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The Optimal Condition of Dry-Heat Stabilization using Oven on Phenolic Content and Antioxidant Activity of Rice Bran: A Meta-Analysis

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ABSTRACT

Rice bran, a beneficial by-product of rice milling, is a rich source of nutrition, containing bioactive compounds such as phenolic compounds and exhibiting high antioxidant activity. Due to these properties, rice bran is a valuable ingredient for functional foods and animal feed. However, its short shelf life caused by rapid rancidity often hinders its use. Dry heating is an effective method to increase the longevity of rice bran. It can be stabilized by heating rice bran to the appropriate temperature, retaining its nutritional value and prolonging its shelf life. This meta-analysis aimed to determine the optimal temperature and time duration for dry-heat stabilization using an oven on Free Phenolic Content (FPC), Bound Phenolic Content (BPC), Total Phenolic Content (TPC), and Antioxidant Activity (AA) of rice bran. A total of 7 articles and 34 experiments were included after applying specified screening criteria. Results indicated that temperature and time duration of dry-heat stabilization had a significant effect ($p<0.05$) on FPC and TPC, but not on BPC and AA. The present study suggests that a temperature range of 105-140°C and heating time of 20-30 min is best for stabilizing rice bran, considering its nutrient quality and extend shelf life. In conclusion, the optimal temperature and time duration of dry-heat stabilization using an oven could stabilize the quality and maintain the phenolic content of rice bran.

Keywords: Stabilized rice bran, Phenolic content, Antioxidant activity, Meta-analysis

Introduction

Rice bran is a by-product obtained from the rice milling process, consisting of the germ and the outer layer of paddy, including the pericarp, seed coat, and aleurone layer (Loypimai, 2015). Rice bran is harvested about 8-10% of the total paddy used (Sharif *et al.*, 2014). Approximately 90% of rice bran production is used as feed (Kahlon, 2009). Rice bran has a high energy and nutrient content, including 21.9 MJ/kg gross energy (Casas, 2019), 11-17% crude protein, 6-14% crude fiber, and 15-22% crude fat (Sharif *et al.*, 2014). Due to its high lipid content and the presence of oxidative and hydrolytic enzymes such as lipase, rice bran is prone to rancidity during storage (Thanonkaew *et al.*, 2012). Lipase is activated as soon as the bran is separated from the rice (Ertürk and Meral, 2019) and can break down lipids into free fatty acids, increasing free fatty acids content of 1-7% per day under favorable conditions (Arora *et al.*, 2015). When the free fatty acid content in rice bran exceeds 5%, rancidity can occur, negatively impacting its quality (Patil, 2016). These factors, including the high lipid content and lipase activity, may limit the use of rice bran as animal feed.

Despite its good nutrient composition, rice bran also contains bioactive compounds that provide health benefits. Rice is rich in phenolic content, which is a general bioactive component in rice bran (Zhao *et al.*, 2018). Phenolic content has antioxidant properties that act as an anti-free radical defense system, protecting against oxidative damage and harmful pathogens (Dai and Mumper, 2010; Abavi *et al.*, 2013). The higher the concentration of the phenolics, the greater the antioxidant activity (Mardiah *et al.*, 2022). Antioxidants can prevent oxidation by interacting with free radicals formed during the early stages of the oxidation process. This interaction prevents the formation of fatty acid radicals and consequently terminates the chain reaction (Waheed *et al.*, 2004).

To preserve the biological quality of rice bran, it is essential to begin the stabilization process soon after the milling process to prevent the hydrolysis of rice bran. This process is necessary to inhibit the release of free fatty acids by inactivating the lipase enzyme, which helps

extend the shelf life of rice bran (Malekian *et al.*, 2000). Various techniques are available for stabilizing rice bran, including physical methods like heat treatment. Heat treatment eliminates harmful microorganisms and affects rice bran's sensory and functional properties, thereby maintaining its quality (Liu, 2018; Meral, 2021). The most widely used method for stabilization is dry heating using a conventional oven (Thanonkaew *et al.*, 2012; Meral, 2021). Dry heating is a simple, convenient, cost-effective, and efficient technology suitable for small and medium-sized businesses and can be scaled up for industrial purposes (Thanonkaew *et al.*, 2012; Sharma, 2014; Yilmaz, 2016).

Many studies have been conducted about the dry heating stabilization of rice bran variations in temperature and heating time. However, the results are diverse; some studies suggest that heating stabilization relatively increases the phenolic content (Ertürk and Meral, 2019; Saji, 2019), while others report contradictory results (Liao, 2022). The variation in results may be influenced by different technologies and operational conditions (Mardiah *et al.*, 2022). Therefore, the objective of the present study is to conduct a meta-analysis of previous studies to determine the effectiveness and optimal conditions of dry-heat stabilization using an oven on phenolic content and antioxidant activity.

Materials and Methods

Searching strategy and screening data

The search for scientific articles published in English on rice bran stabilization using an oven was conducted in March 2023. The article search process included several stages: (1) Searching for specific keywords on various databases, (2) Screening the titles and abstracts to identify relevant articles, (3) Applying specified criteria to select relevant literature, and (4) Conducting a meta-analysis of the selected articles.

The search process used the Publish or Perish software to search for relevant articles on the Scopus and Pubmed databases using the keywords “rice bran” and “stabilization”. The search resulted in 208 potential references. Subsequently, these potential references were screened based on the title and the abstract, resulting in 85 articles. After further review, 123 articles were eliminated because they did not focus on the stabilization process of rice bran.

The screening process continued based on specific criteria, including (1) Literature published in English, (2) Experimental literature (not a review), (3) Free access, (4) Rice bran stabilization using an oven, (5) Discussion of the lipase activity, phenolic content, and antioxidant activity of oven-stabilized rice bran, (6) Data presented in a table (containing standard deviation and several samples or replications). The screening process eliminated 78 articles because they were not published in English and were not freely accessible (9 articles), were not experimental articles (5 articles), were not relevant (not about the dry heating stabilization using an oven, and the variables were not relevant) (58 articles), and did not contain the standard deviation and the number of samples (6 articles). Ultimately, 7 articles and 34 experiments were eligible for the present meta-analysis (Table 1). Details of the literature selection process are presented in the PRISMA-P flowchart (Figure 1).

The relevant data from each article were extracted and collected in a spreadsheet, including article source, temperature and duration time of heating, number of control and experimental samples, mean of control and experimental samples, and standard deviation. The OpenMEE software was used to perform the meta-analysis process. If the meta-analysis resulted in a significant effect, the process continued to analyze the subgroups based on temperature and duration time concerning the variable of interest. Due to the diversity of temperature and duration time data, each subgroup was divided into three ranges. The temperature range included temperatures ≤100°C (70°C, 90°C, and 100°C), temperatures between 105-140°C (105°C, 110°C, 120°C, 130°C, and 140°C), and temperatures ≥150°C (150°C dan 160°C). The duration time range included times ≤15 min (10 and 15 min), times between 20-30 min (20 and 30 min), and times >30 min (40, 60, and 90 min).

Statistical analysis

The effect size was utilized to determine the parameter distance between the control sample and the experimental sample. Hedges' method was chosen because it can calculate the effect size regardless of heterogeneity in sample size, measurement unit, and statistical test results, and it is suitable for estimating the effect of paired treatments (Sanchez-Meca and Marin-Martinez, 2010). In this study, unstabilized rice bran was pooled into the control group, while stabilized rice bran prepared using an oven was combined into the experimental group. The effect size (d) was calculated using the following formula:

$$d = \frac{x^E - x^C}{S} J$$

The X^E is the mean value from the experimental group, while the X^C is the mean value of the control group. J is the correction factor for small sample size, i.e:

$$J = 1 - \frac{3}{(4(N^C + N^E - 2) - 1)}$$

while S is the pooled standard deviation, i.e:

$$S = \sqrt{\frac{(N^E - 1)(s^E)^2 + (N^C - 1)(s^C)^2}{(N^E + N^C - 2)}}$$

Where N^E is the sample size of the experimental group, N^C is the sample size of the control group, S^E is the standard deviation of the experimental group, and S^C is the standard deviation of the control group. The variance of Hedges’d (V_d) is described as follows:

$$V_d = \frac{(N^C + N^E)}{(N^C N^E)} + \frac{d^2}{(2(N^C + N^E))}$$

Table 1. Studies selected for the meta-analysis

No.	Study	Temperature (°C)	Time (min)	FPC	BPC	TPC	AA
1.	Meral (2021) 1	110	20	-	-	-	√
2.	Meral (2021) 2	130	15	-	-	-	√
3.	Meral (2021) 3	150	10	-	-	-	√
4.	Erturk and Meral (2019) 1	120	15	√	√	√	-
5.	Erturk and Meral (2019) 2	140	15	√	√	√	-
6.	Erturk and Meral (2019) 3	160	15	√	√	√	-
7.	Mardiah *et al.* (2022) 1a	70	10	√	√	√	√
8.	Mardiah *et al.* (2022) 2a	70	30	√	√	√	√
9.	Mardiah *et al.* (2022) 3a	70	60	√	√	√	√
10.	Mardiah *et al.* (2022) 4a	90	10	√	√	√	√
11.	Mardiah *et al.* (2022) 5a	90	30	√	√	√	√
12.	Mardiah *et al.* (2022) 6a	90	60	√	√	√	√
13.	Mardiah *et al.* (2022) 7a	110	10	√	√	√	√
14.	Mardiah *et al.* (2022) 8a	110	30	√	√	√	√
15.	Mardiah *et al.* (2022) 9a	110	60	√	√	√	√
16.	Mardiah *et al.* (2022) 10a	160	10	√	√	√	√
17.	Mardiah *et al.* (2022) 11a	160	30	√	√	√	√
18.	Mardiah *et al.* (2022) 12a	160	60	√	√	√	√
19.	Mardiah *et al.* (2022) 1b	70	10	√	√	√	√
20.	Mardiah *et al.* (2022) 2b	70	30	√	√	√	√
21.	Mardiah *et al.* (2022) 3b	70	60	√	√	√	√
22.	Mardiah *et al.* (2022) 4b	90	10	√	√	√	√
23.	Mardiah *et al.* (2022) 5b	90	30	√	√	√	√
24.	Mardiah *et al.* (2022) 6b	90	60	√	√	√	√
25.	Mardiah *et al.* (2022) 7b	110	10	√	√	√	√
26.	Mardiah *et al.* (2022) 8b	110	30	√	√	√	√
27.	Mardiah *et al.* (2022) 9b	110	60	√	√	√	√
28.	Mardiah *et al.* (2022) 10b	160	10	√	√	√	√
29.	Mardiah *et al.* (2022) 11b	160	30	√	√	√	√
30.	Mardiah *et al.* (2022) 12b	160	60	√	√	√	√
31.	Saji *et al.* (2019)	130	20	√	√	-	-
32.	Irakli *et al.* (2020)	150	40	√	√	-	√
33.	Wanyo *et al.* (2014)	120	30	-	-	√	√
34.	Thanonkaew *et al.* (2012)	150	10	-	-	√	-

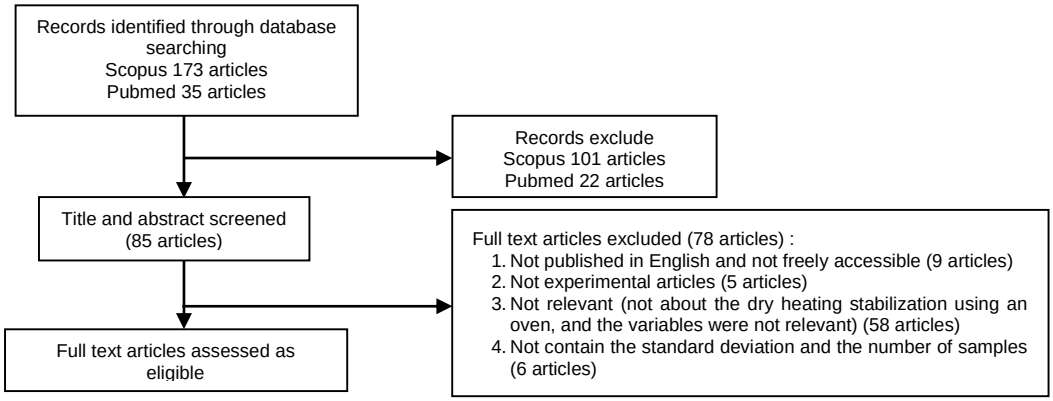

Figure 1. Flow chart of the literature selection process based on PRISMA protocols.

The cumulative effect size (d++) was formulated as follows:

$$d_{++} = \frac{(\sum_{i=1}^{n} W_i d_1)}{(\sum_{i=1}^{n} W_i)}$$

Here, wi represents the inverse of the sampling variance (1/Vd). To measure the accuracy of the effect size, a 95% confidence interval (CI) was used. The formulas mentioned above were obtained from the study by Sanchez-Meca and Marin-Martinez (2010).

To identify publication bias caused by non-significant studies that were not included in the analysis, a Fail-Safe Number (Nfs) was calculated. If Nfs > 5N + 10, then there is substantial evidence of a robust meta-analysis model. The method of Rosenthal (1979) was employed to calculate Nfs, where the smallest sample size from individual studies was used as N. OpenMEE was used to perform all calculations related to the effect size and fail-safe number (Wallace *et al.*, 2017).

Results and Discussion

Publication bias

Before conducting a meta-analysis study, it is important to first identify any biases in the data used in the present study as they can impact the meta-analysis results. The fail-safe N limit in Table 2 was calculated using the formula from Rosenthal (Rosenthal, 1979). According to Table 2, the fail-safe N values for FPC and TPC are above the limit, indicating a robust meta-analysis model with no publication bias. However, the fail-safe N values for

Table 2. Fail-safe N calculation

Response variable	Observed significance level	Target significance level	Fail-safe N	Fail-safe N limit
FPC	<0.0001	0.05	163	155
BPC	0.0006	0.05	84	155
TPC	<0.0001	0.05	208	155
AA	0.0042	0.05	46	155

FPC: free phenolic content, BPC: bound phenolic content, TPC: total phenolic content, AA: antioxidant activity.

BPC and AA are below the limit, suggesting a potential bias that could affect the results. Therefore, the data used for BPC and AA should be interpreted with caution.

Effect of temperature and time duration of dry heating stabilization using oven method

The dry heating method was used to stabilize rice bran due to the basic theory that lipase is unstable and easily inactivated at high temperatures because of protein denaturation (Sharma *et al.*, 2017). The high-temperature treatment can also degrade the cell wall, increasing the release of antioxidant compounds. Table 3 shows that dry heat stabilization using an oven has a significant effect on FPC ($p<0.001$) and TPC ($p<0.05$). However, there was no significant effect ($p>0.05$) on BPC and AA. According to Table 2, the estimated value of FPC and TPC showed a positive trend, indicating that stabilization using an oven can increase the phenolic content. However, the bound phenolic content was not affected. This is because free phenolics are found in plant cell vacuoles, while the bound phenolics are found in plant cell walls such as cellulose and hemicellulose, making it difficult to extract bound phenolics directly (Acosta-Estrada *et al.*, 2014; Shahidi and Yeo, 2016; Pang *et al.*, 2018). Meanwhile, TPC represents the total amount of phenolic compounds including both free phenolic and bound phenolic compounds. The localization of free phenolic dan bound phenolic compounds is shown in Figure 2, and Figure 3 shows the representative of plant cell wall structure and the cross-linking between structural components and phenolic compounds. Specific conditions are required to extract bound phenolics from the cell wall (Meral, 2021). Thereby, dry heat stabilization using an oven does not significantly affect the bound phenolic content. The present study also found that dry heat stabilization using an oven did not significantly affect the antioxidant activity of rice bran. This may be due to the absence of a significant effect on the bound phenolics content. Previous studies reported that free phenolic content showed higher radical scavenging activity compared to bound phenolics (Min *et al.*, 2012; Wanyo *et al.*, 2014). However, it should be noted that bound phenolics are the major phenolic compounds of grains, it's about 250% more than the free phenolics (Ertürk and Meral, 2019; Mardiah *et al.*, 2022). This finding is similar to the result reported by other studies, which showed no significant difference in the antioxidant activity of rice bran with different heating treatments (Wanyo *et al.*, 2014; Siswanti *et al.*, 2019). The publication bias calculated previously predicted no significant effect on BPC and AA.

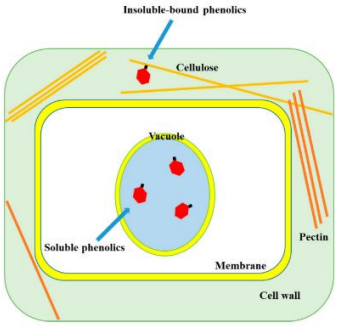

Figure 2. Localization on the free phenolic compound and bound phenolic compound in plant cell. Source: Shahidi and Yeo (2016).

Effect of temperature and duration time of heating on free phenolic content

Free phenolics originate from the plant cell content (vacuoles) and can be produced at higher levels by destroying the plant cell wall through heating. In this study, heating temperatures of ≤ 100°C and ≥150°C had a significant effect ($p<0.05$), while temperatures of 105 – 140°C did not have a significant effect ($p>0.05$) on FPC. However, the present study differs from the literature that there was no significant difference in FPC at temperatures of 105 – 140°C, despite having a relatively high estimated value of increased FPC (Table 4). Mardiah (2022) mentioned that FPC in rice bran increased from a temperature of 90°C and persisted until 160°C, but the rice bran heated at 160°C did not show a significant difference in FPC compared to unstabilized rice bran. The significant increase of FPC at temperatures ≥150°C in this study may be due to the fact that higher temperatures can easily break the cell walls and potentially enhance the levels of bioactive compounds and improve the bioavailability of phenolics (Chen, 2019). However, it also has the potential to damage phenolic compounds and other nutrient compounds, as evidenced by the lower estimated value at temperatures ≥150°C compared to others (Table 4). Phenolic compounds are easily degraded by various factors such as temperature, light, and oxygen, with higher temperatures leading to the destruction of most phenolic compounds (Siswanti *et al.*, 2019). Meanwhile, heating at lower temperatures (<100°C) poses less risk of damaging phenolic content and other nutrient compounds (Table 4), but requires a longer time to increase the free phenolic content. This finding is still debatable. The research findings by Meral (2021) also indicate that the phenolic content of rice bran heated at a temperature of 110°C does not significantly differ compared to those without heating treatment. On

Table 3. Effect of dry heat stabilization using an oven on phenolic compounds, and antioxidant activity of rice bran

			Model results						Heterogeneity		
Response variable	N	Unit	Estimate	Lower bound	Upper bound	SE	*P*-value	τ^2	Q	Het *P*-value	I^2
FPC	29	mg GAE/100 g	0.904	0.458	1.35	0.227	< 0.001	0.301	35.157	0.165	20.357
BPC	29	mg GAE/100 g	0.569	-0.4	1.538	0.494	0.25	4.748	108.283	< 0.001	74.142
TPC	29	mg GAE/100 g	1.014	0.208	1.819	0.411	0.014	3.050	84.336	< 0.001	66.799
AA	28	%	0.651	-0.189	1.491	0.429	0.129	3.566	92.596	< 0.001	69.761

N: number of data, SE: standard error, τ^2: variance of the effect size parameters across the study populations, Q: weighted sum of squared deviations, Het *P*-value: *P*-value for heterogeneity, I^2: heterogeneity level between studies, FPC: free phenolic content, BPC: bound phenolic content, TPC: total phenolic content, AA: antioxidant activity, GAE: gallic acid equivalent.

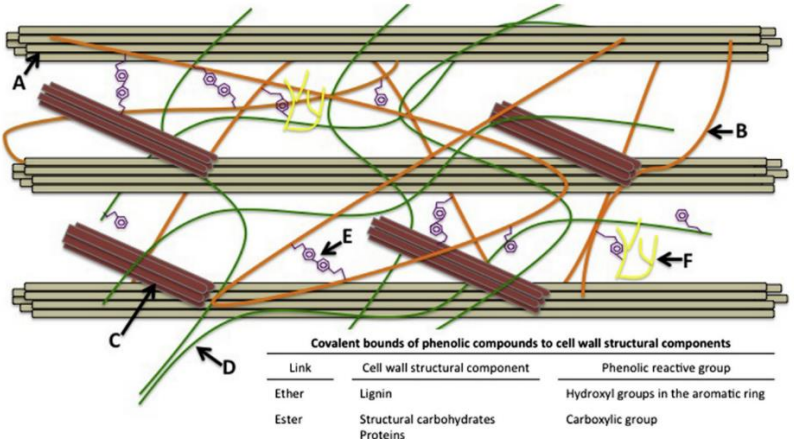

Figure 3. Representation of plant cell wall structure and the cross-linking between structural components and phenolic compounds. A: Cellulose, B: Hemicellulose, C: Structural protein, D: Pectin, E: Phenolic acids, F: Lignin. Source: Acosta-Estrada *et al.* (2014).

the other hand, the research findings by Irakli (2021) indicate that heating rice bran decreases the free phenolic content, while another study by Mardiah *et al.* (2022) shows the opposite. The variation in results could be attributed to various factors, including extraction parameters, variations in varieties, the type of bran used, and environmental conditions during the crop's growth period that might influence the composition subsequently affect the effects of the stabilization treatments (Irakli, 2021). The duration of the heating process can also impact the phenolic content. Based on Table 4, a heating duration time of 20 – 30 min had the most significant influence ($p<0.01$) in increasing FPC compared to a heating time of >30 min. This finding is supported by Loypimai (2009), who reported that increasing temperature and heating duration time can decrease the phenolic content of materials.

Effect of temperature and duration time of heating on total phenolic content

The present study investigated the effect of dry heating stabilization on the total phenolic content, and it was found that there was an inverse relationship between FPC and TPC. The 105 - 140°C temperature range did not significantly affect the FPC. However, based on Table 5, this temperature range had the most significant effect on increasing the TPC ($p<0.001$) compared to other temperature ranges. The inconsistent results in this study may be since most phenolic compounds in rice bran are bonded with the cell wall, which is approximately two times higher than free phenolic content (Yilmaz, 2015). This indicates that there are different temperature effects on the FPC and TPC. Moreover, higher heating temperatures above 150°C can destroy phenolic content, thus decreasing the TPC and FPC (Irakli *et al.*, 2020; Pokkanta, 2022).

Meanwhile according to Table 5, heating for a duration time of ≤ 15 min had the most significant effect ($p<0.05$) on increasing the total phenolic content. This is indicating that a short period of high-temperature heating can already increase the total phenolic content of rice bran. This finding is consistent with the study by Rodchuajeen *et al.* (2016) who found that shorter heating time at high temperatures can decrease the loss of total phenolic content.

Table 4. Sub-group effect of heating temperature and duration time on free phenolic content

Subgroup	Range	Estimate	Lower bound	Upper bound	Std. Error	P-value
Temperature (°C)	≤100	1.05	0.159	1.941	0.454	0.021
	105 - 140	1.043	-0.286	2.371	0.678	0.124
	≥ 150	0.729	0.13	1.328	0.306	0.017
Time duration (min)	≤15	0.653	-0.018	1.324	0.342	0.056
	20 - 30	1.105	0.393	1.817	0.363	0.002
	>30	0.967	-0.129	2.063	0.559	0.084

Table 5. Sub-group effect of heating temperature and duration time on total phenolic content

Subgroup	Range	Estimate	Lower bound	Upper bound	Std. Error	p-value
Temperature (°C)	≤100	1.075	-0.865	3.015	0.99	0.277
	105 - 140	2.104	1.181	3.027	0.471	< 0.001
	≥150	0.299	-0.775	1.374	0.548	0.585
Time duration (min)	≤15	1.855	0.205	3.506	0.842	0.028
	20 - 30	0.39	-0.659	1.44	0.535	0.466
	>30	1.012	-0.479	2.502	0.76	0.183

Conclusions

It was observed that the stabilization process has varying effects depending on the temperature and duration of heating. Dry heat stabilization using an oven affects the free phenolic and total phenolic content but does not impact the bound phenolic content and antioxidant activity of rice bran. The optimal condition for increasing the phenolic content is to heat the bran at high temperatures for a short period or, conversely, heat it at low temperatures for a longer period. Based on these findings, it is recommended to dry heat rice bran using an oven at temperatures ranging from 105°C to 140°C for a duration of 20 to 30 min. This approach effectively preserves the quality and extends the shelf life of rice bran.

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