



## Bioactive compounds and antioxidant activity of rice bran fermented with lactic acid bacteria

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### ABSTRACT

**Aims:** Rice bran has been documented as a rich source of bioactive compounds such as gamma-oryzanol, phenolic acids, phytic acid,  $\beta$ -sitosterol and vitamin E, which offer beneficial health properties and confer antioxidant activity related benefits. The objective of this research is to evaluate the bioactive compounds content of fermented rice bran such as organic acids,  $\gamma$ -oryzanol,  $\alpha$ -tocopherol and phenolic acids using three lactic acid bacteria (LAB), namely *Pediococcus acidilactici*, *Lactococcus lactis* and *Pediococcus pentoseous* in a solid state fermentation.

**Methodology and results:** High performance liquid chromatography (HPLC) was used to analyze the concentration of the active compounds in rice bran. The most abundant organic acids detected in fermented rice bran samples were lactic acid and acetic acid, which showed significant improvement after fermentation. Fermentation of rice bran with *P. acidilactici* showed a two-fold increment in  $\gamma$ -oryzanol and  $\alpha$ -tocopherol compared to unfermented rice bran. A higher concentration of ferulic acid was observed in rice bran fermented with *P. acidilactici* compared to other strains. Meanwhile, coumaric acid concentration in all fermented samples decreased significantly upon fermentation.

**Conclusion, significance and impact of study:** These results indicated that the fermentation with LAB could enhance certain bioactive compounds production and antioxidant activity of rice bran. Therefore, improved rice bran has the potential to be used as an ingredient in functional food and cosmetic formulation.

**Keywords:** Rice bran, bioactive compounds, fermentation, lactic acid bacteria, antioxidant activity

### INTRODUCTION

Rice bran, which is the residue from white rice production, is one of the abundant agricultural by-products in Malaysia. The annual amount of rice bran produced is estimated to be 180,000 metric tons per year. Industrial applications of this agricultural by-product are limited to animal feed and the production of rice bran oil. The chemical composition of rice bran consists of protein, lipid, carbohydrate, crude fiber and vitamin B. Rice bran has high nutritive values (Saunders, 1990) and benefits, such as decreasing the incidence of atherosclerosis disease (Saunders, 1985), lowering blood cholesterol (Kahlon *et al.*, 1994), and preventing cancer, kidney stones and heart diseases (Jariwalla, 2001).

The bioactive compounds that contributed to the promising health-related benefits of rice bran comprise of phenolic acids, flavonoids, gamma oryzanol, tocopherol, tocotrienols,  $\beta$ -sitosterol and phytic acids (Elizabeth, 2011). The main problem limiting the use of these active compounds is their insolubility, due to the bound form. Various methods have been used to enhance the concentration of bioactive compounds in rice bran such as sub-critical water treatment (Pourali *et al.*, 2009), germination with enzymatic treatment (Sungsopha *et*

*al.*, 2009) and microbial fermentation (Oliveira *et al.*, 2012). Several studies showed that fermentation can increase bioactive compounds in rice bran, such as rice bran fermented with *Rhizopus oryzae*, which showed increased phenolic acids and antioxidative activity (Oliveira *et al.*, 2012). A profiling of phytochemicals such as phenolic acids,  $\alpha$ -tocopherol and  $\beta$ -sitosterol in fermented rice bran with *Saccharomyces boulardii* showed that the metabolites are significantly different after fermentation (Elizabeth *et al.*, 2011).

The purpose of this study was to improve the production of bioactive compounds of rice bran by fermentation with lactic acid bacteria (LAB) previously isolated from natural fermentation of rice bran. In this study, we examined how phytochemicals of rice bran are altered through microbial fermentation by evaluating the changes of metabolites in fermented rice bran. Changes in the composition of organic acids,  $\alpha$ -tocopherol,  $\gamma$ -oryzanol and phenolic acids in rice bran fermented with three LAB were investigated. The concentrations of metabolites were analysed using High Performance Liquid chromatography (HPLC). The antioxidant activity was also assessed to evaluate their potential for application in cosmetic and functional food formulations.

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## MATERIALS AND METHODS

### Reagents and chemicals

Standards for the five organic acids (lactic, acetic, kojic, ascorbic and oxalic acids),  $\alpha$ -tocopherol,  $\gamma$ -oryzanol and two phenolic acids (coumaric and ferulic acids), were purchased from Sigma. Ultrapure water was prepared using ultra water Nanopure Diamond and analytical grade reagents were used.

### Microorganism

Microorganisms used in this study were *Pediococcus acidilactici*, *Lactococcus lactis* and *Pediococcus pentoseus* obtained from the Collection of Functional Food Cultures of the Biotechnology Centre, Malaysia Agriculture Research Institute (MARDI). Starter cultures of *P. acidilactici*, *L. lactis* and *P. pentoseus* were kept in lyophilized form and activated in 10 mL of selective media de Man Rogosa Sharpe (MRS) broth and incubated for 24 h at 37 °C. Fresh starter cultures were used for inoculation of rice bran.

### Fermentation process

The rice bran and distilled water were autoclaved (15 min, 121 °C) separately to sterilise. The moisture content of the rice bran was adjusted to 66% with sterilized water aseptically in a conical flask containing 30 g rice bran. The moisture was analysed using a moisture analyzer, and the media was then inoculated with 10% starter culture. Fermentation was carried out at 30 °C for 48 h at static condition and samples were taken for analysis.

### Extraction of active compounds

The extraction of organic acids was carried out using 0.013N H<sub>2</sub>SO<sub>4</sub>. Sample and solvent were mixed with the ratio (1 g: 5 mL) for 1 h in an incubator and shaken at 150 rpm at 30 °C. The mixture was centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through 0.20  $\mu$ m cellulose acetate membrane.

$\gamma$ -Oryzanol and  $\alpha$ -tocopherol contents were determined by HPLC using the method of Sungsopha *et al.* (2009) with slight modification. The fermented rice bran samples (1.0 g) were extracted consecutively in ethanol (5 mL) and water (2 mL). After mixing the mixtures with a vortex for 1 min, KOH (1 mL) was added. The mixture was capped and transferred to a boiling water bath for 25 min. An unsaponified layer was extracted three times, using 10 mL of hexane and ethyl acetate (8:2) mixture. These organic extracts were washed three times with water and evaporated by a rotary evaporator. The residue was dissolved in methanol and filtered with 0.45  $\mu$ m pore size nylon membrane.

The extraction of phenolic acid was carried out according to the method reported by Iqbal *et al.*, (2007). Briefly, 5 g of dry weight of fermented samples were 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 condition. The extracts were filtered through Whatman No.1 filter paper and evaporated to dryness with a reduced pressure at 45 °C, with a rotary evaporator (Buchi, Canada). Extracts were dissolved in 5 mL methanol and used for the analyses of phenolic acids and antioxidant activity.

### HPLC Analysis

The organic acids,  $\alpha$ -tocopherol,  $\gamma$ -oryzanol and phenolic acids in all samples were separated by reversed phase chromatography, which were then detected by absorbance and quantified with external calibration graphs. The organic acids in the sample were separated on a 250 mm x 4.6 mm, Extrasil ODS 5  $\mu$ m column and the detector was set at  $\lambda$ =210 nm and  $\lambda$ =245 nm. The determination of organic acids was carried out at isocratic conditions at 45 °C, using the mobile phase of 50 mM phosphate solution (dissolve 6.8 g potassium dihydrogen phosphate in 900 mL water adjusted to pH 2.8 with sulphuric acid). The flow rate of the mobile phase was set at 0.7 mL/min. The determination of  $\alpha$ -tocopherol and  $\gamma$ -oryzanol was done using reversed phase (4.6 x 100 mm, 3.5  $\mu$ m) column. The detector was set at  $\lambda$ = 325 nm and the separation was carried out in gradient condition using methanol, butanol and water as the mobile phases with a flow rate of 0.5 mL/min.

Meanwhile, for the determination of phenolic acid, a reversed phase (4.6 x 100 mm, 3.5  $\mu$ m) column was used and the detector was set at  $\lambda$ =280 nm, and  $\lambda$ =306 nm. The separation of phenolic acid was made in gradient condition at 30 °C, using a mobile phase made of methanol and acid water (0.1 % acetic acid) and the flow rate of the mobile phase was set at 0.7 mL/min.

### Standard and quantification

Standards with different concentrations were prepared and filtrated using 0.45  $\mu$ m nylon membrane filter. Individual standards of organic acids,  $\alpha$ -tocopherol,  $\gamma$ -oryzanol and phenolic acids were injected separately. The retention time of each individual standard was compared with the retention time of mixed standard solutions for identification purposes. The calibration standard was prepared by injecting different concentrations of mixed standard solutions performed in serial dilution. To determine the contents of organic acids  $\alpha$ -tocopherol,  $\gamma$ -oryzanol and phenolic acids in fermented rice bran, the mixed standard solution stock solution was analysed together with the samples. The quantifications of organic acids,  $\alpha$ -tocopherol,  $\gamma$ -oryzanol and phenolic acids were performed by generating calibration curves, where X= concentration of each standard expressed as microgram per mL; Y= measured peak area of chromatogram (Table 1). All samples were analysed in triplicates.

**Table 1:** Retention time, concentration range of lineal response and correlation coefficient for standard organic acids, phenolic acids,  $\gamma$ -oryzanol and  $\alpha$ -tocopherol.

Standard	Rt (min)	Concentration range ( $\mu\text{g/mL}$ )	Equation	R <sup>2</sup>
Oxalic	3.88	1750-350	Y = 7585.1x-72810	0.9937
Ascorbic	5.29	1750-350	Y = 8327.7x-7497.5	0.9921
Lactic	5.85	15000-3000	Y = 769.58x+4607	0.9939
Acetic	6.33	10000-2000	Y = 253.82x+2571	0.9991
Kojic	11.31	500-100	Y = 56358x-22343	0.9913
$\gamma$ -Oryzanol	30.34	2000-400	Y = 1981.7x+64364	0.9999
$\alpha$ -Tocopherol	1.33	500-100	Y = 1533.2x+31911	0.9878
p-Coumaric	34.72	200-50	Y = 102518x-251678	0.9999
Ferulic	40.11	200-50	Y = 67202x-10017	0.9999

### Determination of total phenolic content (TPC)

The total phenolic content in the fermented extracts was determined by a Folin-Ciocalteu method (Iqbal *et al.*, 2007). An aliquot of 1 mL sample was mixed with 5 mL Folin-Ciocalteu reagent in the test tube. Then, 4 mL of 7.5 % sodium carbonate solution was added and mixed for 30 sec. The tube was left for 2 h at room temperature and away from strong light. The absorbance was read on the UV/visible spectrophotometer (**Varian Cary 50**) at 765 nm against water. The TPC was quantified by plotting a gallic acid calibration curve from 1-100 ppm and expressed as micrograms of gallic acid equivalent (GAE) where 1 ppm is equivalent to 1 microgram GAE per mL of sample extract. The equation for the gallic acid calibration curve was  $Y=0.0108X+0.0449$  (where X= concentration of gallic acid equivalent (GAE) expressed as micrograms of GAE per mL extract; Y= measured absorbance, and the correlation coefficient was  $R^2= 0.9939$

### Analysis of antioxidant activity

#### DPPH radical scavenging activity

The antioxidant activity of the fermented rice bran sample extracts were determined based on the scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The sample extracts (0.5 mL) were added to 2.5 mL of 0.5 mM methanol solution of DPPH and were incubated in the dark at room temperature for 30 min. The absorbance at 517 nm was measured and the inhibition of DPPH radical was calculated in term of percentage (%) using the formula:

$$\text{Percentage inhibition (\%)} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

where,  $A_{\text{control}}$  is the absorbance of the control reaction (containing of 2.5 mL DPPH solution and 0.5 mL methanol) and  $A_{\text{sample}}$  is the sample absorbance. The  $IC_{50}$  values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation.

### 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 the 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  $\pm$  s.d. The coefficient of correlation (R<sup>2</sup>) to determine the relationship between two variables was calculated using MS Excel.

## RESULTS AND DISCUSSION

### Organic acids

LAB strains are known to have a complex requirement for growth, while rice bran contains carbohydrates and other essential nutrients. Three LAB strains, *P. acidilactici*, *P. pentoseous* and *L. lactis* have shown growth in natural rice bran without the addition of other supplements. Hydrolysis, biochemical metabolism and microbial activity in fermented rice bran have resulted in the accumulation of organic acids. The capability of different LAB to produce organic acids during growth has been observed and a heterofermentative metabolic pattern producing mainly lactic acid and acetic acid has been demonstrated.

As shown in Table 2, five organic acids were identified and quantified from the extracts of fermented rice bran with the three LAB strains. Rice bran fermented with *P. acidilactici* produced the highest concentration of lactic, acetic, oxalic and ascorbic acids compared to other strains. The highest concentrations of organic acids observed were lactic and acetic acid in all fermented samples. Lactic and acetic acids contents significantly improved ( $p<0.05$ ) upon fermentation. Meanwhile, oxalic, ascorbic and kojic acids showed insignificant increments ( $p>0.05$ ) in all fermented samples, except for the extracted rice bran fermented with *P. pentoseous*, which showed insignificant reduction ( $p>0.05$ ) of kojic acid compared to unfermented rice bran.

Increased concentrations of organic acids in the fermented samples showed that all three LAB strains are

capable of utilising rice bran as a carbon source and therefore, are amylolytic lactic acid bacteria (ALAB) strains. As most carbohydrates in rice bran are in the form of polysaccharides, the availability of carbon sources for non-amylolytic microorganisms is limited. The increase in metabolites produced such as organic acids indicated possible growth, which complement the finding by Reyed *et al.* (2007) that organic acids content can act as an

indicator for microbial growth. The use of ALAB can eliminate the need for the preliminary step of starch liquefaction by exogenous  $\alpha$ -amylases before fermentation. Exploiting this particular property of some LAB in fermentation will offer opportunities to develop improved fermented products of starchy raw materials.

**Table 2:** Production of organic acids by lactic acid bacteria after 48 h of rice bran fermentation.

Organic acids <sup>1</sup> ( $\mu\text{g/mL}$ )	Extracts			
	Unfermented	<i>P. acidilactici</i>	<i>L. lactis</i>	<i>P. pentoseous</i>
Oxalic	212.01 $\pm$ 12.36 <sup>d</sup>	568.33 $\pm$ 3.77 <sup>d</sup>	376.68 $\pm$ 0.94 <sup>d</sup>	409.80 $\pm$ 0.21 <sup>d</sup>
Ascorbic	34.68 $\pm$ 2.31 <sup>d</sup>	158.43 $\pm$ 3.35 <sup>d</sup>	74.33 $\pm$ 11.75 <sup>d</sup>	162.28 $\pm$ 4.27 <sup>d</sup>
Lactic	Nd	3183.26 $\pm$ 40.88 <sup>c</sup>	2711.03 $\pm$ 170.03 <sup>c</sup>	2884.85 $\pm$ 44.99 <sup>c</sup>
Acetic	1093.30 $\pm$ 55.53 <sup>d</sup>	6334.91 $\pm$ 55.83 <sup>a</sup>	4718.38 $\pm$ 86.78 <sup>b</sup>	4520.01 $\pm$ 28.38 <sup>b</sup>
Kojic	9.29 $\pm$ 0.54 <sup>d</sup>	14.88 $\pm$ 0.20 <sup>d</sup>	16.10 $\pm$ 0.99 <sup>d</sup>	4.67 $\pm$ 1.20 <sup>d</sup>

<sup>1</sup>Anova analyses were performed using Minitab 14 Statistical Software. Each value is expressed as mean $\pm$ 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.

### $\gamma$ -Oryzanol and $\alpha$ -tocopherol

$\gamma$ -oryzanol and  $\alpha$ -tocopherol contents in unfermented rice bran and rice bran fermented with the three strains of LAB are shown in Table 3. The highest concentration of  $\gamma$ -oryzanol was found in rice bran fermented with *P. acidilactici* (1148.38  $\mu\text{g/mL}$ ) and the lowest was found in rice bran fermented with *L. lactis* (522.26  $\mu\text{g/mL}$ ) compared to unfermented rice bran with a value of 954.47  $\mu\text{g/mL}$ .  $\gamma$ -oryzanol concentrations of fermented rice bran extracts varied, where extracts of rice bran fermented with *L. lactis* and *P. pentoseous* showed significant reduction ( $p < 0.05$ ), while rice bran fermented with *P. acidilactici* showed significant improvement ( $p < 0.05$ ) compared to the unfermented sample. Fermentation caused a decrease in  $\gamma$ -oryzanol concentration, except for rice bran fermented with *P. acidilactici*. It is suggested that  $\gamma$ -oryzanol may be broken down to triterpene alcohol or metabolized components, such as metabolite ferulic acid via the activity of microbial ferulate esterases (Handerson *et al.*, 2012). Tocols are natural antioxidant derived from plant material and also found in cereal grains products.

Tocols include tocopherols and tocotrienols, which occur in eight forms. Tocopherols are similar to the tocotrienols in chemical structure and they belong to the vitamin E family (Damien *et al.*, 2013). The analysis of local rice bran used in this study showed that the concentration of  $\alpha$ -tocopherol was higher than the amount documented by Sungsopha *et al.* (2009) which was 62.4  $\mu\text{g/g}$ , which could be due to many factors such as growing environment, storage or postharvest treatment. An increment in  $\alpha$ -tocopherol concentration was observed in all fermented samples compared to unfermented rice bran. Possibly, bound forms of tocols (tocopherol and tocotrienols) are released by enzymatic reaction due to the fermentation with lactic acid bacteria. Bioprocessing methods such as microbial fermentation and enzymatic reaction have been widely used to release bound phytochemicals, which can improve its free forms and bioavailability (Sungsopha *et al.*, 2009; Gani *et al.*, 2012; Damien *et al.*, 2013). Heat treatment has also been reported to improve tocols concentration in oat products (Peterson, 1995).

**Table 3:**  $\gamma$ -Oryzanol and  $\alpha$ -tocopherol concentrations of unfermented and fermented rice bran with LAB at 48 h fermentation.

$\mu\text{g/mL}$	Extracts			
	Unfermented	<i>P. acidilactici</i>	<i>L. lactis</i>	<i>P. pentoseous</i>
$\gamma$ -Oryzanol	954.47 $\pm$ 21.23 <sup>b</sup>	1148.38 $\pm$ 48.20 <sup>a</sup>	522.26 $\pm$ 59.11 <sup>d</sup>	761.82 $\pm$ 22.10 <sup>c</sup>
$\alpha$ -Tocopherol	92.25 $\pm$ 10.06 <sup>c</sup>	182.37 $\pm$ 20.02 <sup>a</sup>	138.37 $\pm$ 15.89 <sup>b</sup>	135.60 $\pm$ 12.45 <sup>b</sup>

Anova analyses were performed using Minitab 14 Statistical Software. Each value is expressed as mean $\pm$ sd. The values in each row with the same letter are not significantly different at the level of 0.05 ( $p > 0.05$ ).

### Phenolic acid composition

A previous study conducted on rice bran found two major phenolic acid compounds in rice bran, which were ferulic and p-coumaric and ferulic acids, were also the most abundant derivatives of hydroxycinnamic acid found in cereal grains (Gani *et al.*, 2012). Phenolic acids compositions in unfermented and fermented rice bran are shown in Table 4. The results of HPLC analysis indicated that fermented rice bran with *P. acidilactici* showed higher concentration of ferulic acid (8.56 µg/mL), but lower concentration of coumaric acid (3.51 µg/mL) after fermentation. As shown in Table 4, a significant increase ( $p < 0.05$ ) of ferulic acid in rice bran fermented with *P. acidilactici* was observed, however, in rice bran fermented with *P. pentoseous* showed insignificant increase ( $p > 0.05$ ). Rice bran fermented with *L. lactis* showed that no ferulic acid was detected. Coumaric acid concentration in all fermented samples significantly decreased ( $p < 0.05$ ) upon fermentation. Ferulic and coumaric acids are the major bound phenolic acids present in the native rice bran. Degradation of bound ferulic acid by the enzyme ferulic acid esterase can be correlated with the increase in ferulic acid. Increase in ferulic acid during fermentation had also been observed by Kim *et al.*, (2012) in rice bran fermented with *Issatchenkia orientalis*. According to

Estrada *et al.*, (2014) microorganism can synthesize enzymes capable of breaking ester links and consequently the released bound phenolic acids may improve the nutraceutical value of cereals and increase the bioavailability due to the improvement of free phenolic acids. The improvement of ferulic acid in fermented samples inoculated with *P. acidilactici*, may be due to ferulic acid esterase enzyme produced by *P. acidilactici* during fermentation. A fermentation can cleave the bonds between phenolic compounds with other materials and release the monomers of phenolic or antioxidants (Anastasia *et al.*, 2102).

Ferulic acid was undetected in rice bran after fermentation with *L. lactis* suggesting that the inter-conversion of liberated ferulic acid to coumaric acid have occurred and was observed by the high concentration of coumaric acid in rice bran fermented with *L. lactis*. A decrease in the concentration of coumaric acid was observed in all fermented samples. This result was similar to that reported by Hedge *et al.*, (2006), Elizabeth *et al.*, (2011) and Kim *et al.*, (2012) who studied phenolic acids in rice bran fermented with *Aspergillus niger*, *Saccharomyces boulardii* and *Issatchenkia orientalis*, respectively.

**Table 4:** Phenolic acids concentration of unfermented rice bran and rice bran fermented with LAB after 48 h fermentation.

µg/mL	Extracts			
	Unfermented	<i>P. acidilactici</i>	<i>L. lactis</i>	<i>P. pentoseous</i>
Ferulic acid	6.19±0.75 <sup>b</sup>	8.56±0.99 <sup>a</sup>	nd	6.96±0.76 <sup>b</sup>
Coumaric acid	10.77±0.52 <sup>a</sup>	3.51±0.14 <sup>c</sup>	7.29±0.21 <sup>b</sup>	3.79±0.24 <sup>c</sup>

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.

### Total phenolic content (TPC) and antioxidant activity

TPC may contribute directly to the effect of antioxidant capacity, therefore, it is necessary to investigate the TPC in the samples. The TPC and antioxidant activity of rice bran fermented with LAB in this study are presented in Table 5 and 6. The TPC of fermented rice bran was expressed as µg gallic acid per mL of samples. The results showed that all fermented extracts had increased TPC compared to the unfermented extract. A significant increase ( $p < 0.05$ ) of TPC in rice bran fermented with *P. acidilactici* and *P. pentoseous* was observed. The highest phenolic content in the extracted rice bran fermented with *P. acidilactici* was up to 246 µg/mL after 48 h of incubation. Fermentation of rice bran affected the total phenolic content. This result was similar as reported by Oliveira *et al.*, (2012), who studied the effect of solid state fermentation on the content of total phenolic compound and antioxidant activity in fermented rice bran with *Rhizopus oryzae*. Studies by Lin *et al.*, (2006) and Lee *et al.*, (2008) proposed that β-glucosidase enzyme and esterases produced by the organism during the

fermentation process was responsible for the polyphenol content enhancement.

The antioxidant activities of fermented extracts were determined based on the scavenging activity toward DPPH. The strongest scavenging effects on the DPPH radical were found in rice bran fermented with *P. acidilactici*. The scavenging effect of the DPPH radical in the unfermented rice bran increased from 66.2% to 82.6% after fermentation with *P. acidilactici*, while the scavenging effects were 77.2% and 71.5% in samples fermented with *L. lactis* and *P. pentoseous*, respectively. The scavenging effect at 200 µg/mL (Table 6) significantly increased ( $p < 0.05$ ) in all fermented extracts samples.

Fermentation with LAB had positive influence on DPPH inhibitory effect, which is in agreement with Oliveira *et al.* (2012), who observed increased DPPH radical scavenging activity of rice bran after fungal fermentation. Fermentation with *P. acidilactici*, *P. pentoseous* and *L. lactis* influenced the increase of bioactive compound in rice bran resulting in an improved antioxidant activity. In order to determine the relationship between TPC and DPPH scavenging activity, a correlation graph was created as shown in Fig. 1. Total

phenolic content and radical scavenging activity of unfermented and fermented rice bran were poorly correlated ( $R^2=0.471$ ). These results strongly suggest that DPPH radical scavenging activity of unfermented and

fermented rice bran may be highly related to the contribution of all bioactive compounds in rice bran and not the specific total phenolic compounds.

**Table 5:** Total phenolics content of unfermented and fermented rice bran with LAB at 48 h fermentation.

µg/mL	Extracts			
	Unfermented	<i>P.acidilactici</i>	<i>L. lactis</i>	<i>P.pentoseous</i>
TPC (GAE)	212.5±0.7 <sup>c</sup>	246±8.5 <sup>a</sup>	214±16.3 <sup>c</sup>	230±15.6 <sup>b</sup>

<sup>1</sup> 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 ( $p>0.05$ )

## CONCLUSION

In summary, our results demonstrated that the fermentation of rice bran with *P. acidilactici*, *P. pentoseous* and *L. lactis* increased the concentration of bioactive compounds such as phenolic acids (ferulic acid), organic acids,  $\gamma$ -oryzanol and  $\alpha$ -tocopherol. The study demonstrated that different LAB strains exhibited varying abilities in enhancing the bioactive compounds. The use of lactic acid bacteria fermentation can enhance the levels of bioactive compounds and improve the functional properties beneficial for health, which could promote the development of products based on rice bran.

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