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COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITIES, COSMECEUTICAL PROPERTIES AND PHENOLIC ACIDS COMPOSITION OF FERMENTED RICE BRAN AND COCONUT TESTA

Dang Lelamurni Abd Razak*, Anisah Jamaluddin, Nur Yuhasliza Abd. Rashid, Shaiful Adzni Sharifudin, Kamariah Long

Biotechnology and Nanotechnology Research Center, Malaysian Agricultural Research and Development Institute, P.O Box 12301, General Post Office, 50774 Kuala Lumpur, Malaysia

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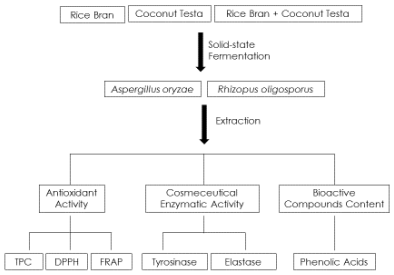
*Corresponding author
danglela@mardi.gov.my

Graphical abstract



Abstract

Malaysia produces large number of agro-industrial products annually including rice and coconut. Along with the production, rice bran and coconut testa are also abundantly generated as by-products. Solid state fermentation (SSF) is a simple biotechnological technique that has been employed for production of bioactive compounds. The use of SSF on agro-industrial by-products is economically viable and helps to reduce the environmental impact of the by-products disposal. In this present study, rice bran (RB), coconut testa (CT) and a combination of rice bran and coconut testa (RBCT) were fermented with single culture of *Aspergillus oryzae* and *Rhizopus oligosporus*. The effect of fungal SSF on the cosmeceutical-related activities and phenolic acid content of the fermented RB, CT and RBCT extracts was determined. The results showed that total phenolic content (TPC) as well as antioxidant activity measured using the ferric reducing ability of plasma (FRAP) of all substrates increased upon fermentation. Contrary to the TPC and FRAP assays results, the radical-scavenging activity of the fermented samples as measured by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, were varied. Tyrosinase inhibition activity was found to be the highest in the extract of RB fermented with *A. oryzae* (56.18%) compared to other extracts. The same extract showed the highest elastase inhibition activity with a value of 60.27%. High performance liquid chromatography (HPLC) analyses showed ferulic, sinapic, caffeic and protocatechuic acids were significantly increased in RB fermented with *A. oryzae*. The results of this study suggest that fungal solid-state fermentation of rice bran may have the potential to be exploited in producing different types of active metabolites that are useful for cosmeceutical industries.

Keywords: Coconut testa, rice bran, fungal fermentation, cosmeceutical, antioxidants.

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1.0 INTRODUCTION

Increased attention has been given to agricultural residues for the production of value-added compounds that might be beneficial for cosmeceutical purposes. Cosmeceutical products are cosmetic products that contain biologically active ingredients having beneficial physiological effects upon consumption. These effects are resulted from enhanced pharmacological actions, which are

lacking in inert cosmetic products [8]. With the increasing concern of awareness, consumers today are moving towards the use of cosmetics from natural resources, such as plants and mushrooms, with no unnecessary chemical ingredients. Substances contributing to the cosmeceutical multi-activities can be found in many natural raw materials, especially of plant origin. Phenolic compounds have been identified as having several cosmecetical-related biological activities such as antioxidant, anti-inflammation and antimicrobial.

Due to environment as well as legislation issues, food and agricultural industries are obliged to find alternative ways to re-utilise its biological wastes [13]. Application of solid-state fermentation (SSF) as inexpensive tool for the effective conversion of by-products to valuable products has existed for many years. Numerous studies have successfully proved the enhancement of nutritional qualities as well as production of many valuable compounds of agricultural by-products through SSF [4,17]. Filamentous fungi of the genus *Rhizopus* and *Aspergillus* have been long used in SSF to produce various types of products [2]. These genera can produce highly digestible proteins without any toxic substance being generated within the controlled environment of fermentation process.

Rice bran and coconut testa are the agricultural by-products from rice (*Oryza sativa*) and coconut (*Cocos nucifera*) processing industries, respectively. Traditionally, most rice bran production was used in the production of fertilizers and as animal feed ingredients [16]. On the other hand, coconut testa is the brown layer at the outmost of the seed, obtained from coconut processing industries. Currently, it is used as ingredient in animal feed. The utilization of both rice bran and coconut testa has been very limited despite their potential as a good source of bioactive compounds. This present study was conducted with the aims to investigate and compare the cosmeceutical potentials of rice bran, coconut testa and a mixture of rice bran and coconut testa fermented with two types of fungi namely, *Aspergillus oryzae* and *Rhizopus oligosporus*.

2.0 METHODOLOGY

2.1 Culture Preparation and Fermentation Procedure

Fungal culture of *A. oryzae* (strain F0017) and *R. oligosporus* (strain F0019) from Collection of Functional Food Culture, MARDI was used in this study. 30 g of rice bran (RB), coconut testa (CT) and a mixture of rice bran and coconut testa (RBCT) (1:1) were weighed in Erlenmeyer flasks and sterilized. 1% of fungal spores (10^6 spores/ml) and 35 ml sterilized distilled water were added and mixed well. Fermentation process was carried out for 12 days at 32°C. Non-fermented RB, CT and RBCT were used as control.

2.2 Determination of Total Phenolic Content and Antioxidant Activities in Fermented RB, CT and RBCT Extracts

2.2.1 Sample Extraction

1 g of each sample 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. The supernatant was filtered and stored until use.

2.2.2 Total Phenolic Content (TPC)

Determination of the total phenolic content in each sample was done according to the Folin-Ciocalteu methodology. 1 ml aliquot of the samples was mixed with 5 ml of Folin-Ciocalteu reagent and 4 ml of 7.5% sodium carbonate solution. The mixture was allowed to react for 2 h in the dark at room temperature. Absorbance was measured at 765 nm using a spectrophotometer and the results were expressed as mg 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 method [3], with some modifications. 150 µl of sample aliquot was mixed with 2850 µl of FRAP solution. The mixture was allowed to react in the dark. After 30 min, absorbance was measured at 593 nm using spectrophotometer. The results were expressed as µg ascorbic acid equivalent (AAE)/gram sample.

2.2.4 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

Radical scavenging activity of the samples was determined according to Thaipong *et al.* [19], with some modifications. 150 µl aliquot of sample was mixed with 2850 µl of fresh DPPH working solution. The mixture was allowed to react in the dark for 30 min. Absorbance was measured at 515nm. Percentage of scavenging activity was determined using the following equation:

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

2.3 Determination of Phenolic Acids Composition in Fermented RB, CT and RBCT Extracts via High Performance Liquid Chromatography (HPLC)

Filtered RB, CT and RBCT extracts were injected for determination of phenolic acid composition according to the method by Robbins and Bean [12] with some modifications. HPLC analyses were carried out using an Alliance Separation Module (Waters 2695) equipped with a photo-diode array detector (Waters 2996) with a reversed-phase analytical column (150 mm x 4.6 mm x Bridge C18, 3.5 µm, Waters). To separate the compounds, the mobile

phase used was 0.1% formic acid (A) and methanol (B) with the flow rate set at 0.7 mL/min. Peak identification was performed by comparing the retention times and the spectral data at 270 and 325 nm to the standard compounds. Quantification of phenolic acids was performed using the calibration curves obtained by injecting known amounts of standard compounds.

2.4 Determination of Enzymatic Inhibition of Fermented RB, CT and RBCT Extracts

2.4.1 Tyrosinase Inhibition Activity

The dopachrome method [1] with some modifications was performed to determine the tyrosinase inhibition activity of fermented RB, CT and RBCT extract using L-3, 4-dihydroxyphenylalanine (L-DOPA) as the substrate. 40 µl of mushroom tyrosinase (31 U/ml), 80 µl of 0.1 M phosphate buffer (pH 6.8) and 40 µl of sample solution were mixed. Sample and blank solutions with and without enzyme were also prepared. 40 µl of 10 mM L-DOPA solution was added as the substrate into every sample and blank. The final mixtures were allowed to react at 25°C in the dark for 5 min. The quantity of dopachrome produced in the reaction mixture was measured at 475 nm using the microplate reader (Versamax).

2.4.2 Elastase Inhibition Activity

Elastase Assay Kit (EnzChek) was used in determination of elastase inhibition activity of fermented RB, CT and RBCT extracts. The assay was performed by mixing 50 µl of sample or positive control with 100 µl of porcine pancreatic elastase (0.5 U/ml) and then incubated in the dark at room temperature. After 15 min, 50 µl of 25 µg/ml DQ™ elastin working solution was added into the mixture and allowed to react for 30 min in the dark at room temperature. Absorbance was measured at 505/515 nm (Ex/Em) using a fluorescent microplate reader.

Inhibition of tyrosinase and elastase activities was calculated using the following equation:

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

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

2.5 Statistical Analysis

Mean values and standard deviations were calculated from the data obtained from triplicate experiments. In determining the significance of the data obtained, One-way Analysis of Variance (ANOVA) test was conducted. Minitab Statistical Software (Version 14) was used for the statistical analysis. Differences with a probability value of < 0.05 were considered to be statistically significant. All data were presented as mean ± standard deviation (SD).

3.0 RESULTS AND DISCUSSION

3.1 Total Phenolic Content and Antioxidant Activities

As shown in Table 1, fermentation using *A. oryzae* and *R. oligosporus* has increased the TPC of all substrates, compared to the non-fermented counterparts. The highest increment of TPC was found in the extracts of RB fermented with both fungi with 5-fold increase compared to the non-fermented counterpart.

Table 1 Total phenolic content of non-fermented and fermented RB, CT and RBCT extracts

Extracts	Total Phenolic Content (mg GAE / g sample)		
	Non-fermented	*A. oryzae*	*R. oligosporus*
RB	1.66±0.61^{d}	7.96±0.18^{a}	7.22±0.28^{a}
CT	0.78±0.12^{d}	1.83±0.14^{cd}	2.41±0.03^{c}
RBCT	1.20±0.32^{d}	4.66±0.24^{b}	4.29±0.27^{b}

[1]Each value is expressed as mean±sd. The values with the same letter are not significantly different at the level of 0.05 (p>0.05). nd = not detected.

FRAP assay measures the reducing potential of antioxidant compounds to react with a ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to produce a colored Fe^{2+}-TPTZ form at 593nm. The reducing antioxidant activity of all samples is shown in Figure 1. Antioxidant activity was the highest in non-fermented and fermented RB extracts, followed by RBCT and CT. According to Oliviera *et al.* [9], fermentation with *R. oryzae* increased the total phenolic content and antioxidant activity of rice bran. Apart from reducing power, other important mechanism of anti-oxidation is scavenging of hydrogen radicals. In this study, DPPH was used to determine the free radical scavenging activity of samples. As shown in Figure 2, it was observed that the radical scavenging activity of RB and RBCT extracts showed no significant differences between the fermented and non-fermented substrates. On the other hand, the scavenging activities of CT extracts showed improvement in fermented CT compared to non-fermented CT.

As reported by Lizcano *et al*. [5], FRAP and DPPH assays employed different mechanisms of reaction that involve different types of radicals. This explained the inconsistency of the antioxidant activity of the tested extracts as both assays determine different aspects of the antioxidant capacity. On the other hand, the Folin-Ciocalteu reagent assay has poor specificity, where the assay is reported to have reaction to other substance such as ascorbic acid

and thiols [15] that could be oxidized by the Folin reagent [18]. Therefore, this assay is not specific to only polyphenolic compounds. It is therefore suggested that both phenolic and non-phenolic compounds are responsible for the radical scavenging activity of the tested extracts.

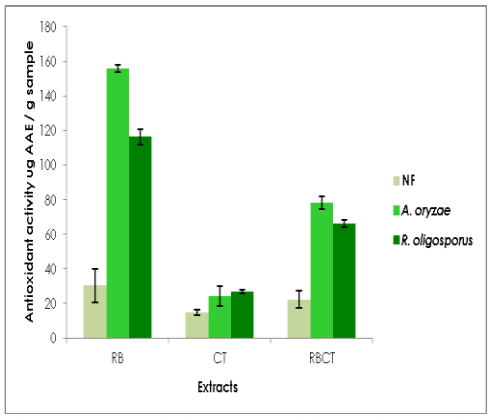

Figure 1 Antioxidant activity of non-fermented and fermented RB, CT and RBCT extracts

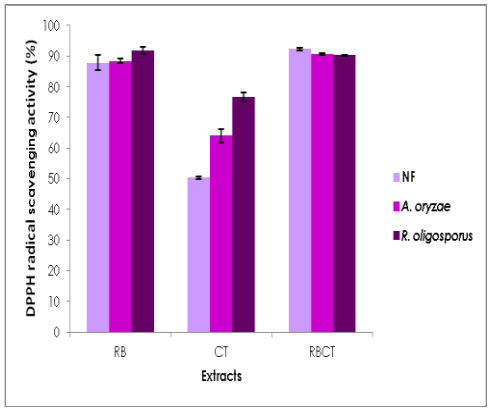

Figure 2 DPPH radical scavenging activity of non-fermented and fermented RB, CT and RBCT

3.2 Enzymatic Inhibition Activities

Tyrosinase and elastase are known to be the major enzymes responsible for skin pigmentation and wrinkle formation, respectively. The inhibitory effects of non-fermented and fermented RB, CT and RBCT extracts on these two enzymes are shown in Table 2 and 3. The most common approach for discovery of the skin anti-pigmentation or whitening agent is by determination of tyrosinase inhibitory property, due to the involvement of tyrosinase as the key enzyme in melanogenesis [11]. The inhibition of tyrosinase activities was significantly different ($p<0.05$) among non-fermented and fermented RB. The highest tyrosinase inhibition activity was found in the extract of RB fermented with *A. oryzae*. Contrary to RB, tyrosinase inhibition activity in RBCT decreased after fermentation with both fungi, while the extract of CT fermented with *R. oligosporus* did not show any activity. Low tyrosinase inhibition of non-fermented RB was consistent with a research conducted by Manosroi *et al*. [6]

Table 2 Tyrosinase inhibition activity of non-fermented and fermented RB, CT and RBCT extracts

Extracts	Tyrosinase Inhibition Activity (%)		
	Non-fermented	*A. oryzae*	*R. oligosporus*
RB	1.69±0.79^{c}	56.18±1.59^{a}	26.22±7.15^{b}
CT	5.56±1.61^{c}	12.39±2.72^{c}	nd
RBCT	13.73±0.85^{c}	12.75±9.36^{c}	9.31±2.94^{c}

[1]Each value is expressed as mean±sd. The values with the same letter are not significantly different at the level of 0.05 ($p>0.05$). nd = not detected.

Elastin is a component of the elastic fibers of the skin dermis [14] that can be degraded by an enzyme called elastase which belongs to the class of proteases. Increased degradation of elastin resulted in a loss of skin elasticity and formation of skin wrinkles. Results presented in Table 3 demonstrate that RB fermented with *A. oryzae* was the most significantly ($p<0.05$) effective against elastase with 7-fold higher inhibition activity than the non-fermented counterpart. All RB extracts demonstrated inhibition activity against both tyrosinase and elastase enzymes. It can be considered therefore that the fermented extracts of RB may have a good potential for applications in development of cosmeceuticals, particularly as whitening and anti-aging agent.

Table 3 Elastase inhibition activity of non-fermented and fermented RB, CT and RBCT extracts

Extracts	Elastase Inhibition Activity (%)		
	Non-fermented	*A. oryzae*	*R. oligosporus*
RB	9.16±0.54^{b}	60.27±9.27^{a}	12.56±0.01^{b}
CT	Nd	15.83±0.07^{b}	nd
RBCT	Nd	nd	nd

[1]Each value is expressed as mean±sd. The values with the same letter are not significantly different at the level of 0.05 ($p>0.05$). nd = not detected.

3.3 Phenolic Acids Composition

Fungi produce different types of enzymes during fermentation such as glycosidase and cellulase which hydrolyses bound-form phenolic compounds resulting in increased concentration of free phenolics. However, upon fermentation, the degradation of phenolic compounds also takes place, causing the loss of phenolic compounds [10]. In this present study, seven components of free phenolic acids were analysed in the non-fermented and fermented RB, CT and RBCT extracts. As displayed in Table 4, 6 out of 7 components were detected in RB fermented with *A. oryzae* extract. Increased quantities of phenolic acids in this extract were detected in ferulic, sinapic, caffeic, syringic and protocatechuic acids.

Table 4 Phenolic acids content of non-fermented (NF) and fermented RB, CT and RBCT extracts

Substrates / Extracts		Phenolic acids[1](µg/ml)						
		Ferulic	ρ-coumaric	Sinapic	Caffeic	Syringic	Protocatec-huic	4-hydroxybe-nzoic
RB	NF	1.88±0.14^{b}	7.33±0.09^{a}	2.52±0.1^{b}	nd	6.04±0.69^{a}	nd	nd
	A. oryzae	10.73±1.14^{a}	5.83±0.05^{b}	3.02±0.3^{a}	2.91±0.03^{b}	7.36±3.55^{a}	17.43±0.32^{a}	nd
	R. oligosporus	1.03±0.93^{b}	5.12±0.02^{b}	nd	4.34±0.07^{a}	nd	nd	13.68±0.69^{a}
CT	NF	nd	nd	nd	nd	nd	12.08±0.49^{b}	4.44±0.21^{b}
	A. oryzae	nd	nd	nd	nd	nd	3.09±0.14^{c}	nd
	R. oligosporus	nd	nd	nd	nd	nd	14.30±0.57^{b}	5.48±0.17^{b}
RBCT	NF	2.26±0.78^{b}	4.22±0.01^{c}	1.17±0.01^{c}	nd	nd	nd	nd
	A. oryzae	1.44±0.05^{b}	5.16±0.01^{b}	0.96±0.04^{cd}	nd	nd	nd	nd
	R. oligosporus	0.6±0.14^{b}	2.85±0.02^{d}	0.7±0.02^{d}	nd	nd	nd	nd

[1] Each value is expressed as mean±sd. The values with the same letter are not significantly different at the level of 0.05 (p>0.05).
nd = not detected.

Ferulic acid content was found to be the highest in RB fermented with *A. oryzae* extract. The same extract also showed the highest TPC (Table 1), antioxidant activity (Figure 2) as well as elastase inhibition activity (Table 3). The results can be attributed to the fact that ferulic acid is a bioactive compound that is commonly used as antioxidant and anti-aging agent in cosmeceutical products. Phenolic compounds are a good antioxidant agent by acting as singlet oxygen quenchers, hydrogen donors as well as reducing agents [7]. However, synergistic effect of other biological components of RB, CT and RBCT might have contributed to the antioxidant activity as well as inhibition activity of both tyrosinase and elastase of these extracts.

4.0 CONCLUSIONS

The present investigation showed that among the three substrates, the extracts of RB showed better antioxidant activities, cosmeceutical potentials and phenolic acids content compared to CT and RBCT. Fungal SSF of rice bran with *A. oryzae* has significantly improved the biological functionalities of rice bran. The results of this study suggest that fungal SSF of rice bran may have the potential to be exploited in producing different types of active metabolites that are useful for cosmeceutical industries.

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