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FULL LENGTH ARTICLE

Cosmeceutical potentials and bioactive compounds of rice bran fermented with single and mix culture of *Aspergillus oryzae* and *Rhizopus oryzae*

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Abstract In the present study, rice bran, one of the most abundant agricultural by-products in Malaysia, was fermented with single and mixed cultures of *Aspergillus oryzae* and *Rhizopus oryzae*. The fermented rice bran extracts were tested for their functional properties and compared to the non-fermented counterparts. Antioxidant activities as well as phenolics and organic acid contents were evaluated. Skincare-related functionalities were also tested by evaluating tyrosinase and elastase inhibition activities. Tyrosinase inhibition activity, measured to determine the anti-pigmentation effect of extracts, was found to be the highest in the extract of rice bran fermented with *A. oryzae* (56.18%) compared to other extracts. In determining the anti-aging effect of fermented rice bran extracts, the same extract showed the highest elastase inhibition activity with a value of 60.52%. Antioxidant activities were found to be highest in the mix-cultured rice bran extract. The results of phenolic and organic acid content were varied; the major phenolic acid detected was ferulic acid with a value of 43.19 μg/ml in the mix-cultured rice bran extract. On the other hand, citric acid was the major organic acid detected, with the highest content found in the same extract (214.6 mg/g). The results of this study suggest that the fermented rice bran extracts may have the potential to be further exploited as ingredients in cosmetics as well as in antioxidant-rich products.
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1. Introduction

Rice bran is a brown layer presented between rice grains and the outer husk of paddy, which is rich in proteins, oil and other nutrients. It is one of the major by-products of rice production and among the most abundant agricultural wastes in Malaysia. It has huge potential to be exploited as a substrate for

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production of value-added products using biotechnological tools such as fermentation and can be applied in the food, health and cosmetics industries (Pourali et al., 2010).

Solid-state fermentation (SSF) is a biotechnological process used as an alternative way to further improve the biological functionalities of compounds in many types of substrates such as agricultural by-products. SSF is reported to have some advantages over submerged fermentation (SmF) such as higher fermentation productivity, and the higher end-concentration of products, among others (Hölker et al., 2004). SSF may lead to higher yields and productivities or better product characteristics of valuable compounds such as enzymes, organic acids and many other bioactive compounds. It is widely known that biochemical changes that occur during fermentation can definitely affect the substrate's properties such as bioactivity and digestibility (Katina et al., 2007a). A study conducted by Lateef et al. (2008) found that SSF enhanced the nutritional qualities and antioxidant activities of different agro-solid wastes, such as palm kernel cake, cocoa pod husk and cassava peel.

Among the potential microorganisms that can be used in the fermentation of agricultural wastes, fungi have been known for their ability to produce enzymes that degrade the cell wall of plants and may improve the chemical composition and bioactivity of the substrates used. Fungi of the genus *Rhizopus* and *Aspergillus* have been long used in SSF to produce various types of products. These genera can produce highly digestible proteins without any toxic substance being generated within the controlled environment of fermentation process. *Aspergillus* sp. have long been used in the production of a traditional but highly nutritious tempeh and have also been reported to be used in the production of pectinases (Dartora et al., 2002). Production of aromatic volatile compounds using *Rhizopus* strains grown on agro-industrial wastes was studied by Christen et al. (2000).

A number of fermentation studies have been performed on rice bran throughout the years. Rice bran has been repeatedly used in bacterial fermentation for the production of lactic acid (Li et al., 2012; Gao et al., 2008; Tanaka et al., 2006; Watanabe et al., 2013). Rice bran has also been used as a substrate in the production of biomass (Oshoma and Ikenebomeh, 2005), and enzymes such as protease (Ali and Vidhale, 2013), cellulase (Rajesh et al., 2012) and amylase (Grover et al., 2013), as well as secondary metabolites such as griseofulvin (Saykhedkar and Singhal, 2004). Oliviera et al. (2011) reported changes in the lipid and phospholipid composition of rice bran after solid-state fermentation, while another study was conducted on the phenolic acid content and antioxidant activities of fermented rice bran (Schmidt et al., 2014), with both studies using *Rhizopus oryzae*. On the other hand, *Aspergillus oryzae* has been used in the production of protease through the fermentation of rice bran (Chutmanop et al., 2008). To date, and to the best of our knowledge, there is no report on the study of solid state fermentation of rice bran with a mixed culture of *A. oryzae* with *R. oryzae*.

The metabolic synergisms among fungi can be exploited through the use of mixed cultures in the solid-state fermentation process. Thus, this study was conducted with the objective to investigate and compare the potential bioactivities of rice bran fermented with two different starter organisms, *R. oryzae* and *A. oryzae*, in single and mixed cultures. In this study, the antioxidant potential, and organic acid and phenolic acid contents of fermented rice bran extracts were evaluated. Their skincare-related functionalities were also tested by evaluating tyrosinase and elastase inhibition activities. These functional properties of fermented rice bran were assessed with the aim to evaluate their potential for application in the cosmeceutical and functional food industries.

2. Materials and methods

2.1. Culture preparation and fermentation procedure

A. oryzae (strain F0017) and *R. oryzae* (strain F0013) from Collection of Functional Food Culture, MARDI, were maintained on potato dextrose agar media (PDA). A fungal culture at their active sporulating stage, which was 7-days old, was used in this study.

Rice bran (30 g) was weighed in 250 ml Erlenmeyer flasks and 35 ml of distilled water was added to the rice bran. The substrates were then subjected to autoclaving (120 °C for 20 min). Next, 1% of fungal spores (10^6 spores/ml), of both single and mixed cultures, were added into each flask, mixed well using a sterile spatula and incubated at 32 °C for 12 days. The samples were then harvested and dried at 50 °C for 24 h. Each experiment was performed in triplicate. Non-fermented rice bran was used as a control.

2.2. Determination of total phenolic content and antioxidant activities in fermented rice bran extracts

2.2.1. Sample extraction

For this, 1 g of fermented and unfermented samples was mixed with 10 ml of distilled water and boiled for 15 min. All samples were then centrifuged at 10,000 rpm for 15 min and the supernatant was filtered. The filtrates were then kept at −20 °C for further analysis.

2.2.2. Total phenolic content (TPC)

The Folin–Ciocalteu methodology was used to determine the total phenolic content in each sample. A 1 ml aliquot of the samples was allowed to react with 5 ml of Folin–Ciocalteu reagent and 4 ml of 7.5% sodium carbonate solution for 2 h at room temperature and in dark condition. Absorbance was measured at 765 nm using a spectrophotometer and the results were expressed as μg gallic acid equivalent (GAE)/gram sample.

2.2.3. Ferric Reducing Ability of Plasma (FRAP) assay

The FRAP assay was performed according to the Benzie and Strain (1996) method, with some modifications. Fresh FRAP working solution of this assay was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ (2,4,6-tripyridyl-s-triazine) solution and 2.5 ml $FeCl_3{\cdot}6H_2O$ solution, which was warmed at 37 °C prior to use. Then, 150 μl of sample aliquot was allowed to react with 2850 μl of FRAP solution for 30 min in the dark. Absorbance was measured at 593 nm using a spectrophotometer. A standard curve was prepared by using different concentrations of ascorbic acid ranging from 0 to 200 ppm. The change in the absorbance of FRAP solution at different concentrations of ascorbic acid over a period of 30 min was

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measured and plotted. The results were expressed as mg ascorbic acid equivalent (AAE)/gram sample.

2.2.4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Determination of radical scavenging activity of the samples was performed according to Thaipong et al. (2006), with some modifications. Here, a 150 µl aliquot of sample was allowed to react with 2850 µl of fresh DPPH working solution for 30 min in the dark. Percentage of scavenging activity was determined using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

2.3. Determination of bioactive compounds in fermented rice bran extracts via High Performance Liquid Chromatography (HPLC)

2.3.1. Sample extractions

The extraction of organic acids was carried out using 0.013 N H_2SO_4. Samples and solvents were mixed for 1 h in an incubator shaker, 150 rpm, at 30 °C. The mixture was centrifuged at 10,000 rpm for 20 min. The supernatant was then filtered through 0.20 µm cellulose acetate membrane. The extraction of phenolic acids was carried out according to the method reported by Iqbal et al. (2005). Briefly, 5 g of dry weight was extracted with 25 ml of 70% methanol for 2 h in an electrical shaker, at 30 °C. Further extraction was done twice with 20 ml of 80% methanol containing 0.15% HCl, under the same set of conditions. The addition of 0.15% HCl aimed to maximize the removal of more phenolic acids out of the tested extracts. The extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness with reduced pressure at 45 °C, by Rotary Evaporator (Buchi). Extracts were dissolved with 5 ml methanol and used for the analyses of phenolic acids.

2.3.2. Standards quantification

Standards with different concentrations were prepared and filtered using a 0.45 µm nylon membrane filter. Individual standards of organic acids, α-tocopherol, γ-oryzanol and phenolic acids were injected separately. The retention time of each individual standard was compared with the retention time of mixed standard solution for identification purposes. The calibration standard was prepared by injecting different concentrations of mixed standard solutions made by serial dilution. To determine organic acid, α-tocopherol, γ-oryzanol and phenolic acid contents in fermented rice bran, the mixed standard solution was analyzed together with samples. Quantifications of organic acids, α-tocopherol, γ-oryzanol and phenolic acids were done by generating calibration curves. All samples were analyzed in triplicates.

2.3.3. HPLC analysis

Organic and phenolic acids in samples were determined using a HPLC Alliance Separation Module (Waters 2695) equipped with a diode array detector (Waters 2996). The organic and phenolic acids in the sample test solution were separated by reversed phase chromatography and detected by absorbance before being quantified with external calibration graphs. The organic acids in the sample were separated on a 250 mm × 4.6 mm, Extrasil ODS 5 µm column based on the method by Nour et al. (2010). For the simultaneous detection, the detector was set at $\lambda = 210$ nm and $\lambda = 245$ nm. The determinations of organic acids were made in isocratic conditions at 30 °C, using a mobile phase made of 50 mM phosphate solution (6.8 g potassium dihydrogen phosphate dissolved in 900 ml water: pH value adjusted to pH 2.8 with sulfuric acid). The flow rate of the mobile phase was 0.7 ml/min. α-tocopherol and γ-oryzanol were determined using reversed phase (4.6 × 100 mm, 3.5 µm) column. The detector was set at $\lambda = 325$ nm.

On the other hand, for phenolic acids, aliquot of sample solution (10 µl) was separated using a reverse-phase analytical column (150 mm × 4.6 mm × Bridge C18, 3.5 µm, Waters) with the temperature controlled at 25 °C, according to the method by Robbins and Bean (2004) with some modifications. The mobile phase consisted of mobile phase A (0.1% formic acid) and mobile phase B (methanol), with a flow rate of 0.7 ml/min. Peak identification was performed by comparing the retention times and the UV spectra at 280 and 325 nm with authentic compounds. Calibration curves obtained by injecting known amounts of pure compounds as external standards were used to quantify the compounds.

2.4. Determination of enzymatic inhibition of fermented rice bran extracts

2.4.1. Tyrosinase inhibition activity

Tyrosinase inhibition activity was determined using the dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate, according to the method by Alam et al. (2011) with minor modifications. Here, 40 µl of mushroom tyrosinase (31 U/ml), 80 µl of 0.1 M phosphate buffer (pH 6.8) and 40 µl of the test sample solution were mixed (called sample solution with enzyme). Sample solutions without enzyme were also prepared by repeating all previous steps but with no extracts added. With no test sample solution added, blank solutions with and without enzyme were also prepared. We also prepared positive controls of 1 mg/ml kojic acid solutions, with and without enzyme. Here, 40 µl of 10 mM L-DOPA solution was added as the substrate into every sample and blank. These assay mixtures were incubated at 25 °C for 5 min. The amount of dopachrome produced in the reaction mixture was measured at 475 nm using the microplate reader (Versamax). Percentage of tyrosinase inhibition activity was calculated as follows:

$$\%\ \text{tyrosinase inhibition} = \frac{(A - B) - (C - D)}{(A - B)} \times 100$$

Note:

A = absorbance of blank solution with enzyme,
B = absorbance of blank solution without enzyme,
C = absorbance of sample solution with enzyme,
D = absorbance of sample solution without enzyme.

2.4.2. Elastase inhibition activity

The elastase inhibition activity was measured with EnzChek Elastase Assay Kit (Invitrogen Life Technologies Inc., USA)

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according to the manufacturer's recommendations. The elastase inhibition assay was done by mixing 50 μl of sample or positive control with 100 μl of porcine pancreatic elastase (0.5 U/ml). The mixture was then incubated at room temperature away from light for 15 min. About 50 μl of 25 μg/ml DQ™ elastin working solution was added into the mixture, which served as a substrate. The mixture was then incubated for 30 min at room temperature in the dark and measured at 505/515 nm (Ex/Em) using a fluorescent microplate reader. Percent inhibition of elastase activity was calculated as follows:

$$\% \text{ elastase inhibition} = \frac{(A - B) - (C - D)}{(A - B)} \times 100$$

Note:

A = absorbance of blank solution with enzyme,
B = absorbance of blank solution without enzyme,
C = absorbance of sample solution with enzyme,
D = absorbance of sample solution without enzyme.

2.5. Statistical analysis

Mean values and standard deviations were calculated from the data obtained from triplicate experiments. One-way Analysis of Variance (ANOVA) test was used to determine significant differences between variables using Minitab (Version 14) Statistical Software. Differences with a probability value of < 0.05 were considered significant. All data were reported as mean ± standard deviation (sd).

3. Results

3.1. Radical scavenging and antioxidant activities of water and methanol extracts of non-fermented and fermented rice bran

The antioxidant potential of all fermented samples, assayed by the FRAP method, was significantly increased ($p < 0.05$) compared to the non-fermented samples. As displayed in Fig. 1, the highest antioxidant activity was detected in mix-cultured rice bran with the value of 160.88 μg AAE/g sample and 98.35 μg AAE/g sample in water and methanol extracts, respectively. All fermented rice bran extracts displayed significant ($p < 0.05$) differences of antioxidant activity between water and methanol extracts, whereby water extracts showed higher antioxidant activity than methanol extracts. However, for non-fermented rice bran, the difference between each extract was not only insignificant ($p > 0.05$), but also the lowest activity of all samples with the value of 30.22 μg AAE/g sample and 30.93 μg AAE/g sample in water and methanol extracts, respectively.

Another antioxidant screening method that determines the proton radical scavenging action of antioxidant compounds, DPPH radical scavenging assay, showed contradictory results compared to those obtained in the FRAP assay. As displayed in Fig. 2, DPPH radical scavenging activity in all fermented samples was not significantly improved ($p > 0.05$) upon fermentation except for rice bran fermented with *R. oryzae*, which showed the highest radical scavenging activity of 93.41% and 90.26% in water and methanol extracts, respectively. The lowest radical scavenging activity was detected in non-fermented rice bran, with the value of 87.82% in water extract and 87.51% in methanol extract. However, these values were not significantly ($p > 0.05$) different from *A. oryzae*- and mix cultured-fermented rice bran.

3.2. Enzymatic inhibition activity of non-fermented and fermented rice bran extracts

Non-fermented and fermented rice bran extracts were tested for their skincare-related functionalities by evaluating tyrosinase and elastase inhibition activities. Tyrosinase inhibition activity, measured to determine the anti-pigmentation effect of extracts, was found to be significantly ($p < 0.05$) improved in all fermented samples, compared to non-fermented counterpart (Table 1). The highest tyrosinase inhibition activity was detected in rice bran fermented with *A. oryzae* extract with the value of 56.18%.

On the other hand, in determining the anti-aging effect of fermented rice bran extracts, elastase inhibition activity of both non-fermented and fermented rice bran extracts was evaluated. Similar to the results of tyrosinase inhibition activity, rice bran fermented with *A. oryzae* showed the highest elastase

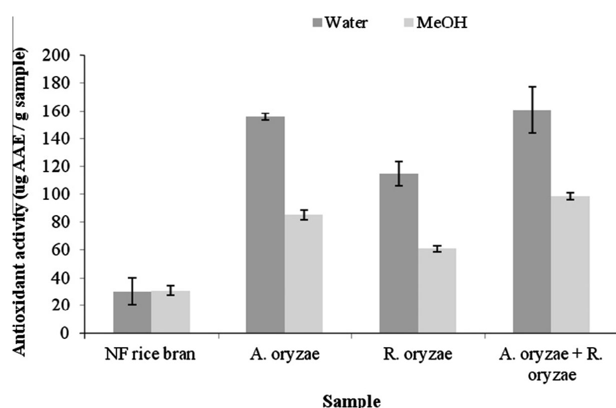

Figure 1 Antioxidant activity of water and methanol extracts from non-fermented rice bran and rice bran fermented with *A. oryzae*, *R. oryzae* and a mixed culture of *A. oryzae* and *R. oryzae*.

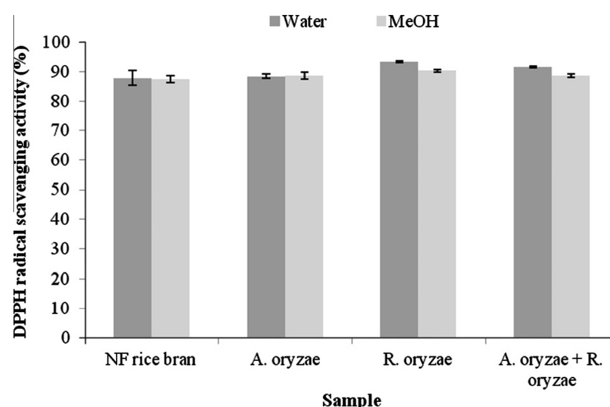

Figure 2 DPPH radical scavenging activity of water and methanol extracts from non-fermented rice bran and rice bran fermented with *A. oryzae*, *R. oryzae* and a mixed culture of *A. oryzae* and *R. oryzae*.

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Table 1 Enzymatic inhibition activity of non-fermented and fermented rice bran extracts.

Enzymatic inhibition activity (%)[a]	Extracts			
	Non-fermented	*A. oryzae*	*R. oryzae*	*A. oryzae* + *R. oryzae*
Tyrosinase	1.65 ± 0.57[d]	56.18 ± 1.12[a]	23.41 ± 0.31[b]	18.31 ± 2.44[c]
Elastase	9.15 ± 0.39[c]	60.52 ± 6.57[a]	11.87 ± 0.71[c]	32.87 ± 6.72[b]

[a] ANOVA analyses were performed using Minitab 14 Statistical Software. Each value is expressed as mean ± sd. The values in each row with the same letter are not significantly different at the level of 0.05 ($p > 0.05$). nd = not detected.

inhibition activity, with the value of 60.52%. However, not all fermented rice bran extracts showed a significant increase in elastase inhibition activity, whereby the *R. oryzae*-fermented rice bran extract showed non-significant ($p > 0.05$) improvement with only 11.87% compared to 9.15% in non-fermented rice bran extract.

3.3. Phenolic acid content of methanol extracts of fermented and non-fermented rice bran using HPLC method

To determine the changes of free phenolic acids in rice bran during fermentation, 8 types of phenolic acids (ferulic, ρ-coumaric, sinapic, vanillic, caffeic, syringic, protocatechuic and 4-hydroxybenzoic) were analyzed by HPLC. As shown in Table 2, the results of phenolic acid content in methanol extracts of fermented rice bran were varied. Methanol extracts of rice bran fermented with a single culture of *A. oryzae* showed the presence of 6 types of phenolic acids, with a significant ($p < 0.05$) increase of ferulic, caffeic and protocatechuic acids compared to non-fermented rice bran. ρ-Coumaric, sinapic and syringic acids were also present, but in amounts that were not significantly ($p > 0.05$) different from non-fermented rice bran. The extract of rice bran fermented with *R. oryzae* also showed the presence of 6 types of phenolic acids, with the exception of ferulic and ρ-coumaric acids. Interestingly, a high amount of ferulic acid (43.19 μg/ml) was detected in the extract of rice bran fermented with mixed cultures of *A. oryzae* and *R. oryzae,* but only 2 types of phenolic acids were detected in this extract and the other was sinapic acid. Vanillic, caffeic, protocatechuic and 4-hydroxybenzoic acids were not detected in non-fermented rice bran. Sinapic was the only phenolic acid detected in all samples and displayed substantial content increase in *R. oryzae*- and mix-cultured fermented rice bran. Interestingly, 4-hydroxybenzoic was only detected in rice bran fermented with the single culture of *R. oryzae* with the value of 10.57 μg/ml.

3.4. Organic acids content of water extracts of fermented and non-fermented rice bran using HPLC method

A total of 5 types of organic acid were evaluated in non-fermented and fermented rice bran samples, namely oxalic, citric, succinic, kojic and acetic acids. As shown in Table 3, all organic acids were detected in non-fermented and *A. oryzae*-fermented rice bran extracts, while 4 types of organic acids were detected in mix-cultured fermented rice bran with the exception of succinic acid. On the other hand, oxalic and acetic acids were detected in all samples. Citric acid showed the most substantial improvement in *A. oryzae*- and mix-cultured-fermented rice bran extracts, with more than 10-fold increase compared to non-fermented rice bran. All organic acids detected in fermented rice bran extracts showed significant ($p < 0.05$) increment compared to the non-fermented sample, with the exception of oxalic acid in *R. oryzae*-fermented rice bran extract.

4. Discussion

The results of phenolic and organic acids content in the present study can largely be explained by the fact that levels of bioactive compounds can be modified by the metabolic activity of microbes, enzyme hydrolysis and biochemical metabolism during fermentation. However, the enhancement of bioactive compounds content and antioxidant activities varied for each microorganism used. Also, as described by Hölker et al. (2004), metabolic synergisms among fungi during fermentation can be exploited during fermentation but results of investigations are usually difficult to interpret. As displayed in Table 2, vanillic, caffeic, protocatechuic and 4-hydroxybenzoic acids were not detected in non-fermented rice bran but were present in fermented rice bran, contrary to the report by Schmidt et al. (2014), which detected those compounds in non-fermented rice bran. The same author also reported to have detected the presence of ferulic and ρ-coumaric acids in *R. oryzae*-fermented rice bran, which were absent in our study. It is suggested that these contradictory results can be attributed to the differences in the substrate origins as well as experimental conditions, such as the variety of rice bran, fermentation conditions and extraction method. This suggestion is supported by Martins et al. (2011), who stated that the variation of results can be influenced by many factors and strongly depends on the type of substrate, the fungus used as well as the fermentation conditions.

Structural breakdown of cell walls induced by fermentation may occur, leading to the liberation and/or synthesis of various bioactive compounds (Katina et al., 2007b). For example, in the present study, the existence of ρ-coumaric acid content in non-fermented and *A. oryzae*-fermented rice bran extracts as well as caffeic acid in *A. oryzae*- and *R-oryzae*-fermented rice bran is suggested to be attributed to the fact that ferulic acid can be bio-transformed into smaller compounds. During fermentation, enzymes such as amylases, xylanases and proteases derived from both the substrates and fungi contribute to the modification of substrates' composition. Zheng and Shetty (2000) supported this suggestion, by stating that an improvement in phenolic acids content usually relates to the action of fungal enzymes such as β-glucosidase, α-amylase and laccase, along with other enzymes, which play essential roles in the immobilization of bioactive phenolic compounds during solid-state fermentation. On the other hand, Ju et al.

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Table 2 Phenolic acids content of non-fermented and fermented rice bran extracts.

Phenolic acids[a] (μg/ml)	Extracts			
	Non-fermented	*A. oryzae*	*R. oryzae*	*A. oryzae* + *R. oryzae*
Ferulic	1.88 ± 0.1[c]	10.73 ± 0.807[b]	nd	43.19 ± 4.95[a]
ρ-coumaric	7.33 ± 0.13[a]	5.83 ± 0.09[b]	nd	nd
Sinapic	2.52 ± 0.08[b]	3.02 ± 0.22[b]	10.31 ± 0.47[a]	10.49 ± 1.64[a]
Vanillic	nd	nd	16.69 ± 0.53[a]	nd
Caffeic	nd	2.91 ± 0.02[b]	4.35 ± 0.05[a]	nd
Syringic	6.05 ± 0.49[b]	7.36 ± 2.51[b]	10.19 ± 1.48[a]	nd
Protocatechuic	nd	17.44 ± 0.23[a]	16.47 ± 0.69[a]	nd
4-hydroxybenzoic	nd	nd	10.57 ± 0.21[a]	nd

[a] ANOVA analyses were performed using Minitab 14 Statistical Software. Each value is expressed as mean ± sd. The values in each row with the same letter are not significantly different at the level of 0.05 ($p > 0.05$). nd = not detected.

Table 3 Organic acids content of non-fermented and single and mix-cultured fermented rice bran extracts.

Organic acids[a] (mg/g)	Extracts			
	Non-fermented	*A. oryzae*	*R. oryzae*	*A. oryzae* + *R. oryzae*
Oxalic	2.73 ± 0.07[b]	7.60 ± 1.20[a]	2.86 ± 0.31[b]	8.07 ± 1.35[a]
Citric	17.14 ± 0.64[b]	209.61 ± 11.45[a]	nd	214.58 ± 12.10[a]
Succinic	1.21 ± 0.02[b]	24.14 ± 2.83[a]	nd	nd
Kojic	0.04 ± 0.01[b]	0.41 ± 0.08[a]	nd	0.34 ± 0.01[a]
Acetic	2.37 ± 0.06[c]	16.35 ± 0.79[b]	24.89 ± 26.42[a]	17.13 ± 0.82[b]

[a] ANOVA analyses were performed using Minitab 14 Statistical Software. Each value is expressed as mean ± sd. The values in each row with the same letter are not significantly different at the level of 0.05 ($p > 0.05$). nd = not detected.

(2009) argued that microbial oxidation, reduction or degradation of phenolic compounds by fermenting microbes contributes to the decreased phenolic acid content in fermented samples.

Generally, each organic acid has its own production pathway, but some organic acids share the same pathway (Saber et al., 2010). Fungi, in particular Aspergilli, are widely known for their potential to overproduce a variety of organic acids (Karaffa et al., 2001). With respect to the tested fungi, *A. oryzae* was found to be more effective than *R. oryzae* and the mixed culture in the total organic acid produced on rice bran. *Aspergillus* sp. are known to produce citric acid in high productivity and high yield by the fermentation of simple sugar (Tsao et al., 1999), thus explaining the high content of citric acid in single *A. oryzae*- and mix-cultured-fermented rice bran.

In our study, it was found that the antioxidant activity of fermented rice bran, as measured by FRAP assay, was not correlated (data not shown) with the radical scavenging activity of those samples. The contradictory results of both antioxidant-measuring experiments are suggested to have been caused by different mechanisms of both assays, which led to different observations of antioxidant activity, whereby FRAP measures the reducing ability of the extracts tested while the DPPH assay measures the scavenging ability of radical compounds. These findings were supported by Sun and Ho (2005), as well as by Aguilar-Garci et al. (2007), who stated that the FRAP and DPPH assay methods operate on different reaction mechanisms, accounting for the finding that they are not mutually equivalent. Also, according to Fukumoto and Mazza (2000), methods used for measuring antioxidant activity are extremely dependent on the conditions used and the substrates or products monitored in the assays; therefore, the same results should not be expected. The comparable DPPH radical scavenging activity in non-fermented and fermented rice bran extracts could be due to the high radical scavenging activity of bioactive compounds readily in rice bran, such as α-tocopherol and γ-oryzanol (Xu et al., 2001).

Phenolic compounds may act as antioxidants by several different mechanisms, including free radical scavenging, chelation of metal ions and the inhibition of pro-oxidant enzymes (Amorati et al., 2006). In plants, phenolics are usually found in conjugated forms through the hydroxyl group. It is suggested that the increase in antioxidant activity and phenolic content in fermented rice bran can be attributed to hydrolytic enzymes, such as β-glucosidase, which are produced by fungi. This was explained in a report by Bhanja et al. (2009) which stated that these enzymes act upon the substrate and increase the availability of free hydroxyl groups on the phenolic structure, thus increasing the content of free phenolics and subsequently the antioxidant activity of the substrate. As displayed in Table 2, only 2 types of phenolic acids were detected in mix-cultured rice bran, sinapic and ferulic acids, when compared to other extracts. Interestingly, as shown in Fig. 1, the antioxidant activity of all of the extracts tested was shown to be in the ascending order of non-fermented < *R. oryzae* < *A. oryzae* < mix-cultured rice bran for both water and MeOH extracts. Based on these results, due to the substantial amount of ferulic acid detected in mix-cultured rice bran extracts, it is possible to suggest that ferulic acid plays a major role in reducing ferric to ferrous in the FRAP assay as well as in scavenging the free radical compound in the DPPH assay. This suggestion is supported by a

report from Srinivasan et al. (2007), which indicates that ferulic acid is a potent antioxidant compound and a highly effective scavenger of free radicals. The substantial content of citric acid in the mix-cultured rice bran extract compared to other extracts tested (Table 3) may also be attributed to the high antioxidant activity of this extract. Citric acid is a well-known secondary antioxidant which functions as a synergist to primary antioxidants such as phenols and also oxygen scavengers. On the other hand, the overall antioxidant capacity in an extract is not necessarily indicated by the potent antioxidant compounds present (Leong and Shui, 2002). The extracts were complex mixtures of many compounds which can show different results by different methods of antioxidant assays. The concentration of compounds should also be taken into account, as it is well known that antioxidant compounds, such as phenolic acids, have concentration-dependent antioxidant or pro-oxidant activities (Yoshino and Murakami, 1998). However, the concentration-dependent activity of specific compounds only works in certain range of concentration. As demonstrated by Fukumoto and Mazza (2000), most phenolic compounds had pro-oxidant activity at low concentration. A study conducted by Maurya and Devasagayam (2010) has found that ferulic and caffeic acids start behaving as pro-oxidant above a specific concentration limit.

Elastase and tyrosinase are the two key enzymes that are well-known for being involved in skin deterioration. Elastase is an enzyme that is capable of degrading elastin, an extracellular protein that provides elasticity to the skin connective tissue. It has been reported that repetitive exposure to UV radiation from the sun accelerates the synthesis of elastase leading to a loss of skin elasticity and consequently the formation of skin wrinkles (Maity et al., 2011). Tyrosinase is a key enzyme for melanin biosynthesis in plants as well as in microorganisms and mammalian cells (Mayer, 1987). Kojic acid, among other well-known tyrosinase inhibitors such as hydroquinone and azelaic acid, has been reported and tested as a cosmetic and pharmaceutical ingredient to be used to prevent melanin overproduction in epidermal layers of the skin (Miyazawa et al., 2003). The inhibition of these enzymes is contributed by many types of compounds in the extracts tested and the synergism that exists between the compounds as well as their respective concentration. The highest inhibition of both elastase and tyrosinase was exhibited by the *A. oryzae*-cultured rice bran extract. Ferulic acid and syringic acid were detected in this extract, whereby these two compounds have been reported to have anti-elastase property and thus may have worked synergistically. Hydroxybenzoic acids such as syringic acid and hydroxycinnamic acids such as ferulic acid are well known to have anti-aging properties (Mukherjee et al., 2011). A study by Maity et al. (2011) revealed that syringic acid from the extract of *Tagetes erecta* showed comparable elastase inhibition activity to oleanolic acid, a compound that has been widely used as an anti-wrinkle ingredient in cosmetic formulations (Nishimori et al., 1997). According to a report from Miyazawa et al. (2003) on an extract from black rice bran, protocatechuic acid showed strong tyrosinase inhibition activity. Thus, the existence of kojic and protocatechuic acids in the fermented rice bran extracts tested may have contributed to its comparatively higher tyrosinase inhibition activity than non-fermented counterparts. Low tyrosinase inhibition activity of non-fermented rice bran extract was concomitant with a report from Manosroi et al. (2010) that showed low activity in rice bran extract, which was due to the low content of compounds having a specific configuration to inhibit the tyrosinase enzyme and also low contents of chelating agents.

5. Conclusions

Solid-state fermentation of rice bran with single and mixed culture of *A. oryzae* and *R. oryzae* improved the biological functionalities of rice bran. Antioxidant activities, phenolic and organic acids content as well as skin-related enzyme inhibition activities were improved upon fermentation. The results of this study suggest that fungal solid-state fermentation of rice bran may have the potential to be used as a tool and be exploited in producing different types of active metabolites that are useful for many types of food- and cosmetic-related industries.

Conflict of interest

The authors declare that there are no conflicts of interest.

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