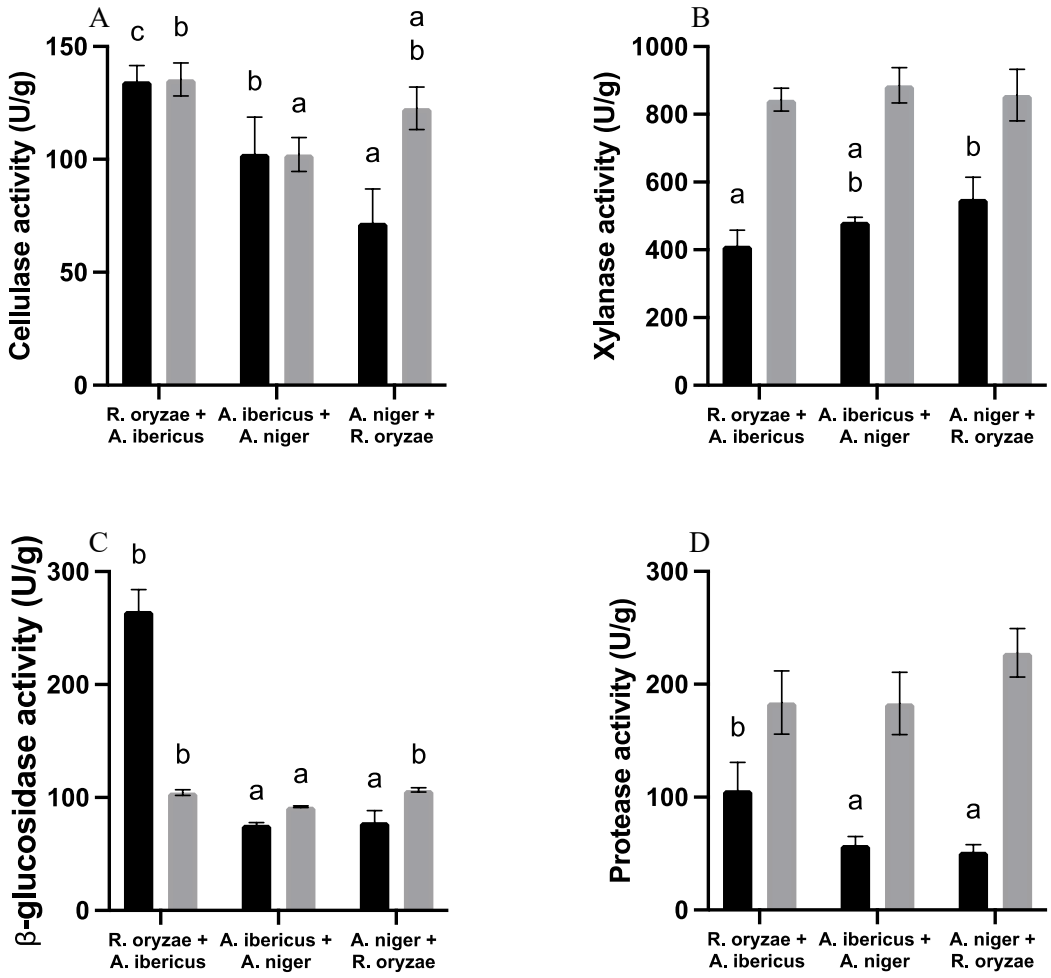
659 **FIGURES**

660 **Figure 1:**



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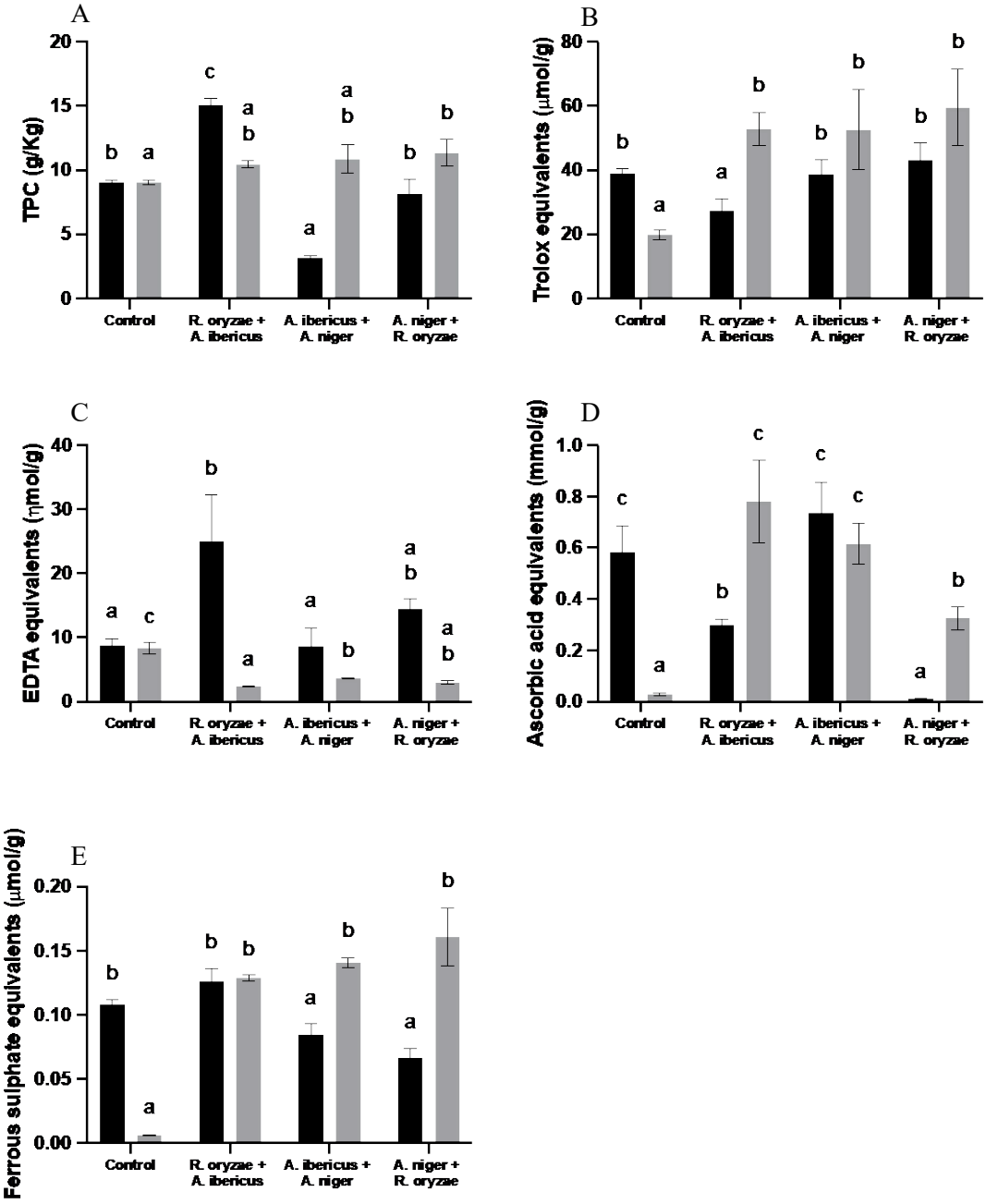
663 **Figure 2:**



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