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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). Oliveira 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 FeCl₃·6H₂O 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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