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Original

Phenolic compounds and antioxidant activity in fermented rice *(Oryza sativa)* bran

Compostos fenólicos e atividade antioxidante em farelo de arroz (Oryza sativa) fermentado

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Abstract

This study investigated the content of total phenolic compounds and antioxidant activity in fermented rice bran in order to evaluate the effect of solid state fermentation on these properties. The process was performed with the fungus *Rhizopus oryzae* CTT 1217 in tray reactors at 30 °C for 120 hours. Samples of fermented rice bran were collected every 24 hours. Antioxidant property was evaluated by the diphenyl-1-picrylhydrazyl radical scavenging method and through the inhibition of enzymatic oxidation and lipid peroxidation of olive oil. The methanol extract of the biomass obtained at 96 hours of fermentation inactivated 50% of free radical in 15 minutes. The same extract reduced the peroxide value in the olive oil by 57% after 30 days of storage. The aqueous extract of the biomass obtained at 120 hours was the most efficient inhibitor of the darkening reaction catalyzed by peroxidase.

Keywords: *inhibition of oxidation; antioxidants; bioprocess.*

Resumo

Este trabalho investigou o conteúdo de compostos fenólicos totais e a atividade antioxidante em farelo de arroz fermentado, com o objetivo de avaliar o efeito da fermentação em estado sólido nessas propriedades. O processo foi realizado com o fungo *Rhizopus oryzae* CTT 1217 em reatores de bandeja a 30 °C por 120 horas. Amostras de farelo de arroz fermentado foram coletadas a cada 24 horas. A capacidade antioxidante foi avaliada pelo método do sequestro do radical livre difenil-1-picrilhidrazil e também por meio da inibição da oxidação enzimática e da peroxidação lipídica do óleo de oliva. O extrato metanólico produzido com a biomassa de 96 horas sequestrou 50% do radical livre em 15 minutos. O mesmo extrato reduziu o índice de peróxido no óleo em 57% após 30 dias de armazenamento. O extrato aquoso obtido da biomassa de 120 horas foi o inibidor mais eficiente da reação de escurecimento enzimático catalisada pela peroxidase.

Palavras-chave: *inibição da oxidação; antioxidantes; bioprocesso.*

1 Introduction

Antioxidants are molecules capable of reducing or preventing other molecules from oxidizing, while biological antioxidants may be defined as substances that, when in small concentrations compared to that of an oxidizable substrate, delay or prevent oxidation of that substrate (MOON; SHIBAMOTO, 2009). Due to the risks of consuming synthetic antioxidants, research studies into natural products containing antioxidant activity have increased with the aim of replacing them or applying associations that reduce the toxic effect (AUNGULU et al., 2007; MENDIOLA et al., 2010).

Among the several classes of naturally occurring antioxidant substances, phenolic compounds have drawn particular attention because they inhibit lipid peroxidation (KRISTINOVA et al., 2009) and lipo-oxygenation in vivo. That is mainly due to the reducing properties of the chemical structures that enable neutralization or sequestration of free radicals, as well as the chelation of transition metals, thus avoiding the phase of inhibiting the spread of oxidative processes. Vegetable tissues are good sources of these compounds, often containing simple phenols, phenolic acids (derived from benzoic and cinnamic acid), coumarin, flavonoids, stilbenes, condensed and hydrolysable tannings, lignins, and lignans (MOON; SHIBAMOTO, 2009; MELO; GUERRA, 2002).

Among the sources of phenolic compounds, rice (*Oryza sativa*) should be highlighted because it is one of the most produced and consumed cereals in the world and plays an important role in the diet-health relation containing distinct phenolic compounds, tocopherols, tocotrienols, and g-oryzanol mainly associated with the pericarp (IQBAL; BHANGER; ANWAR, 2005). However, grain polishing reduces the concentration of phenolic compounds in the endosperm, , which remain in the bran where they can be bounded to carbohydrates, fatty acids or proteins making the hydrolysis process important to obtain maximum yield of the phenolic acids (ZHOU et al., 2004; WOJDYŁO; OSZMAINSKI, 2007).

Solid state fermentation (SSF) is an alternative for the production of phenolic compounds and enhance antioxidant activity in foods. A 2008 study detected increased bioactive

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compound content in aged rice solid-state fermented with *Cordyceps sinensis* (ZHANG et al., 2008). The increased antioxidant potential of several raw materials by fermentative processes has also been widely studied since different hydrolytic enzymes can be produced directly from solid substrate and can, simultaneously, be used to release phenolic compounds (RANDHIR; VATTEM; SHETTY, 2004; LEE; HUNG; CHOU, 2008). In a study on the bioprocessing of beans to prepare koji using SSF with different food-grade filamentous fungi (in particular *Aspergillus* sp. and *Rhizopus* sp.), Lee, Hung and Chou (2008) found enhancement in the antioxidant properties of the beans, which could be related to the increase in the phenol and anthocyanin contents (LEE; HUNG; CHOU, 2008).

The purpose of this study was to evaluate the availability of compounds with antioxidant properties in solid state fermented rice bran with *Rhizopus oryzae* aiming at extracting and applying them as preservatives and/or using them as functional ingredients in food formulations.

2 Materials and methods

2.1 Substrate

The substrate used for fermentation was rice bran obtained from IRGA (Rio Grandense Rice Institute). Preparation of the bran consisted of standardizing its granulometry between 0.35 and 0.70 mm and keeping it at –10 °C until use.

2.2 Solid State Fermentation

Rhizopus oryzae CTT 1217 fungus was isolated from rice bran and identified in the Laboratory of Microbiology at the Food Processing Center of Passo Fundo University (UPF), RS, Brazil. Cultures were kept at 4 °C in potato-dextrose agar (PDA) medium. The spores were scraped from the slopes into Tween 80 (0.2%) aqueous emulsion. The same medium was used for spores' incubation for 7 days at 30 °C until new and complete fungi sporulation in the culture. Spore suspension for fermentation was achieved with the addition of 50 mL of Tween 80 (0.2%) aqueous emulsion to the fungus culture and spore release by scraping with a Drigalski handle. The spore concentration was estimated by enumeration in a Neubauer counting chamber.

Fermentation was carried out in tray bioreactors measuring $29 \times 17 \times 5.5$ cm^3. Rice bran substrate (100 g) was covered, sterilized, and placed on 1.5 cm-deep trays in the bioreactors

Prior to fermentation , 45 mL of saline solution (KH_2PO_4 2 g.L^{-1}, $MgSO_4$ 1 g.L^{-1}, NH_2CONH_2 1.8 g.L^{-1} in HCl 0.4 N) and spore suspension were added giving the medium an initial concentration of 4.0×10^6 spores.g^{-1} (BADIALE-FURLONG; CACCIAMANI; GARDA-BUFFON, 2007).

The medium was homogenized by adding sterile water for moisture adjustment to 50% taking into consideration the saline solution and spore suspension volumes already added. The trays were covered with sterile cotton cloth, which enabled aeration during incubation, and were kept in an incubator oven at 30 °C for 120 hours. For the characterization of antioxidant compounds, the samples were collected every 24 hours at the beginning of fermentation (zero time).

2.3 Antioxidant compound extraction and quantification

An aqueous extract with antioxidant compounds was obtained from 20 g of rice bran and 60 mL of methanol under stirring in a shaker for 1 hour. The extract was filtered through a paper filter and washed three times with 10 mL of hexane. The methanol extract was transferred to a flat-bottomed flask, and the solvent was evaporated in a rotary evaporator at 50 °C. The residue was dissolved with 70 mL water under agitation in an ultrasonic bath. The aqueous extract was clarified with barium hydroxide 0.1 M and zinc sulfate 5%, left at rest for 20 minutes, centrifuged at 2800 × g for 10 minutes, and filtered into a 100 mL flask (SOUZA et al., 2009). The same procedure was used with methanol extract, and the solvent was subsequently evaporated.

Aliquots of 0.3, 0.5, 0.8, and 1 mL of fermented and non-fermented bran extracts were arranged in a series of tubes completing the volume to 1 mL with water. Next, 4.5 mL of alkaline solution was added (Na_2CO_3 2%, $CuSO_4$ 2%, and sodium and potassium tartrate 4% to the proportion 100:1:1). The mixture was left to rest for 15 minutes in a water bath at 37 °C. A total of 0.5 mL of Folin-Ciocalteu reagent (diluted 1:2 with water) was added and it was left to rest for a further 15 minutes at 37 °C. The absorbance units (AU) were read using a spectrophotometer (VARIAN/CARY 100) at 750 nm. Total phenolic compounds were quantified by a ferulic acid standard curve with concentrations ranging from 1.7 to 12.2 mg.mL^{-1}.

2.4 Inactivation of radical 1, 1-diphenyl-picrilhidrazil (DPPH)

The DPPH free radical consumption by the biomass extracts was evaluated by the absorbance unit (AU) reduction in the spectrophotometer UV-Vis (VARIAN/CARY 100) at 515 nm using ferulic acid solution as the positive control (SOUSA et al., 2007).

The DPPH calibration curve was prepared from a methanolic solution with the concentration of 9.7×10^{-4} M, from which the dilutions 2.4×10^{-5}; 3.8×10^{-5}; 4.8×10^{-5}; 5.5×10^{-5} and 7.7×10^{-5} M were made and to which 3.0 mL of DPPH methanol solution (5.2×10^{-5} m), 0.9 mL of methanol and 0.1 mL of the studied antioxidants were added. The reactive mixture was kept at room temperature without light exposure, and the change of color from violet to yellow was measured after 15, 30, 45, and 60 minutes of reaction. The AU reductions were converted into percentage of antioxidant activity according to the following Equation 1 (HUANG; OU; PRIOR, 2005).

$$\%Inhibition = \frac{AU_{control} - AU_{sample}}{AU_{control}} \quad (1)$$

2.5 Enzymatic oxidation inhibition

Peroxidase enzyme was obtained from the solution of 20 g of pink tomato pulp (*Solanu tuberosum*) with 100 mL of phosphate buffer solution under stirring in a blender for 2 minutes,

followed by filtration. An aliquot of 10 mL of supernatant was added to 20 mL of acetone. The supernatant was discharged, and the precipitated protein was suspended with EDTA 0.25 M.

An enzymatic reaction occurred at 30 °C and pH 6.0 for 10 minutes in reactors with 1.5 mL of phosphate buffer solution of pH 6.0 added with 1.0 mL of potato enzymatic extract and hydrogen peroxide 0.08%.

The 1.0% guaiacol solution was used as the substrate of the reaction, which ranged from 0.1 mL to 0.5 mL. Aqueous extracts with phenolic compounds were added as potential inhibitors of the reaction, and in the control experiment, the phenolic extract was replaced with distilled water. The AU was measured with a spectrophotometer at 470 nm (VARIAN/CARY 100). Distinct substrate (guaiacol) concentrations were employed, and the results were plotted on a Lineweaver and Burk graph to estimate Km and Vmax in the presence and absence of an inhibitor.

2.6 Assessment of antioxidant activity in the lipid system

Six sets of reactors were prepared; each set was composed of ten 250 mL Erlenmeyer, each pair corresponding to a specific storage time of 0, 7, 14, 21, and 30 days. Five g of olive oil virgin was weighed with analytical precision (0.0000 g) in each Erlenmeyer flask.Fifty mL of methanolic solution was added to the first set (blank).

Fifty mL of a ferulic acid solution containing 10.5 $mg.mL^{-1}$ (0.11 $mg.g_{oil}^{-1}$) was added to the second set (Standard). Fifty mL of phenolic extract of defatted rice bran 80.7 $mg_{total\ phenols}mL^{-1}$ (0.81 $mg.g_{oil}^{-1}$) was added to the third set (NF). To the fourth, fifth, and sixth sets of experiments (FB1, FB2, and FB3) 50, 150, and 250 mL of phenolic extract from fermented rice bran (FB) for 96 hours with 170 $mg_{total\ phenols}.mL^{-1}$ (1.7; 5.1 and 8.5 $mg.g_{oil}^{-1}$) were added, respectively. After adding the reagents, the flasks were homogenized at 150 rpm in a horizontal shaker for 20 minutes. The peroxide values of each treatment were determined (ASSOCIATION…, 2000) prior to the experiment. The remaining reactors were sealed with cellophane paper and stored in direct sunlight for 3 hours and in indirect light for 7 hours. The peroxide values of the 2 flasks of each treatment were determined every 7 days up to 30 days. The mean value spent on titration was used for the peroxide value estimate, expressed as $meq_{peroxide}.Kg_{sample}^{-1}$.

2.7 Statistical analysis

Determinations were made in triplicate and the data evaluated through analysis of variance (ANOVA); the means were compared among each other by the Tukey test at a 5% significance level.

3 Results and discussion

3.1 Total phenolic compound contents

Total phenolic compound (TPC) contents in non-fermented (NF) rice bran during fermentation were expressed as $\mu g_{ferulic\ acid}.g_{bran\ db}^{-1}$, measured in time intervals, and in the aqueous and methanol extracts they were expressed as $mg_{ferulic\ acid}.mL_{bran\ extract}^{-1}$. These values are shown in Table 1. The TPC of non-fermented rice bran presented content of 480 $mg_{ferulic\ acid}.g_{bran}^{-1}$, and fermented rice bran prepared as substrate at an interval of 0 hours presented TPC content of 910 $mg_{ferulic\ acid}.g_{bran}^{-1}$. The increase in the total phenolic compound content can be explained by the ability of fungi to degrade lignocellulosic materials due to their highly efficient enzymatic system.

Fungi have two types of extracellular enzymatic system: the hydrolytic system, which produces hydrolases that are responsible for polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades and opens phenyl rings (SÁNCHEZ, 2009). During fermentation, several enzymes are produced, such as a-amylase, b-glycosidase and xylanase, directly in the substrate with a consequent release of phenols (BHANJA; KUMARI; BANERJEE, 2009) evidenced by the gradual increase observed for 72 hours. The decrease in the TPC contents after that period probably resulted from degradation, as shown by other authors (HEGDE et al., 2006).

Rice bran is an abundant source of phenolic compounds such as ferulic acid, which can be found in large amounts in insoluble form (62%) bound to polysaccharides with polysaccharides (ADOM; LIU, 2002; HEGDE et al., 2006). In a broad screening study with 320 samples of brown rice with different coloring degrees, TPC values were found between 290 and 5830 $mg_{eq.gallic\ acid}.g_{grain}^{-1}$ (GOFFMAN; BERGMAN, 2004).

Table 1. Total phenolic compounds in non-fermented bran and throughout fermentation.

Sample	Rice bran $\mu g_{ferulic\ acid}.g_{bran\ db}^{-1}$	Methanolic extract $\mu g_{ferulic\ acid}.mL^{-1}$	Aqueous extract $\mu g_{ferulic\ acid}.mL^{-1}$
NF	480.0 ± 20.0^{e}	80.7 ± 16.4^{a}	88.0 ± 6.6^{b}
FB 0 h	910.0 ± 84.0^{b}	97.0 ± 2.6^{a}	107.0 ± 8.9^{b}
FB 24 h	2200.0 ± 200.0^{d}	220.0 ± 2.0^{c}	260.0 ± 12.6^{d}
FB 48 h	1900.0 ± 140.0^{cd}	210.0 ± 13.3^{c}	220.0 ± 4.8^{c}
FB 72 h	1460.0 ± 50.0^{a}	170.0 ± 7.2^{b}	180.0 ± 2.0^{a}
FB 96 h	1540.0 ± 200.0^{ac}	170.0 ± 9.5^{b}	160.0 ± 10.5^{a}
FB 120 h	1200.0 ± 80.0^{ab}	140.0 ± 3.6^{d}	160.0 ± 2.0^{a}

- mean±SD; NF = non-fermented rice bran, FB = fermented rice bran for 0, 24, 48, 72, 96 and 120 hours; Similar letters in the same column imply means without statistical difference at 95% reliability.

3.2 Antioxidant activity of extracts

The results showed that FB 96 hours extract exhibited the highest antioxidant activity, followed by FB 48 hours to 120 hours extract. The lowest inhibitions were detected with NF and FB 0 hours. Table 2 presents TPC concentrations in methanol extracts and the DPPH percentage consumed in the distinct reaction times.

In the 15 minutes interval, FB 96 hours extract with 4.3 $mg_{phenols}.mL^{-1}$ significantly reduced the DPPH concentration with a 95% reliability level, while the others required intervals longer than 15 minutes to reach such an inhibition value. Ferulic acid (2.6 $mg_{phenols}$.mL) used as standard and FB 24 hours (5.5 $mg_{phenols}mL^{-1}$) extract significantly reduced the DPPH concentration in 15 minutes by 37% and 36%, respectively, despite the difference in the concentration of total phenols.

According to Singh et al. (2010), soybean products fermented by SSF with *Trichoderma harzianum* showed stronger antioxidant activity than unfermented products, which was probably related to the markedly higher contents of phenolic acids, flavonoids, and aglycone isoflavone with more free hydroxyl groups achieved during SSF.

In addition to increasing the antioxidant activity of certain foods, bioconversion of phenolic compounds by SSF may also cause other changes in food properties that influence human health. An example of this is the SSF of mung beans (also known as green beans) with *Rhizopus oligosporus*. This process has been demonstrated as being able to mobilize the conjugate forms of phenolic precursors naturally found in mung beans and improves their health-linked functionality. According to Randhir and Shetty (2007), SSF of mung beans significantly increased the phenolic content enhancing the antioxidant activity of the beans.

3.3 Inhibition of enzymatic oxidation

Figure 1 shows the inhibitory effect of aqueous phenolic extracts of NF and fermented brans under the darkening reaction of guaiacol in distinct concentrations catalyzed by enzymatic extract of peroxidase from potato. The oxidation inhibition by extracts is represented by the reduction of the AU at 470 nm.

Catalytic darkening was inhibited in all experiments with a 95% reliability level in relation to the reaction without any inhibitor, and FB 120 hours extract presented higher inhibition,

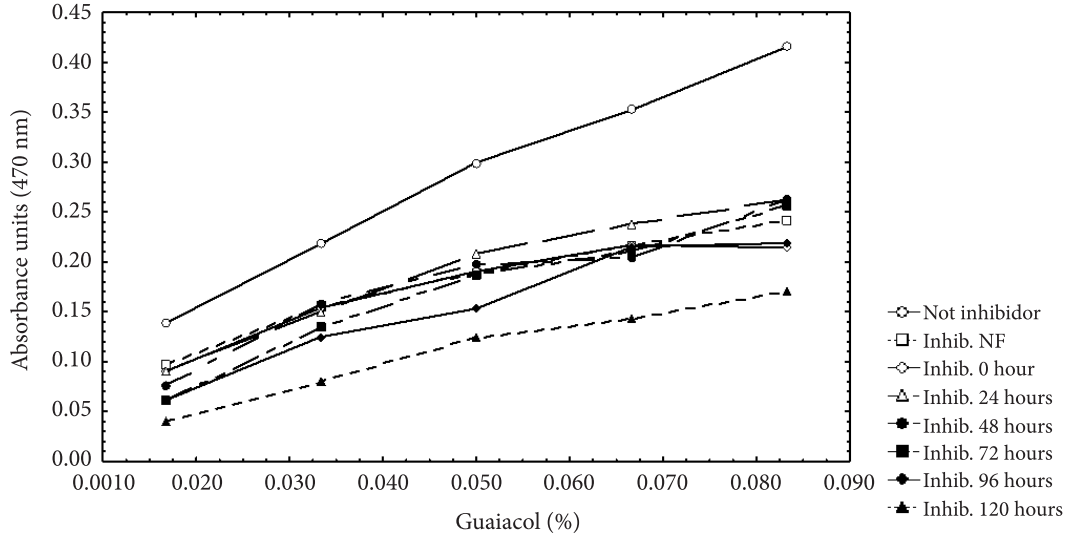

Figure 1. Enzymatic darkening in the presence of not fermented rice bran (NF) and fermented rice bran extracts.

Table 2. Total phenolic compounds and DPPH percentage consumed by the extracts.

Sample	μg_{phenol}/mL	DPPH consumed (%)			
		15min	30min	45min	60min
NF	2.0	29 ± 0.4[a]	32 ± 0.5[c]	34 ± 0.8[b]	35 ± 0.8[b]
FB 0 h	2.4	29 ± 0.4[a]	32 ± 1.7[c]	34 ± 0.3[b]	35 ± 0.4[b]
FB 24 h	5.5	36 ± 0.6[b]	44 ± 1.0[ab]	46 ± 1.3[a]	49 ± 0.9[a]
FB 48 h	5.3	47 ± 1.4[d]	54 ± 0.7 [d]	58 ± 0.8[d]	59 ± 3.1[d]
FB 72 h	4.3	42 ± 0.9[c]	42 ± 2.5[ab]	47 ± 2.2[a]	50 ± 0.3[a]
FB 96 h	4.3	50 ± 2.9[d]	59 ± 1.7[e]	64 ± 0.8[e]	66 ± 0.9[e]
FB 120 h	3.5	42 ± 1.6[c]	46 ± 1.9[b]	48 ± 1.5[a]	50 ± 0.5[a]
Ferulic acid	2.6	37 ± 3.1[b]	39 ± 0.4[a]	42 ± 1.5[c]	42 ± 0.8[c]

- mean±SE; NF = non-fermented rice bran, FB = fermented rice bran for 0, 24, 48, 72, 96 and 120 hours; Similar letters in the same column imply means without statistical difference at 95% reliability.

i.e., 59% of absorbance reduction in the reaction with 0.08% guaiacol solution.

Although other studies have demonstrated antioxidant activity of phenolic compounds from several sources, the mechanism of such activity is not always clarified (MARINOVA; YANISHLIEVA, 2003). In this study, the Michaelis-Menten relation was applied to the Lineweaver-Burk plots in order to identify the inhibition mechanism of guaiacol oxidation by peroxidase. Figure 2 shows the Lineweaver-Burk linearity relations in the presence and absence of extracts. The Km and Vmax estimated values are shown in Table 3.

The maximum reaction speed of enzymatic darkening decreased in the presence of extracts from 0 hours and 48 hours, followed by a km increase. That behavior suggests that at the beginning of fermentation, the extracts exhibit competitive type of inhibition characterized by increased Km and relative Vmax constancy. As fungal biomass increases, there is a tendency for non-competitive type inhibition characterized by increased Km and Vmax, especially after 72 hours of the process, suggesting that with ongoing fermentation other compounds are produced and irreversibly affect enzymatic activity.

3.4 Inhibition of lipid oxidation

Methanol extracts from fermented bran were employed in the lipid system to assess their antioxidant power by peroxide determination for 30 days. The olive oil used in the experiment had an average peroxide value of 5.8 $mEq_{peroxide}.kg_{sample}^{-1}$. The estimated results in $mEq_{peroxide}.kg_{sample}^{-1}$ of all experiments are shown.

According to the results obtained, it can be seen that the peroxide values were not changed in any treatment during 7 days of storage for treatments with FB 2 and 3, such values were maintained for 14 days. In the control experiment, the maximum peroxide content was found on the 21st day of storage, which did not occur in the other experiments. The treatment with FB 3 extract presented the highest inhibition of peroxide formation reducing its compound by 60% in 21 days and by 34% in 30 days.

Other authors who have used natural extracts with phenolic compounds of coriander and garlic in sunflower oil also reported an inhibition of lipid oxidation evidenced by reduced peroxide formation when submitted to storage at room temperature or under heat (IQBAL; BHANGER, 2007; ANGELO; JORGE,

Table 3. Km values and maximum speeds of darkening enzymatic reactions in the presence of phenolic compounds of fermented rice bran.

Reaction	Km (% guaiacol)	Vmax (AU/min)
Not Inhibitor	0.073	0.074
NF Inhibitor	0.049	0.038
0 h Inhibitor	0.053	0.038
24 h Inhibitor	0.081	0.053
48 h Inhibitor	0.127	0.068
72 h Inhibitor	0.592	0.228
96 h Inhibitor	0.208	0.083

AU = absorbance unit.

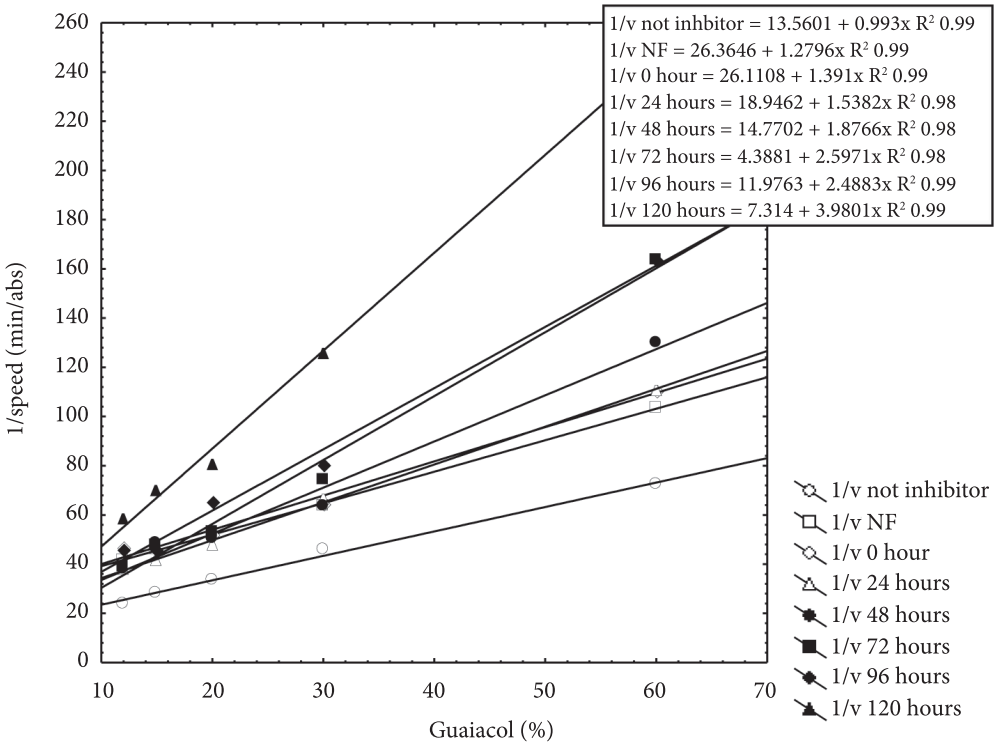

Figure 2. Lineweaver-Burk relation to peroxidase in presence and absence of phenolic extracts. NF- Not fermented rice bran.

2008). However, compounds obtained from biomass have not been reported.

Throughout the experiments, the control showed evidence of oxidation caused by light incidence in the lipid system, which was confirmed by a significant increase in the peroxide value.

All phenolic extracts showed a reduction in the peroxide formation with the lowest percentage observed on the 14th day, with a mean reduction of 59% among the extracts. The extract with the highest phenolic concentration (FB3) was the most efficient in the inhibition of peroxide formation presenting a reduction of 4.9%, 61.0%, 60.0%, and 34.3% in relation to the controls on the 7th, 14th, 21st, and 30th days, respectively.

4 Conclusion

Solid state fermentation was shown to be an effective method to intensify rice bran antioxidant properties. After evaluating other nutritional properties, fermented rice bran can be used for the preparation of different foods based on its functionality. This process could be the key for the availability of bioactive compounds. After 24 hours of fermentation, it was possible to increase phenols in the extracts by 58.6% from the biomass of *Rhizopus oryzae* (2200 $mg_{ferulic\ acid}.g_{bran}^{-1}$).

The rice bran phenolic extract fermented for 96 hours with 4.3 $mg_{ferulic\ acid}.mL^{-1}$ reduced the DPPH concentration by 50% in 15 minutes. Aqueous phenolic extract of rice bran fermented for 120 hours was the most efficient inhibitor in the darkening reaction catalyzed by peroxidase. Finally, rice bran phenolic extract fermented for 96 hours reduced peroxide development in olive oil by 34% after 30 days of storage.

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References

ADOM, K. K.; LIU, R. H. Antioxidant activity of grains. **Journal of Agricultural and Food Chemistry,** v. 50, n. 23, p. 6182-6187, 2002 PMid:12358499. http://dx.doi.org/10.1021/jf0205099

ANGELO, P. M.; JORGE, N. Avaliação do óleo de girassol adicionado de antioxidantes sob estocagem. **Ciência e Tecnologia de Alimentos,** v. 28, n. 2, p. 498-502, 2008. http://dx.doi.org/10.1590/S0101-20612008000200035

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis of AOAC International**. 14th ed. Washington: AOAC, 2000. p. 1141

AUNGULU, G. et al. Activity of gaseous phase steam distilled propolis extracts on peroxidation and hydrolysis of rice lipids. **Journal of Food Engineering**, v. 80, p. 850-858, 2007. http://dx.doi.org/10.1016/j.jfoodeng.2006.08.007

BADIALE-FURLONG, E.; CACCIAMANI, J. L. M.; GARDA-BUFFON, J. Fermentação fúngica: Enriquecimento protéico e degradação de micotoxinas em farelo de cereal contaminado com aflatoxina B1 e ocratoxina A. **Brazilian Journal of Food Technology**, v. 10, n. 4, p. 233-239, 2007.

BHANJA, T.; KUMARI, A.; ANERJEE, R. Enrichment of phenolics and free radical scavenging property of wheat koji prepared with two filamentous fungi. **Bioresource Technology**, v. 100, p. 2851-2866, 2009. PMid:19232489. http://dx.doi.org/10.1016/j.biortech.2008.12.055

GOFFMAN, F. D.; BERGMAN, C. J. Rice kernel phenolic content and its relationship with antiradical efficiency. **Journal of the Science of Food and Agriculture**, v. 84, n. 10, p. 1235-1240, 2004. http://dx.doi.org/10.1002/jsfa.1780

HEGDE, S. et al. Degradation of cereal bran polysaccharide-phenolic acid complexes by Aspergillus niger CFR 1105. **Food Chemistry**, v. 96, p. 14-19, 2006. http://dx.doi.org/10.1016/j.foodchem.2005.01.050

HUANG, D.; OU, B.; PRIOR, R. L. The chemistry behind antioxidant capacity assays. **Journal of Agricultural and Food Chemistry**, v. 53, p. 1841-1856, 2005. PMid:15769103. http://dx.doi.org/10.1021/jf030723c

IQBAL, S.; BHANGER, M. I. Stabilization of sunflower oil by garlic extract during accelerated storage. **Food Chemistry**,v. 2, p. 246-254, 2007. http://dx.doi.org/10.1016/j.foodchem.2005.09.049

IQBAL, S.; BHANGER, M. I.; ANWAR, F. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. **Food Chemistry**, v. 2, p. 265-272, 2005. http://dx.doi.org/10.1016/j.foodchem.2004.09.024

KRISTINOVA, V. et al. Antioxidant Activity of Phenolic Acids in Lipid Oxidation Catalyzed by Different Prooxidants. **Journal of Agricultural and Food Chemistry**, v. 57, p. 10377-10385, 2009. PMid:19817371. http://dx.doi.org/10.1021/jf901072t

LEE, I. H.; HUNG, Y. H.; CHOU, C. C. Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. **International Journal of Food Microbiology**, v. 121, p. 150-156, 2008. PMid:18031859. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.09.008

MARINOVA, E. M.; YANISHLIEVA, N. V. Antioxidant activity and mechanism of action of some phenolic acids at ambient and high temperatures. **Food Chemistry**, v. 81, p. 189-197, 2003. http://dx.doi.org/10.1016/S0308-8146(02)00411-9

MELO, E. A.; GUERRA, N. B. Ação antioxidante de compostos fenólicos atualmente presentes em alimentos. **Boletim Sociedade Brasileira de Ciência e Tecnologia-SBCTA**, v. 36, p. 1-11, 2002.

MENDIOLA, J. et al. Design of Natural Food Antioxidant Ingredients through a Chemometric Approach. **Journal of Agricultural and Food Chemistry**, v. 58, p. 787-792, 2010. PMid:20025217. http://dx.doi.org/10.1021/jf901951z

MOON, J. K.; SHIBAMOTO, T. Antioxidant assays for plant and food components. **Journal of Agricultural and Food Chemistry**, v. 57, p. 1655-1666, 2009. PMid:19182948. http://dx.doi.org/10.1021/jf803537k

RANDHIR, R.; VATTEM, D.; SHETTY, K. Solid-state bioconversion of fava bean by *Rhizopus oligosorus* for enrichment of phenolic antioxidants and L-DOPA. **Innovative Food Science and Emerging Technlogies**, v. 5, p. 235-244, 2004. http://dx.doi.org/10.1016/j.ifset.2004.01.003

RANDHIR, R.; SHETTY, K. Mung beans processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcer management**. Innovations Food Science and Emerging Technology**, v. 8, p. 197-204, 2007. http://dx.doi.org/10.1016/j.ifset.2006.10.003

SÁNCHEZ, C. Lignocellulosic residues: Biodegradation and bioconversion by fungi. **Biotechnology Advances**, v. 27,

p. 185-194, 2009. PMid:19100826. http://dx.doi.org/10.1016/j.biotechadv.2008.11.001

SINGH, H. B. et al. Solid-state cultivation of *Trichoderma harzianum* NBRI-1055 for modulating natural antioxidants in soybean seed matrix. **Bioresource Technology**, v. 101, p. 6444-6453, 2010 PMid:20363120. http://dx.doi.org/10.1016/j.biortech.2010.03.057

SOUSA, C. M., M. et al. Fenóis totais e atividade antioxidante de cinco plantas medicinais. **Quimica Nova**, v. 2, p. 351-355, 2007.

SOUZA, M. M. et al. Study on the extracting conditions of phenolic compounds from onion *(Allium cepa L.)*. **Revista Instituto Adolfo Lutz**, v. 68, n. 2, p. 192-200, 2009.

WOJDYŁO, A.; OSZMAINSKI, J. Comparison of the content phenolic acid, a-tochopherol and the antioxidant activity in oat naked and weeded. **Journal of Environmental, Agricultural and Food Chemistry**, v. 4, p.1980-1988, 2007.

ZHANG, Z. et al. Chemical composition and bioactivity changes in stale rice after fermentation with Cordyceps sinensis. **Journal of Bioscience and Bioengineering**, v. 106, n. 2, p. 188-193, 2008. PMid:18804063. http://dx.doi.org/10.1263/jbb.106.188

ZHOU, Z. et al. The distribution of phenolic acids in rice. **Food Chemistry**, v. 87, n. 3, p. 401-406, 2004 http://dx.doi.org/10.1016/j.foodchem.2003.12.015