

# Effect of fermentation process on bioactive compounds and nutritional value of rice bran: a meta-analysis investigation

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## Abstract

Rice bran is a valuable byproduct of the rice milling process, used as a food ingredient and possesses numerous health benefits and antioxidants. The fermentation process has been utilized to enhance the nutritional quality of rice bran. The objective of this study was to review and compare various research findings on the impact of fermentation on rice bran's bioactive compounds, nutritional quality, and antioxidant activity. A total of 225 articles were obtained from databases, published from 2010 up to May 2020. There were thirteen eligible articles suited to further analyses using systematic review and meta-analysis. The results showed that rice bran fermentation significantly ( $p < 0.05$ ) enhanced the levels of ash, protein and crude fibre. There were significant differences ( $p < 0.05$ ) in the levels of total phenolic content and antioxidant activity after fermentation using *Rhizopus oligosporus* and *R. oryzae*. The rice bran fermentation increased ferulic acid, sinapic acid, and syringic acid, and decreased p-coumaric acid. Caffeic acid and vanillic acid were only detected in fermented rice bran, with amounts of 4.86  $\mu\text{g/mL}$  and 16.41  $\mu\text{g/mL}$ , respectively. In summary, the fermentation process could be an option to enhance bioactive compounds, nutritional, and antioxidant activity of rice bran.

## 1. Introduction

Rice processing involves several milling stages to produce the edible final product. Rice bran is a byproduct of the milling process, which produces milled rice as the main product (68-72%) and byproducts as husks (20%) and rice bran (8-12%) (Bodie *et al.*, 2019). Several studies have reported on the potential of rice bran to be used as food ingredient because it contains high amount of nutritional and bioactive compound, as amino acid (tryptophan, histidine, isoleucine, leucine, tyrosine, lysine, phenylalanine, threonine, methionine, valine, alanine, cysteine, asparagine, glutamic acid, glycine, arginine, serine, proline), micronutrient (magnesium, calcium, phosphorus, manganese, and B vitamins),  $\gamma$ -oryzanol, tocopherol, tocotrienol, polyphenols, phytosterol, dan carotenoid (Ryan, 2011; Li *et al.*, 2016). These components have health benefits for our body as antioxidants and prevent various chronic diseases. Several studies on the functional properties of rice bran include preventing atherosclerosis, anti-diabetic, anti-cancer, and anti-inflammatory (Islam *et al.*, 2011; Perez-Tertero *et al.*, 2017).

Fermentation has been widely used to enhance the nutritional value and improve the quality of rice bran

because it can extend shelf life, improve taste, increase bioactive compounds, reduce antinutrients (phytic acid), and increase protein (Oliveira *et al.*, 2010; Cheng *et al.*, 2016; Srivastava, 2018). The effects of fermentation on bioactive compounds in the rice bran have been documented in numerous studies with inconsistent results. This could be due to several things, such as different fermentation conditions, the rice cultivars used, and the complexity of the biotransformation of bioactive compounds by different microorganisms. Several studies showed that fermented rice bran has a higher content of bioactive compounds and better functional properties compared to non-fermented rice bran. There is a breakdown of complex compounds that bind to lignocellulose or polysaccharides in the fermentation process, increasing phenolic compounds for health benefits such as antioxidant activity, helping prevent cell aging and disease (Oliveira *et al.*, 2012; Cabuk *et al.*, 2018). During the fermentation process, microbes produce enzymes that can hydrolyse complex bioactive compound into free forms (Oliveira *et al.*, 2012). Phenolic acids are present in free and bound forms. This compound can be divided into two sub-groups: hydroxybenzoic and hydroxycinnamic acid. Hydroxycinnamic acids are generally found in cereal

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grains, which are derived from ferulic, p-coumaric, caffeic, and sinapic acids. The ferulic acid is the most abundant found in the outer part of cereal grain. Meanwhile, hydroxybenzoic acids mainly originated from *p*-hydroxybenzoic, protocatechuic, syringic, and vanillic acid; they are found in red fruit, black radish, and onion in low concentration. Phenolic acids have strong antioxidant properties, so it plays role in preventing degenerative diseases such as cancer, diabetes, cardiovascular disease, and inflammation (Kupski *et al.*, 2012; Kumar and Goel, 2019).

The fermentation process can improve the sensory quality of rice bran. Based on the research of Ardiansyah *et al.* (2021), the fermentation process can increase the sensory acceptance of rice bran. Cempo Ireng rice bran and Inpari 30 rice bran fermented with *R. oligosporus* for 72 h, were more acceptable to the panellists compared to the benchmark because the samples had dominant taste and aroma attributes acceptable to the panellists. Rice bran fermented using *R. oryzae* for 24 h can increase total phenolic content (TPC) up to 5 times and inhibit DPPH free radicals up to 87% compared to non-fermented rice bran (Kupski *et al.*, 2012). The TPC of rice bran fermented using *Monascus purpureus* for 12 h only increased by 4.22% (Jamaluddin *et al.*, 2016). However, several studies have revealed that the antioxidant activity or bioactive compound also decreases as the fermentation time increases. As an example, in the research of Schmidt *et al.* (2014) the TPC of rice bran fermented using *R. oryzae* for 120 h increased from 2.4 mg/g to 5.1 mg/g. However, the antioxidant activity of fermented rice bran decreased compared to that of non-fermented rice bran.

Based on the explanation above, there were several differences in research results regarding the effect of the fermentation process on the content of bioactive compounds and antioxidant activity in rice bran, so this study was conducted to statistically determine the effect of the fermentation process on the nutritional value, bioactive compounds, and antioxidant activity of rice bran. The use of the meta-analysis method in this study is expected to be useful in providing answers to the differences in results.

## 2. Materials and methods

### 2.1 Literature search and strategy

A meta-analysis statistically combines the results of multiple similar studies to provide pooled effect estimates derived from extracted data to draw conclusions in a systematic review. This approach is typically conducted on extensively researched studies, ensuring a substantial literature base. A literature search

was conducted to identify studies assessing the impact of fermentation on the enhancement of nutritional components (protein, lipid, ash, and crude fibre), bioactive compounds (TPC, ferulic acid, sinapic acid, syringic acid, p-coumaric acid, caffeic acid, and vanillic acid), and antioxidant activity.

Two strategies were employed to search for relevant literature. First, four reputable international electronic databases were utilized: Science Direct, PubMed, Directory of Open Access Journals, and Google Scholar. Searches were performed using combinations of the following keywords to identify potentially relevant studies published from 2010 to May 2020: “Rice” or “Rice bran” or “By-product rice milling” and “Fermentation” or “Fermented rice bran” and “Nutrition component” or “Physicochemical” or “Proximate compositions” or “Nutritional value” and “Bioactive compound” or “Phytochemical” or “Phenolic content” and “Antioxidant activity” or “Antioxidant properties”. Second, a manual search was conducted through citation tracking. References cited by retrieved articles were examined, and the reference lists of these articles were reviewed to identify all relevant studies.

### 2.2 Inclusion and exclusion criteria

Studies that met the following inclusion criteria underwent initial identification and screening: original research articles published in English, full text availability, use of fermented and non-fermented rice bran or solely fermented rice bran samples, and for meta-analysis, availability of mean values and standard deviation (SD) or graphs convertible into quantitative data.

The exclusion criteria included the following: studies with data that could not be reliably extracted, case reports or poster abstracts, and review studies.

### 2.3 Data extraction and analysis

Each study was reviewed manually to gather pertinent data, which were organized into a structured table listing the article title, author(s), publication year, analysed parameters, fermentation methods, microbial types, sample details, mean  $\pm$  SD values, units of measurement, and additional relevant data.

During the second screening, articles were further assessed based on fermentation duration, types of starter cultures, and quantitative data availability. For the assessment of antioxidant activity, selection was restricted based on the testing methodology used, but not on fermentation duration. Additionally, no restrictions were imposed on rice cultivars or fermentation techniques.

Quantitative data from selected articles were compiled to calculate combined effect estimates using Confidence Intervals (CI). The reported data included the mean; 95% CI, LL to UL (lower and upper limit of CI). CI analysis was conducted to convey the precision of the measurement using a confidence level of 95%, indicating a 95% confidence that the true value falls within the calculated range (Choudhary and Garg, 2013).

The CI analysis was performed using Microsoft Excel 2007, and the results were presented in the form of a forest plot. For each nutritional value parameter (ash, protein, lipid, and crude fibre), p-values and  $I^2$  values were evaluated using STATA 16.0 for Windows 64-bit x86-64 (2019) to determine heterogeneity between treatments.  $I^2$  values with numbers 0–30%, <30% to 60%, and over 60% indicate low, medium, and high heterogeneity, respectively. The significance values for TPC and antioxidant activity parameters were evaluated using a T-test: Two-Sample Assuming Equal Variances with a p-value < 0.05. The data for each proximate parameter are presented as a combined figure from the forest plot evaluated using Microsoft Excel 2007, along with a table of p-values and heterogeneity calculations using STATA 16.0 for Windows 64-bit x86-64. Meanwhile, the data for TPC and antioxidant activity parameters are presented solely in the form of a forest plot.

### 3. Results and discussion

#### 3.1 Search results and data screening

The database search using keywords yielded 225 articles. After removing duplicates and screening titles or abstracts, 42 articles remained, which were then read and reviewed in full. Following a thorough evaluation, 13 full-text articles met the research criteria, resulting in 11 articles being used for further calculation using CI, while three articles underwent statistical review. This systematic review and meta-analysis study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, published in 2009. The flowchart of the research study is illustrated in Figure 1.

There are three types of fungi that were used as starter cultures in the fermentation process across the thirteen selected papers: *R. oryzae* (nine articles), *R. oligosporus* (four articles), and *M. purpureus* (three articles). This study limited the types of starter cultures to ensure comparability across cases.

#### 3.2 Overview of included studies

The findings from the three studies included in this systematic review are summarised in Table 1. These studies, published from 2010 to 2020, utilized *M.*

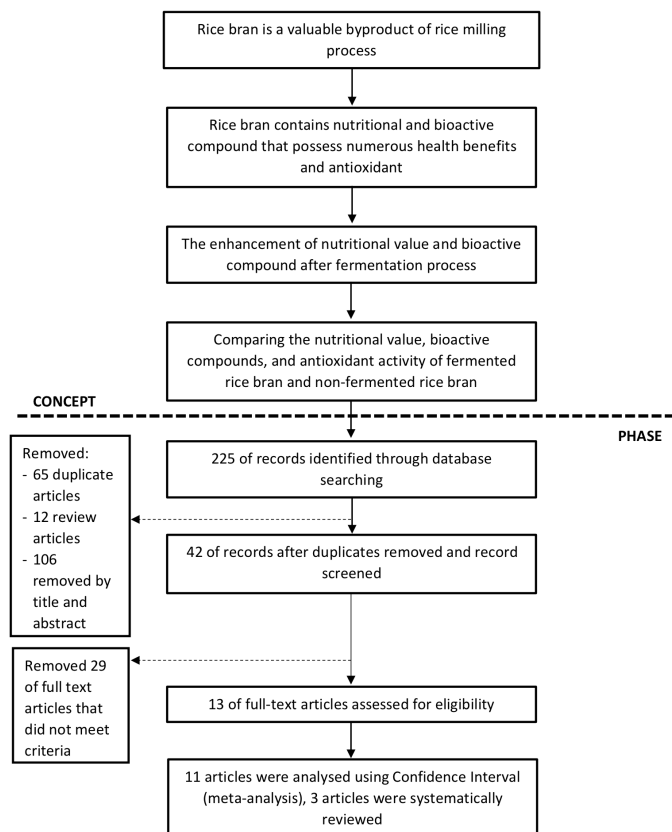


Figure 1. Flow chart of the research study.

*purpureus* as a starter culture.

The data indicated that the ferulic acid content in fermented rice bran increased from  $1.88 \pm 0.14$  to  $9.72 \pm 0.93$   $\mu\text{g/mL}$ , representing a five-fold increase following the fermentation process. The p-coumaric acid content in fermented rice bran decreased from  $7.33 \pm 0.99$  to  $7.01 \pm 0.06$   $\mu\text{g/mL}$ , reflecting a decrease of approximately 4.36% after fermentation. The sinapic acid content in fermented rice bran increased from  $2.52 \pm 1$  to  $4.68 \pm 1.26$   $\mu\text{g/mL}$ , indicating a two-fold increase post-fermentation. The syringic acid content in fermented rice bran increased from  $6.04 \pm 0.69$  to  $10.4 \pm 1.51$   $\mu\text{g/mL}$ , representing a 1.5-fold increase after the fermentation process. Meanwhile, caffeic and vanillic acids were detected following the fermentation of rice bran ( $4.86 \pm 0.14$   $\mu\text{g/mL}$  and  $16.41 \pm 0.43$   $\mu\text{g/mL}$ , respectively).

The results suggest that ferulic, p-coumaric, sinapic, and syringic acids were detected in both non-fermented and fermented rice bran. The content of ferulic, sinapic, and syringic acids increased with fermentation, while p-coumaric acid decreased. Notably, neither vanillic acid nor caffeic acid was found in non-fermented rice bran; both were present after fermentation. The enhancement of ferulic, sinapic, and syringic acids may be attributed to the release of these compounds following fermentation, resulting from the structural breakdown of the cell wall, which facilitates the synthesis of various bioactive compounds (Wang et al., 2019). Conversely,

Table 1. Results of systematic review of three included studies.

Parameter test	Type of microbes	Mean value		Units	References
		NFRB	FRB		
Ferulic acid	<i>M. purpureus</i>	1.88±0.14	9.72±0.93	µg/mL	Razak et al. (2015)
	<i>M. purpureus</i>	1.88±0.14	9.72±0.93	µg/mL	Jamaluddin et al. (2014)
	<i>M. purpureus</i>	1.88±0.14	9.72±0.93	µg/mL	Jamaluddin et al. (2016)
p-coumaric acid	<i>M. purpureus</i>	7.33±0.99	7.01±0.06	µg/mL	Razak et al. (2015)
	<i>M. purpureus</i>	7.33±0.99	7.01±0.06	µg/mL	Jamaluddin et al. (2014)
	<i>M. purpureus</i>	7.33±0.99	7.01±0.06	µg/mL	Jamaluddin et al. (2016)
Sinapic acid	<i>M. purpureus</i>	2.52±0.1	4.68±1.26	µg/mL	Razak et al. (2015)
	<i>M. purpureus</i>	2.52±0.1	4.68±1.26	µg/mL	Jamaluddin et al. (2014)
	<i>M. purpureus</i>	2.52±0.1	4.68±1.26	µg/mL	Jamaluddin et al. (2016)
Syringic acid	<i>M. purpureus</i>	6.04±0.69	10.4±1.51	µg/mL	Razak et al. (2015)
	<i>M. purpureus</i>	6.04±0.69	10.4±1.51	µg/mL	Jamaluddin et al. (2014)
	<i>M. purpureus</i>	6.04±0.69	10.4±1.51	µg/mL	Jamaluddin et al. (2016)
Caffeic acid	<i>M. purpureus</i>	nd	4.86±0.14	µg/mL	Razak et al. (2015)
	<i>M. purpureus</i>	nd	4.86±0.14	µg/mL	Jamaluddin et al. (2014)
	<i>M. purpureus</i>	nd	4.86±0.14	µg/mL	Jamaluddin et al. (2016)
Vanillic acid	<i>M. purpureus</i>	nd	16.41±0.43	µg/mL	Razak et al. (2015)
	<i>M. purpureus</i>	nd	16.41±0.43	µg/mL	Jamaluddin et al. (2014)
	<i>M. purpureus</i>	nd	16.41±0.43	µg/mL	Jamaluddin et al. (2016)

NFRB: non-fermented rice bran, FRB: fermented rice bran

the corresponding decrease in p-coumaric acid can be attributed to the hydrolysis and degradation of phenolic compounds.

Enzymes such as protease, xylanase, and amylase, produced during fermentation, play a vital role in breaking down chemical bonds, leading to the release of bound phenolics (Adebo and Medina-Meza, 2020). The integration of esterified phenolic acids in the plant cell wall can be released through acid or alkali hydrolysis. The addition of a hydroxyl group to cinnamic acid by the action of monooxygenase converts it to p-coumaric acid. Furthermore, p-coumaric acid undergoes hydroxylation and oxymethylation to produce caffeic and ferulic acids, respectively (Alvarado et al., 2002; Kumar and Goel, 2019). This is evidenced by the decrease in p-coumaric acid and the presence of caffeic acid after fermentation. Caffeic acid is a potentially strong antioxidant with various benefits, including anti-cancer, anti-inflammatory, and antioxidant properties. The changes in

the profile of phenolic acids caused by fermentation depend on the type of substrate, the microorganism used, and the fermentation conditions (Martins et al., 2011).

### 3.3 Results of meta-analysis

This meta-analysis assessed the impact of fermentation on the nutritional and bioactive composition of rice bran, a byproduct known for its valuable nutrient and phytochemical content. The results presented in Figures 2-6 and 8 revealed that the fermentation process led to an increase in nutritional value (ash, protein, and crude fiber), TPC, and antioxidant activity, while there was a slight reduction in lipid content, although this difference was not statistically significant.

#### 3.3.1 Ash

Data calculations for the ash parameter were obtained from three studies that met criteria (Oliveira et

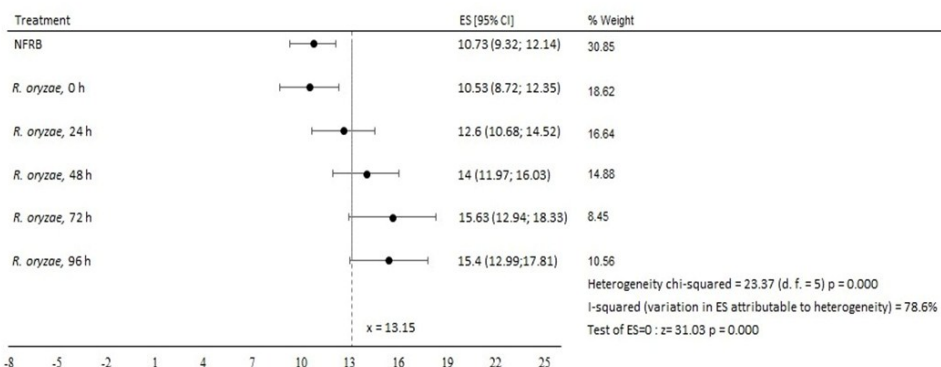


Figure 2. Forest plot of the effect of rice bran fermentation on ash content.

al., 2010; Kupski *et al.*, 2012; Ribeiro *et al.*, 2017), with fermentation periods of 0 h, 24 h, 48 h, 72 h, and 96 h. The results of the CI calculation (Figure 2) indicated a significant increase in ash content during rice bran fermentation, particularly after 24 h (12.6; 95% CI, 10.68 to 14.52;  $p = 0.000$ ). Overall, there was high heterogeneity and a significant effect between treatments ( $I^2 = 78.6\%$ ;  $p < 0.05$ ).

The mean ash content increased approximately 1.17–1.45 times in fermented rice bran. The increase in ash content indicates a rise in minerals within the substrate (Ogodo *et al.*, 2017). A higher ash content was observed after 72 h of fermentation, with an increase of 45.67%, possibly due to the inherent nature of fungal growth. During fermentation, mycelial synthesis contributes to the increase in ash content (Ribeiro *et al.*, 2017). According to Hasan *et al.* (2014), an increase in nutritional value during the fermentation process can occur through at least three mechanisms. The first mechanism involves microorganisms that are both catabolic and anabolic, breaking down complex compounds and synthesizing complex vitamins and other growth factors. The second mechanism involves the release of nutrients locked within plant structures and cells. The fermentation process, particularly by certain bacteria, yeast, and molds, can decompose indigestible coatings and cell walls both chemically and physically. The third mechanism involves the enzymatic degradation of substances that are indigestible by humans into simpler sugars and sugar derivatives, such as cellulose, hemicellulose, and related polymers. The cellulose-containing substrate in fermented foods can be improved for human consumption through the action of microbial enzymes (Potter and Hotchkiss, 2006).

The wide range of CI is primarily due to the heterogeneity among the articles used and the small sample size ( $n = 3$ ). The CI width indicates the level of uncertainty in estimating population parameters, where a smaller range of CI indicates greater accuracy (Miao and Chiou, 2008; Sedgwick, 2014). The size of the CI range is influenced by the confidence level ( $\alpha$ ), sample size,

standard error (SE) or standard deviation (SD) present in the study, and data variability (Sedgwick, 2014; Hazra, 2017; Hespanhol *et al.*, 2019). A wide SE can lead to an increasingly broad range of CIs; however, as the sample size increases, the CI width decreases (Sedgwick, 2014).

### 3.3.2 Protein

Data calculations for the protein parameter were evaluated from three studies that met criteria (Oliveira *et al.*, 2010; Kupski *et al.*, 2012; Ribeiro *et al.*, 2017), with fermentation durations of 0 h, 24 h, 48 h, 72 h, and 96 h. The results of the CI calculation indicated that protein content significantly increased during rice bran fermentation, particularly after 24 h (18.4; 95% CI, 15.68 to 21.12;  $p = 0.000$ ). High heterogeneity and a significant effect between treatments were observed ( $I^2 = 93.7\%$ ;  $p < 0.05$ ).

Fermentation enhances protein digestibility, nutritional value, protein content, carbohydrate accessibility, and amino acid balance and reduces antinutritional factors (Alka *et al.*, 2012). The protein content of fermented rice bran (Figure 3) showed an increase as fermentation time progressed. The highest protein content was observed after 96 h of fermentation, with an increase of 82%. The wide range of CI was due to the heterogeneity between data from the articles used and the small sample size ( $n = 3$ ). The mean value for zero-time fermented rice bran was comparable to that of non-fermented rice bran.

The development of fungal biomass increased protein content (Kupski *et al.*, 2012). Additionally, microorganisms such as yeast, fungi, algae, and bacteria can produce single-cell protein (SCP) by utilising various carbon sources. SCP is rich in protein and vitamins and contributes to the total protein content (Najafpour, 2006).

Rice bran protein offers numerous health benefits and potential for development in the food processing sector. Rice bran has been reported as a source of hypoallergenic proteins, comprising albumins (37%),

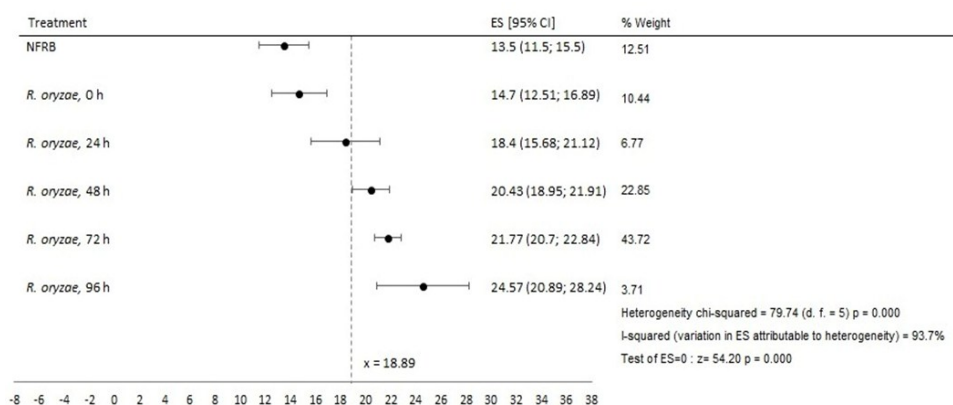


Figure 3. Forest plot of the effect of rice bran fermentation on protein.

globulins (36%), glutelins (22%), and prolamins (5%). The protein efficiency ratio (PER) of rice bran is 2.0–2.5 compared to 2.5 for casein, and it is approximately 90% digestible (Wang *et al.*, 1999). The lysine content (3–4%) and amino acid composition in rice bran contribute to its superior protein quality compared to other cereal bran, making it suitable for children's growth and development (Khan *et al.*, 2011).

### 3.3.3 Lipid

Data calculations for the lipid parameter were evaluated from three studies that met criteria (Oliveira *et al.*, 2010; Kupski *et al.*, 2012; Ribeiro *et al.*, 2017), with fermentation durations of 0 h, 24 h, 48 h, 72 h, and 96 h. The results of the CI calculation indicated that lipid content decreased after 48 h of fermentation (17.33; 95% CI, 11.35 to 23.31;  $p = 0.972$ ). Low heterogeneity and an insignificant effect between treatments were observed ( $I^2 = 0.0\%$ ;  $p > 0.05$ ).

The forest plot (Figure 4) illustrated an insignificant ( $p > 0.05$ ) decrease in lipid content in fermented rice bran after 48 h of fermentation. The initial decrease occurred in 48 h, with a reduction of 6.53%, and the highest decrease reached 12.46% in 96 h of fermentation. The wide range of CI is due to the heterogeneity among the data of the articles used and the small sample size ( $n = 3$ ). The reduction in lipid content may be attributed to the utilisation of lipid by microorganisms for chitin synthesis (Oliveira *et al.*, 2010). Furthermore, the

biochemical and physiological changes during the fermentation process require carbohydrates and lipids present in the substrate to be used as carbon sources (Afify *et al.*, 2011).

### 3.3.4 Crude fibre

Data calculations for the crude fibre parameter were evaluated from three studies that met criteria (Oliveira *et al.*, 2010; Kupski *et al.*, 2012; Ribeiro *et al.*, 2017), with fermentation periods of 0 h, 24 h, 48 h, 72 h, and 96 h. The results of the CI calculation indicated that crude fibre content significantly increased during rice bran fermentation, particularly after 24 h (8.37; 95% CI, 5.27 to 11.46;  $p = 0.000$ ). High heterogeneity and a significant effect between treatments were observed ( $I^2 = 89.9\%$ ;  $p < 0.05$ ).

The forest plot (Figure 5) demonstrated a significant ( $p > 0.05$ ) increase in fibre content in fermented rice bran. The highest increase occurred after 96 h of fermentation, reaching up to two-fold compared to non-fermented rice bran. There was no significant difference in the mean value at 48 h and 72 h, with CI values remaining in the same range. The wide range of CI is due to the heterogeneity between data from the articles used and the small sample size ( $n = 3$ ). The intrinsic production of chitin by fungi caused an increase in fibre content (Oliveira *et al.*, 2010). Fungi produce various types of polysaccharides, which may be associated with membranes and cell walls or are intracellular. The types

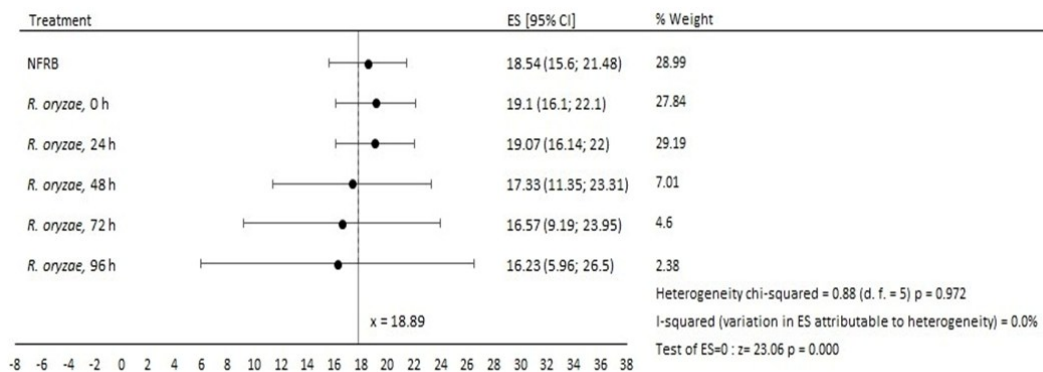


Figure 4. Forest plot of the effect of rice bran fermentation on lipid.

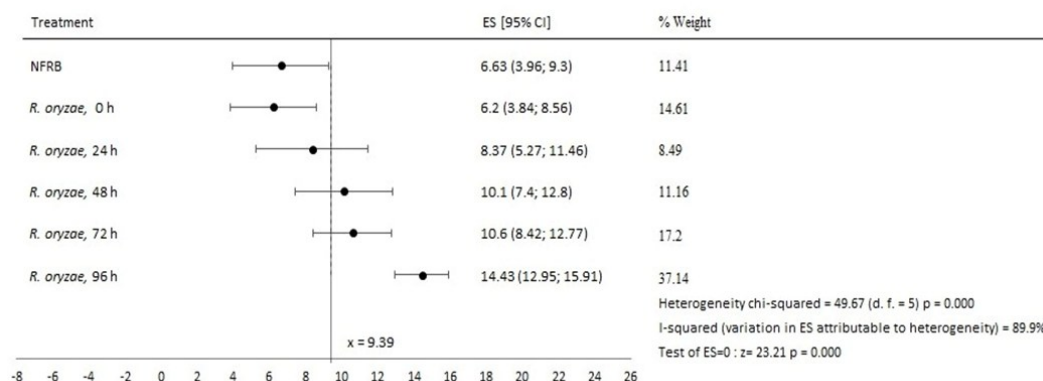


Figure 5. Forest plot of the effect of rice bran fermentation on crude fibre.

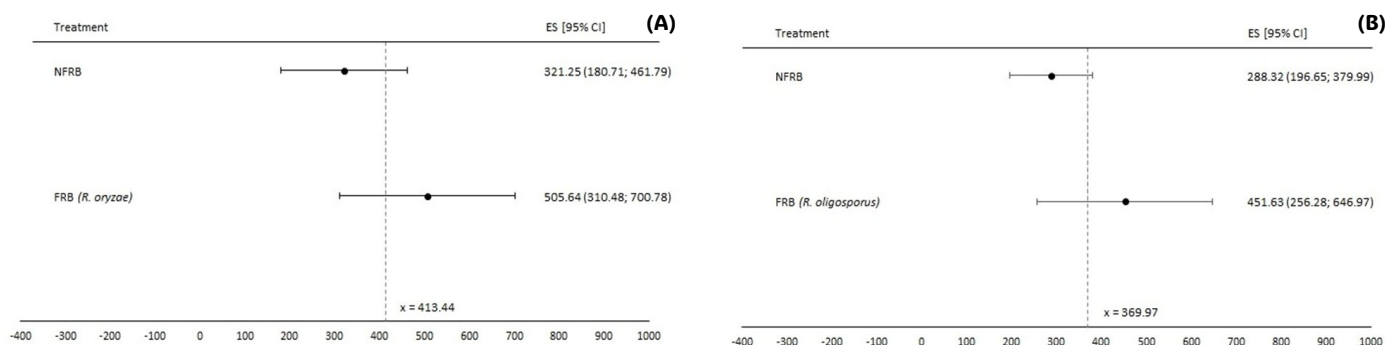


Figure 6. Forest plot of the effect of rice bran fermentation using (a) *R. oryzae* and (b) *R. oligosporus* on TPC.

of fungi polysaccharide are chitin and cellulose (Griffin, 1994). Chitin is a polysaccharide composed of N-acetylglucosamine (Gachhi and Hungund, 2018) and provides structural strength to the exoskeletons of fungal cell walls (Eleih-Ali-Komli and Hamblin, 2016).

### 3.3.5 Total phenolic content

Data from seven studies were evaluated (Razak et al., 2015; Ribeiro et al., 2017; Zulfafamy et al., 2018; Noviasari et al., 2019; Ardiansyah et al., 2019; Souza et al., 2019; Janarny and Gunathilake, 2020), with one study using *R. oryzae* and *R. oligosporus* as starter cultures, three studies using *R. oryzae*, and three studies using *R. oligosporus* with 96 h of fermentation time. The results of the CI calculation indicated that TPC significantly increased during rice bran fermentation using *R. oryzae* and *R. oligosporus* (505.64; 95% CI, 310.48 to 700.78 and 451.63; 95% CI, 256.28 to 646.97, respectively). A significant effect was observed between treatments ( $p = 0.03$ ).

Phenolic compounds in rice generally include derivatives from two groups, hydroxycinnamic acids (ferulic acid, sinapic acid, and isoferulic acid) and benzoic acids (gallic acid, protocatechuic acid, 2,5-dihydroxybenzoic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, and p-coumaric acid) (Zhang et al., 2010; Kupski et al., 2012; Pang et al., 2018). The TPC of the fermented rice bran (Figures 6.a and 6.b) demonstrated an increase, both when fermented using *R. oryzae* and *R. oligosporus*, amounting to approximately 57% more than non-fermented rice bran. The changes in phenolic compound content during fermentation are influenced by the substrate, type of microorganism, and fermentation conditions (Martins et al., 2011). Additionally, each rice variety contains different amounts of phenolic compounds. Several studies have indicated that pigmented rice has a higher TPC than white rice (Pang et al., 2018; Janarny and Gunathilake, 2020).

Phenolic compounds are commonly present as chains of hydrolysable tannins and lignin or linked to cell wall structural components such as cellulose and

proteins via ester linkages (Martins et al., 2011). The increase in phenolic compounds is primarily due to the release of bound phenolics that are attached to cell wall components, facilitated by the activity of fungi's extracellular enzymes (phytase, lignocellulolytic,  $\beta$ -glucosidase,  $\alpha$ -amylase, decarboxylase, esterase, hydrolase) (Nkhata et al., 2018; Omarini et al., 2019; Noviasari et al., 2019; Adebo and Medina-Meza, 2020; Janarny and Gunathilake, 2020). As described by Cabuk et al. (2018), the relaxation of the lignocellulosic matrix during fermentation can release bound phenolic compounds. These bioactive compounds offer various health benefits, including antioxidant, anti-cancer, and anti-inflammatory properties.

### 3.3.6 Antioxidant activity

Various methods have been employed to analyse antioxidant activity in rice bran. Approximately 83.33% of the total articles that passed the first screening discussed the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method for analysing antioxidant activity in rice bran (Figure 7). The DPPH method is commonly used due to its simplicity, speed, sensitivity, and minimal sample requirements. Moreover, DPPH radical is more stable than those used in other methods (Kedare and Singh, 2011).

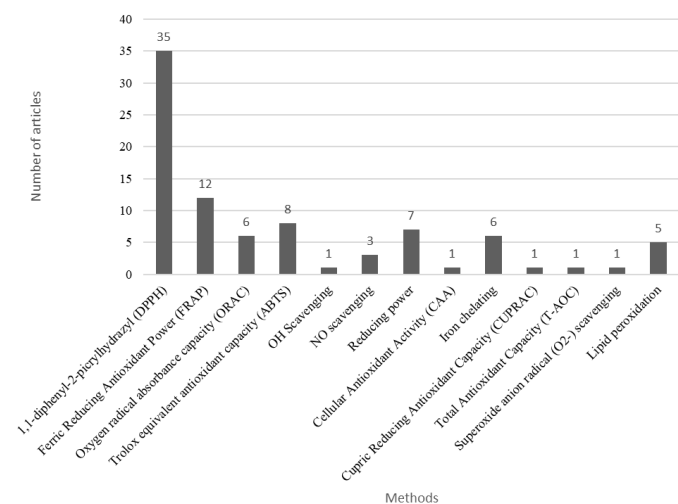


Figure 7. Number of articles based on methods of measuring antioxidant activity.

Table 2. Antioxidant activities of fermented rice bran in various fermentation methods.

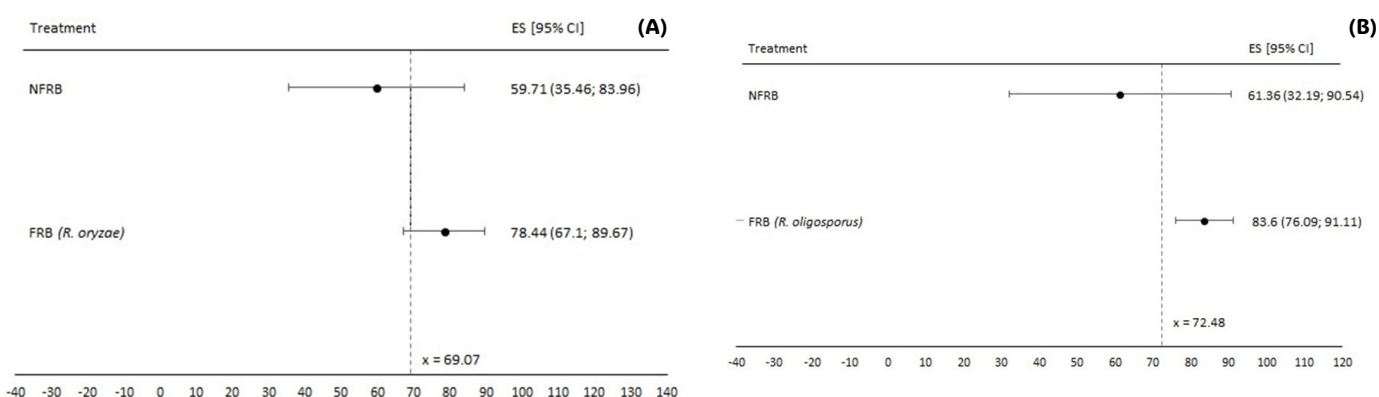
Rice varieties	Fermentation methods and type of microbes	Antioxidant assay	Results	References
21 rice bran varieties (Segyejinmi, Chindeul, Seolgaeng, Gopum, Sunpum, Heonpum, Haedam, Chujum, Wolbaek, Haepum, Anda, Danmi, Goami2, Dasan1, Misomi, Goami4, Ilpum, Migwang, Samkwang, Jungsaenggold, and Haiami)	The submerged liquid fermentation system; <i>Lentinula edodes</i>	DPPH and ORAC	- DPPH radical scavenging activities ranged from 29.23% to 65.13% and from 33.53% to 71.30% in non-fermented and fermented rice brans - The ORAC assay ranged from 220.93 to 641.02 $\mu\text{M TE/g}$ and from 328.13 to 1101.31 $\mu\text{M TE/g}$ in non-fermented and fermented rice brans	Jung et al. (2017)
	Solid State Fermentation (SSF); <i>R. oryzae</i> CTT 1217	DPPH	The rice bran phenolic extract fermented for 96 h with 4.3 mg ferulic acid. $\text{mL}^{-1}$ reduced the DPPH concentration by 50%, 59%, 64%, 66% in 15, 30, 45, and 60 min.	Oliveira et al. (2012)
	Lactic acid fermentation; <i>Pediococcus acidilactici</i> , <i>Lactococcus lactis</i> and <i>Pediococcus pentoseus</i>	DPPH	DPPH radical scavenging activities in the non-fermented rice bran increased from 66.2% to 82.6% after fermentation with <i>P. acidilactici</i> , while the scavenging effects were 77.2% and 71.5% in samples fermented with <i>L. lactis</i> and <i>P. pentoseus</i>	Rashid et al. (2015)
	SSF; <i>R. oryzae</i>	DPPH	The fermentation after 96 h inhibited the DPPH radical by 87%	Kupski et al. (2012)
	SSF; <i>Aspergillus oryzae</i> (strain F0017) and <i>R. oryzae</i> (strain F0013)	DPPH and FRAP	- The highest antioxidant activity was detected in mix-cultured ( <i>A. oryzae</i> + <i>R. oryzae</i> ) rice bran, with the value of 160.88 lg AAE/g sample and 293 98.35 lg AAE/g sample in water and methanol extracts by the FRAP method. - DPPH radical scavenging activity in all fermented samples was not significantly improved ( $p > 0.05$ ) upon fermentation, except for rice bran fermented with <i>R. oryzae</i> , which showed the highest radical scavenging activity of 93.41% and 90.26% in water and methanol extracts.	Razak et al. (2017)
	SSF; <i>R. oligosporus</i> (strain F0020) and <i>M. purpureus</i> (strain F0061)	DPPH and FRAP	- The highest antioxidant activity was detected in mix sample ( <i>R. oligosporus</i> + <i>M. purpureus</i> ) rice bran, with the value of 144.03 $\mu\text{g AAE/g}$ sample in water extract by the FRAP method. - DPPH radical scavenging activity of rice bran fermented with <i>R. oligosporus</i> showed the highest radical scavenging activity of 91.75% and 90.19% in water and methanol extracts.	Razak et al. (2015)

Table 2 (Cont.). Antioxidant activities of fermented rice bran in various fermentation methods.

Rice varieties	Fermentation methods and type of microbes	Antioxidant assay	Results	References
Cempo Ireng	SSF; <i>R. oryzae</i> and <i>R. oligosporus</i>	DPPH and FRAP	<p>- The highest DPPH radical scavenging capacity is found in 96h mix sample (<i>R. oryzae</i> + <i>R. oligosporus</i>), with the value of 11.64 mg TE/g dry weight sample for the methanolic extract and <i>R. oryzae</i> 72 h for 70% ethanolic extract, with the value of 16.02 mg TE/g dry weight sample.</p> <p>- The results of the FRAP assay showed the highest antioxidant capacity was found in the <i>R. oryzae</i> 72 h sample for the methanolic fraction, with the value of 7.93 mg TE/g dry weight sample, and the mix sample (<i>R. oryzae</i> + <i>R. oligosporus</i>) 96h for the ethanolic fraction with the value of 13.30 mg TE/g dry weight sample.</p>	Zulfafamy et al. (2018)
Cempo Ireng	SSF; <i>R. oligosporus</i>	DPPH	<p>- The DPPH free radical scavenging activity in non-fermented rice bran was 26.28±4.16%, which significantly increased (<math>p &lt; 0.05</math>) to 76.91±0.06% after 96h of fermentation.</p>	Noviasari et al. (2019)
	SSF; <i>M. purpureus</i>	DPPH and FRAP	<p>- Antioxidant potential evaluated using FRAP analysis exhibited an enhancement in fermented substrates from 30.22±9.57 mg AAE/g sample to 61.21±4.50 mg AAE/g sample.</p> <p>- The radical scavenging activity evaluated using DPPH assay showed a decrease in fermented rice bran from 87.82±2.41% to 85.14±0.12%.</p>	Jamaluddin et al. (2016)
	Heat-stabilized, defatted rice bran; <i>Bacillus subtilis</i> , <i>B. amyloliquefaciens</i> , <i>B. licheniformis</i> , and <i>B. pumilus</i>	DPPH	<p>Antioxidant activity of fermented samples increased until reaching a maximum at 96h, with a value 79.7%.</p>	Webber et al. (2014)
	SSF; <i>Monascus pilosus</i>	DPPH, ABTS, iron chelating activity, FRAP	<p>- The DPPH radical scavenging activity of the fermented sample did not significantly (<math>p &gt; 0.05</math>) compare with NFRB.</p> <p>- The ABTS radical scavenging activity increased 20% after fermentation.</p> <p>- Iron chelating activity increased from 32% to 55% after fermentation at a concentration of 0.5 mg/mL.</p> <p>- Reducing power increased about 1.6-fold after fermentation at a concentration of 2 mg/mL.</p>	Cheng et al. (2016)

Table 2 (Cont.). Antioxidant activities of fermented rice bran in various fermentation methods.

Rice varieties	Fermentation methods and type of microbes	Antioxidant assay	Results	References
Four varieties (Bg352, Bw367, Bg406, and H4)	SSF; <i>R. oryzae</i>	DPPH, FRAP, and the term total antioxidant capacity (T-AOC)	<p>- The DPPH radical scavenging activity of all four rice varieties increased about 31.53%-83.87% with fermentation.</p> <p>- Reducing the power of Bw367 and Bg352 has increased with fermentation by 58.31% and 33.69%, meanwhile reducing the power of Bg406 and H4 has decreased with fermentation by 82.66% and 83.66%.</p> <p>- The total antioxidant capacity of Bw367 and Bg352 has increased with fermentation by 38.78% and 44.32%, meanwhile the total antioxidant capacity of Bg406 and H4 has decreased by 69.09% and 21.07%.</p>	Janarny and Ghunatilake (2020)
Three varieties (Inpari 6, Inpari 30, and Inpara 1)	SSF; <i>R. oligosporus</i>	DPPH	The DPPH radical scavenging activity of the samples increased with fermentation, the highest increase was in the Inpari 30 variety (incubation time 72 h), with the value of 83.71±0.61%.	Ardiansyah et al. (2019)
	SSF and high hydrostatic pressure treatment; <i>Issatchenkia orientalis</i> MFST1	DPPH and linoleic acid (lipid) peroxidation	The DPPH radical scavenging activity of the control (hot-water extraction of FRB) and CHFRB sample (high hydrostatic pressure treatment of FRB with complex enzymes) showed similar scavenging activities of 85.15% and 86.59% at 1 mg/mL, respectively, while the HFRB sample (high hydrostatic pressure treatment of FRB without enzymes) produced a lower activity of 74.53% at 1 mg/mL.	Kim and Han (2012)
BR-IRGA 417 variety	SSF; <i>R. oryzae</i>	DPPH and linoleic acid (lipid) peroxidation	The phenolic extract of fermented rice bran showed slow inhibition of the DPPH radical, presenting an EC <sub>50</sub> value of 250 mg/g <sub>DPPH</sub> .	Schmidt et al. (2014)

Figure 8. Forest plot of the effect of rice bran fermentation using (a) *R. oligosporus* and (b) *R. oryzae* on antioxidant activity.

Each study that passed the first screening (42 papers) was reviewed manually to present the results of the antioxidant activity of fermented rice bran across various fermentation methods (Table 2). Many studies reported an increase in antioxidant activity with fermentation; however, some studies indicated a decrease in antioxidant activity following fermentation.

Data were evaluated from six studies (Oliveira et al., 2012; Kupski et al., 2012; Razak et al., 2015; Razak et al., 2017; Noviasari et al., 2019; Ardiansyah et al., 2019), with three studies using *R. oryzae* and three studies using *R. oligosporus* as starter cultures. The results of the CI calculation indicated that antioxidant activity significantly increased during rice bran fermentation using *R. oryzae* and *R. oligosporus* (78.44;

95% CI, 67.1 to 89.67 and 83.6; 95% CI, 76.09 to 91.11, respectively). A significant effect was observed between treatments ( $p = 0.01$ ).

The results (Figure 8.a and 8.b) showed that antioxidant activity significantly increased during rice bran fermentation using *R. oryzae* and *R. oligosporus*. The mean value of antioxidant activity increased by 31.3% and 35.8% in 96 h fermented rice bran using *R. oryzae* and *R. oligosporus*, respectively. The wide range of CI is due to the heterogeneity between data from the articles used and the small sample size. The free and bound phenolics significantly contributed to antioxidant activity. Phenolic compounds donate electrons and hydrogen to free radicals, activate endogenous antioxidant mechanisms, and act as metal chelators (Ghasemzadeh and Ghasemzadeh, 2011; Juurlink et al., 2014; Sevgi et al., 2015). Moreover, Adom and Liu (2002) reported that 71% of the bound phytochemicals in rice play a role as contributors to total antioxidant activity. The antioxidative potential of rice bran was not only associated to any single compound but the combined effect of dietary fibers, phenolic compounds, and other bioactive compounds present in the rice bran (Martins et al., 2011).

#### 4. Conclusion

The present study revealed that the fermentation process affected the nutritional value of rice bran, increasing ash by 45.67%, increasing protein by 82%, increasing crude fibre by two-fold, and reducing lipid by 12.46%. In addition, fermentation caused changes in profile phenolic acids i.e. ferulic acid, sinapic acid, syringic acid, p-coumaric acid, caffeic acid, and vanillic acid. There was a significant increase in TPC and antioxidant activity in fermented rice bran, almost two-fold than non-fermented rice bran, mainly due to the release of bound phenolics to free form. The antioxidative potential of rice bran was associated with the combined effect of dietary fibers, phenolic compounds, and other bioactive compounds present in the rice bran. The fermentation was shown to be an effective method to enhance rice bran nutritional value, bioactive compound, and antioxidant properties.

#### Conflict of interest

The authors declare no conflict of interests.

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