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Research article

Lipase activity, phenolics content and antioxidant activity of rice bran stabilized using natural versus forced convective drying

Zahara Mardiah[a,b,†], Dian Shofinita[a,†], Johnner P. Sitompul[a,†,*]

[a] *Department of Chemical Engineering, Institute of Technology Bandung, Bandung 40132 Indonesia*

[b] *Ministry of Agriculture, Jakarta 1255, Indonesia*

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Abstract

Importance of the work: Some studies show that thermal stabilization may degrade the phenolic components in rice bran, while others indicates the opposite.

Objectives: To evaluate the effects of natural convective stabilization (NCS) and forced convective stabilization (FCS) on rice bran stability.

Materials & Methods: Freshly milled rice bran was distributed uniformly on aluminum pans to a thickness of 0.8 cm. Then, the pans were placed in a preheated oven. Thermal stabilization was performed at 70°C, 90°C, 110°C or 160°C with time intervals of 10 min, 30 min or 60 min for each type of stabilization. The lipase activity, total phenolics content (TPC) and antioxidant activity were determined

Results: Stabilization treatments that began at 90°C or higher lowered the lipase activity in rice bran. The operating conditions of 160°C for 60 min produced the most significant decrease in lipase activity and the highest improvement in the TPC and antioxidant activity. The enhancement of total phenolics and antioxidant activity of rice bran was more effective using FCS compared to NCS. FCS decreased the lipase activity by 73.2%, while increasing the TPC and antioxidant activity by 12.5% and 8.8%, respectively. Ultra-high-performance liquid chromatography analysis revealed the presence of a peak found only in FCS rice bran. This peak might be a marker of adequate rice bran stabilization.

Main finding: Forced convective stabilization may be considered as an alternative approach for effectively inhibiting lipase activity in rice bran, while increasing its phenolics content and antioxidant activity. Thus, this approach can improve the nutritional value and economic viability of rice bran.

† Equal contribution.
* Corresponding author.
E-mail address: Sitompul@che.itb.ac.id (J.P. Sitompul)

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Introduction

Rice bran has acquired considerable attention in the functional food sector due to bioactive compounds that help metabolic processes in the body and provide health benefits (Sharif et al., 2014; Chakraborty and Budhwar, 2018). Rice bran is high in phenolics that can be extracted and utilized in the production of nutritional supplements (Goufo and Trindade, 2014). Phenolic acids are the most prevalent bioactive components in rice bran (Aparecida et al., 2012; Zhao et al., 2018). Phenolics possess antioxidant properties and have been shown to be beneficial in preventing cancer (Dai and Mumper, 2010), lowering the risk of cardiovascular disease and, lowering the risk of type 2 diabetes (Morton et al., 2000), promoting weight loss (Hsu and Yen, 2008), enhancing the immune system and acting as an anti-inflammatory (Ambriz-pérez et al., 2016).

Despite its great biological value, rice bran is frequently wasted in favor of animal feed. Once removed from paddy grain, the rice bran cells rupture, allowing lipids to interact with highly reactive lipases, resulting in lipid oxidation and rancidity (Irakli et al., 2018). The commercial process of rice milling produces low-value goods due to rice bran containing lipase, which hydrolyzes and oxidizes lipids (Thanonkaew et al., 2012). As this process persists throughout storage, rice bran must be stabilized immediately after milling to avoid lipolysis (Ramezanzadeh et al., 1999). Stabilization is highly beneficial in preventing the release of free fatty acids by inactivating lipase; thus, its shelf life is extended, and commercialization for human consumption is enabled (Malekian et al., 2000; Patel, 2012).

Rice bran can be stabilized chemically or physically. Chemical stabilization was attempted by reducing the pH of the rice bran to 4.0, but this approach was deemed ineffective (Akhter et al., 2015). On the other hand, physical stabilization is accomplished by ultraviolet (UV) irradiation, ultrasound, or heat application, such as hot-air heating, ohmic heating, microwave heating, or infrared heating (Buranathai et al., 2015). Hot-air heating is a cost-effective and efficient technology that is well suited for small and medium-sized enterprises (Thanonkaew et al., 2012; Yilmaz, 2016). Apart from inactivating rice bran lipase, heat treatment also destroys microorganisms and preserves the nutritious value of rice bran (Liu et al., 2018). In creating a novel rice bran-based product, it is very important to inactivate lipase while retaining the bioactive nutrients; therefore, it is important to understand how stabilization affects the phenolics concentration and antioxidant capacity (Vallabha et al., 2015). According to Setyaningsih et al. (2016) and Pace et al. (2018), heating phenolic compounds to 70°C resulted in a 3–12.5% in the phenolics content. Some studies have suggested that stabilization can cause damage to phenolic compounds (Liao et al., 2020), but other research suggests that the opposite is true (Ertürk and Meral, 2019; Saji et al., 2020). The variation in findings may be related to various stabilizing technologies and their operating conditions. Thanonkaew et al. (2012) applied natural convective stabilization and discovered that rice bran oil produced contained more nutrition than unstabilized rice bran. However, the effect on the bioactive content in the rice bran has not been studied.

Several researchers have conducted studies on rice bran stabilization using hot-air drying (Aparecida et al., 2012; Thanonkaew et al., 2012; Bhosale and Vijayalakshmi, 2015; Yu et al., 2020); however, understanding of the effects of temperature and time is still limited. Operating conditions have a significant impact on the outcomes of rice bran stabilization; hence, a thorough understanding of this is essential. In addition, there is no evidence in the published literature of studies comparing hot-air stabilization using forced and natural convection. Therefore, the current study aimed to evaluate the influence of thermal stabilization on the lipase activity, phenolics content and antioxidant activity of rice bran. Specifically, the study assessed the effect of natural and forced convective stabilization under a variety of operating conditions.

Materials and Methods

Sample preparation

The sample used was Ciherang rice variety obtained from the Indonesian Center for Rice Research in West Java, Indonesia. The rice was milled (Satake THU 35A mini husker) to remove the husks. Then, a Satake TM-05 mini polisher was used to obtain the rice bran. The rice bran was sieved through a 60-mesh sieve to ensure a consistent particle size. The unstabilized rice bran was kept at 0°C until further treatment.

Determination of proximate composition

The proximate composition of unstabilized rice bran was analyzed in accordance with the procedures of the Association

of Official Agricultural Chemists (2006). In brief, the moisture content was determined by drying at 105°C until constant weight. Ash content was determined using a muffle furnace at 550°C. Protein was determined using the Kjeldahl method with 5.95 as the conversion factor. Lipid content was determined using the Soxhlet extraction method with hexane as the solvent. The total carbohydrate content was calculated by subtraction.

Thermal stabilization

Thermal stabilization was accomplished by dry-heating in a natural convective oven and a forced convective oven. A blower-equipped oven (Binder FD-53) was utilized for forced convective stabilization (FCS). The air velocity over each sample tray was 1.2 m/s. natural convective stabilization (NCS) used an oven without a blower (Memmert U40). Unstabilized rice bran was placed on an aluminum tray and spread evenly to a thickness of 0.8 cm. Then, the pans were placed in a preheated oven and heated at temperatures of 70°C, 90°C, 110°C or 160°C for 10 min, 30 min or 60 min. The stabilized rice bran was kept in firmly sealed amber glass vials at 0°C until further treatment.

Lipase activity (% hydrolysis)

Lipase activity analysis was carried out according to Buranathai et al. (2015) with modifications. Briefly, 1 g of rice bran was weighed and 200 µL of MiliQ water were added. The sample was mixed with a spatula until equally dispersed before being set aside for 2 hr. Afterwards, 1 g olive oil was added and the sample was covered. The samples were kept for 24 hr in an incubator (Heidolph Unimax 1000) at 37°C. Then, 0.2 g of the sample was taken and 1.5 mL of a combination of diethyl ether and methanol (2:1) was added, along with three drops of Phenolphthalein indicator. The material was titrated with 2 mM NaOH until the color of the solution became pink (correlated with pH 9). The percentage of hydrolysis was determined using Equation 1:

$$\% \text{ Hydrolysis} = \frac{\text{Volume NaOH of sample}}{\text{Volume NaOH of control}} \times 100\% \quad (1)$$

where all volumes are measured in milliliters.

Extraction of free phenolics and bound phenolics

Free phenolics extraction

The free phenolics were extracted using the method described by Ti et al. (2014) with modifications. Briefly, 1 g of rice bran was weighed and then combined with 10 mL of ethanol 80%. The sample was immersed in an ultrasonic bath (Elma S 180 H) with ultrasonic power of 200 W and 37 kHz for 30 min. The filtrate and supernatant were separated in a centrifuge (Hitachi Himac CS 120GXII) at 3,622×*g* for 15 min. The supernatant was collected and stored in a bottle with a tight lid and referred to as free phenolics extract.

Bound phenolics extraction

The bound phenolics were extracted using a method described by Ti et al. (2014) with modifications. The filtrate was dried for 24 hr at room temperature using filter paper and the dried filtrate was collected. Then, the filtrate was weighed to 0.1 g, hydrolyzed with 2 mL of NaOH 2 M and placed in an ultrasonic bath (Elma S 180 H) with ultrasonic power of 200 W and a frequency of 37 kHz for 20 min. Then, 2 mL of HCl 2 M was added to the mixture to neutralize the NaOH, followed by 4 mL ethanol 80%. The mixture was centrifuged (Hitachi Himac CS 120GXII) at 3622×*g* for 15 min. The supernatant was kept in a vial with a tight lid and referred to as bound phenolics extract.

Total phenolics content

The total phenolics content (TPC) was determined using a modified colorimetric method (Bolea et al., 2016), where 80 µL of the extract was diluted with 2mL of distilled water, followed by 200 µL of Folin-Ciocalteu 0.25 N reagent (Sigma-Aldrich). After 3 min, 1 mL of 7.5% Na_2CO_3 was added to the mixture. The mixture was incubated in a dark chamber at room temperature for 2 hr. A UV-visible spectrum (VIS) spectrophotometer (Shimadzu UV-2550) was used to measure the absorbance of the mixture at a wavelength of 765 nm. Ethanol 80% was used as a blank. The TPC was calculated using a gallic acid calibration curve in the concentration range 0.1–2 mg/mL and reported as milligrams of gallic acid equivalents (GAE)/100 g dry weight (DW) of rice bran.

Antioxidant activity

The antioxidant activity of the rice bran extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test modified from Gujral et al. (2012). In total, 50 µL of the

sample were combined with 3.95 mL of freshly made DPPH radical solution 0.1 mM. The mixture was incubated in the dark at room temperature for 60 min. The blanks and controls were prepared similarly to the samples, except that ethanol 80% was used in place of DPPH in the blanks and as a substitute for samples in the controls. A UV-VIS spectrometer (Shimadzu UV-2550) was used to measure the absorbance of the mixture at 515 nm. The antioxidant activity was determined using Equation 2:

$$\text{Antioxidant activity (\% inhibition)} = 100 - \left(\frac{\text{Abs sample} - \text{Abs blank}}{\text{Abs control}} \times 100\right) \quad (2)$$

where Abs sample is the absorbance of the sample, Abs blank is the absorbance of the blank and Abs control is the absorbance of the control.

Ultra-high-performance liquid chromatography

Chromatographic analysis of the rice bran extracts was conducted using ultra-high-performance liquid chromatography (UHPLC; PerkinElmer Flexar FX-20). The column used was a Syncronis™ C18 (Thermo Scientific) 50 mm × 2.1 mm with a 1.7 µm particle size. The column temperature was maintained at room temperature (30 ± 2°C) throughout the uHPLC operation. Detection was performed at 280 nm and 320 nm using a photodiode array (PDA) detector. The mobile phase consisted of MeOH (solvent A) and miliQ water (solvent B) with the following gradient: 0–1 min, 0–0.1% A; 1–9 min, 0.1–0.2% A; 9–12 min, 100% A. The injection volume of the sample was 20 µL at a flow rate of 0.3 mL/min. The *Chromera* software was used to process the chromatogram data.

Statistical analysis

Statistical analysis was carried out using the data analysis tool pack included with the Microsoft Excel program and the Statistical Tool for Agricultural Research (STAR 2.0.1). Each experiment was duplicated, and results were presented as mean ± SD. The effects of the thermal stabilization on the rice bran were determined using a univariate analysis of variance with a significance threshold of 95% ($p < 0.05$) and Duncan's multiple range test. The coefficient of determination (R^2) was applied to determine the adequacy of data fitting. Multiple regression analysis was performed using a linear model in the *XLStat 2021.2.2* software package.

Results and Discussion

The moisture content was in the range of 9–10% and comparable to research by Sirikul et al. (2009) and Sunphorka et al. (2012), though the protein content was less, but there were more lipids (24.4 g/100g), as shown in Table 1.

Table 1 Proximate composition of unstabilized rice bran (mean±SD); Each data point was derived from two replicates.

Proximate composition	g/100g rice bran
Moisture content	9.75±0.02
Ash	7.35±0.09
Protein	8.6±0.5
Lipids	24.4±0.3
Carbohydrate	49.9±0.11

Effect of thermal stabilization on lipase activity

Lipase is an enzyme present in rice bran that has an important role in the bran's shelf life. The lipase enzyme hydrolyzes bran lipids to liberate short-chain fatty acids. The effect of thermal stabilization on rice bran lipase activity on various operating conditions was investigated by evaluating the lipase capacity to hydrolyses lipids.

A change in color of the rice bran was seen when it was stabilized at 160°C, with the color becoming more brownish. However, the rice bran generated a pleasant odor and did not smell burnt. A preliminary study indicated that while the color of the rice bran stabilized at 160°C and turned brownish, the color of the rice bran extract remained consistent with the color of the rice bran extract generated from rice bran stabilized at a lower temperature (data not shown). Therefore, a temperature of 160°C was still deemed suitable. A stabilization temperature higher than 160°C produced bran with a blackened appearance and an unpleasant burnt odor. Due to these adverse organoleptic changes, the current investigation was conducted at a temperature limit of 160°C. The color of the stabilized bran at various temperatures is presented in Fig. 1.

As shown in Table 2, when the stabilization temperature and time increased, the lipase activity in the rice bran decreased. Stabilized rice bran had significantly lower lipase activity than unstabilized rice bran in all treatment variations, except at 70°C for 10 min. The lipase activities of FCS and NCS were 68.5% and 75.4%, respectively, at 90°C, and 56.3% and 57.0% at 110°C, respectively. These results indicate that stabilization temperatures of up to 110°C suppressed the lipase activity by

Fig. 1 Rice bran color after thermal stabilization for 60 min at various temperatures using natural convective stabilization (NCS) and forced convective stabilization (FCS)

almost 50%. Rice bran lipase activity decreased more rapidly at 160°C, whereas the FCS and NCS lipase activities were 26.8% and 28.2%, respectively. Therefore, a high stabilization temperature was more effective at inhibiting the lipase activity. There was no significant difference between NCS and FCS in their ability to inhibit lipase activity (Table 2).

However, no treatment was able to totally inactivate lipase as was confirmed by several other studies. For example, stabilizing rice bran in the microwave for 2.5 min at 700 W decreased lipase activity by 63% (Ertürk and Meral, 2019). Rice bran stabilized using an extruder and low-temperature radiofrequency resulted in decreased lipase activity by 69% and 71%, respectively (Liao et al., 2020). The lipase activity reduction in the current study was higher than those results, as the lipase activity could be lowered by 73%.Kim et al. (2014) stated that rice bran heated at 100°C for 1 hr could maintain its free fatty acid level below 5% for 25 wk of storage. Furthermore, they suggested using extensive heating to inactivate any residual enzymes and bacteria prior to storage. According to Tao et al. (1993), a free fatty acid concentration of less than 5% is acceptable, while rice bran with an free fatty acid percentage greater than 5% is classified as unfit for human consumption.

Table 2 Effect of thermal stabilization using natural convective stabilization (NCS) and forced convective stabilization (FCS) on rice bran lipase activity and phenolics content (mean±SD); Each data point was derived from two replicates

Treatment	Temperature (°C)	Time (min)	Lipase activity (% hydrolysis)	Free phenolics (mg GAE/100g DW)	Bound phenolics (mg GAE/100g DW)	Total phenolics (mg GAE/100g DW)
	Unstabilized		100.0±0.1^{a}	1707.3±48.1^{gh}	5770.4±242.4^{ef}	7477.7±290.5^{ef}
FCS	70	10	97.7±1.1^{a}	1773.1±45.5^{defgh}	6203.2±179.3^{de}	7976.3±133.8^{de}
		30	95.4±4.4^{a}	1705.5±28.3^{gh}	6990.0±683.7^{bc}	8695.4±711.9^{bc}
		60	66.9±7.6^{defgh}	1755.1±55.5^{efgh}	6616.8±284.7^{cd}	8371.9±229.2^{cd}
	90	10	83.8±3.3^{b}	1753.5±23.2^{efgh}	7375.8±600.5^{b}	9129.3±577.3^{b}
		30	76.9±4.4^{bcd}	1757.7±31.7^{efgh}	6925.7±341.8^{bc}	8683.5±310.1^{bc}
		60	68.5±1.1^{cdefg}	1739.9±53.5^{efgh}	8247.2±173.2^{a}	9987.1±119.9^{a}
	110	10	67.6±13.1^{b}	1734.2±0.4^{fgh}	6617.7±5.6^{cd}	8351.9±5.2^{cd}
		30	60.6±3.3^{bc}	1900.7±113.2^{bc}	4988.7±48.4^{hi}	6889.3±161.7^{fgh}
		60	56.3±10.9^{bcd}	1958.5±68.5^{ab}	6657.1±125.7^{cd}	8615.6±194.1^{bcd}
	160	10	62.0±2.2^{cdefg}	1838.0±16.4^{cdef}	6787.3±404.6^{bcd}	8625.3±421.0^{bc}
		30	42.3±1.1^{i}	1805.0±20.9^{cdefg}	6454.6±60.1^{cd}	8259.6±81.0^{cd}
		60	26.8±2.2^{j}	1874.9±46.7^{bcd}	6537.3±218.7^{cd}	8412.1±265.3^{cd}
NCS	70	10	100.0±0.0^{a}	1842.8±10.1^{cde}	4443.7±174.0^{i}	6286.4±163.9^{h}
		30	82.4±1.0^{b}	1782.4±24.2^{defg}	5018.7±92.4^{hi}	6801.1±68.2^{gh}
		60	66.2±6.0^{defgh}	1763.1±32.9^{efgh}	5126.6±58.1^{gh}	6889.7±25.2^{fgh}
	90	10	95.8±4.0^{a}	1675.8±8.4^{h}	5679.4±15.8^{efg}	7355.1±24.2^{fg}
		30	68.3±3.0^{cdefg}	1820.2±35.5^{cdef}	5153.8±139.7^{gh}	6974.0±104.2^{fg}
		60	75.4±7.0^{bcdef}	1837.0±45.2^{cdef}	5518.6±246.9^{fgh}	7355.5±292.1^{fg}
	110	10	60.6±10.0^{fgh}	1814.9±26.9^{cdef}	5099.6±214.7^{gh}	6914.4±187.8^{fg}
		30	64.1±7.0^{efgh}	2020.0±6.6^{a}	5422.2±308.7^{fgh}	7442.2±315.3^{ef}
		60	57.0±1.0^{gh}	1823.4±72.3^{cdef}	4952.7±178.5^{hi}	6776.1±106.1^{gh}
	160	10	69.0±5.0^{bcdef}	1838.0±51.8^{cdef}	6659.9±180.7^{cd}	8497.9±232.6^{bcd}
		30	53.3±2.0^{hi}	1820.3±11.0^{cdef}	6731.7±179.0^{cd}	8551.9±189.9^{bcd}
		60	28.2±3.0^{j}	1773.0±33.0^{defgh}	6613.4±145.7^{cd}	8386.4±178.7^{cd}

GAE = gallic acid equivalents; DW = dry weight

Mean values within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different

The resultant linear equation explained the effect of stabilization time and temperature factors on lipase percentage hydrolysis as:

$$\% \text{ Hydrolysis} = 121.9 - 0.36\,T - 0.49\,t \text{ (for NCS) } R^2 = 0.97$$
$$\% \text{ Hydrolysis} = 131.4 - 0.42\,T - 0.44\,t \text{ (for FCS) } R^2 = 0.94$$

where % hydrolysis denotes the capacity of lipase in rice bran to convert lipids to free fatty acids, T denotes the stabilization temperature (in degrees Celsius), and t denotes the stabilization time (in minutes).

Effect of thermal stabilization on rice bran phenolics content

The TPC of a sample can be determined by its reducing ability using the Folin-Ciocalteu reagent. This technique offers numerous advantages, including rapidity, reproducibility and low cost (Moore and Yu, 2008). Rice bran contains both free and bound phenolic compounds. Free phenolics are found in plant cell vacuoles, while bound phenolics are found in the plant cell wall matrix (Shahidi and Yeo, 2016). Because bound phenolics are covalently linked to the cell wall matrix, it is not possible to extract them directly; thus, in the current study, hydrolysis of bound phenolics was performed using an alkaline solution (Zhang et al., 2010; Pang et al., 2017).

As shown in Table 2, the content of the bound phenolics in the rice bran was higher than for the free phenolics. Unstabilized and stabilized rice bran contained 1,675–1,958 mg GAE/100g DW of free phenolics and 4,443–8,247 mg GAE/100g DW of bound phenolics, indicating that the bound phenolics content in the rice bran was 250% more than the free phenolics. These findings confirmed other reports that the majority of phenolic compounds in grains are bonded (Acosta-Estrada et al., 2014; Ertürk and Meral, 2019).

In general, the results showed that the stabilized rice bran had a higher free phenolics content than the unstabilized rice bran. The free phenolics content of the NCS rice bran was in the range 1,675–1,837 mg GAE/100g DW, whereas for the FCS rice bran, the range was 1,705–1,958 mg GAE/100g DW. The free phenolics content of the NCS rice bran started to increase at 90°C, but it was no longer significantly different from that of the unstabilized rice bran after heating at 160°C for 60 min. On the other hand, the free phenolics of the FCS rice bran increased significantly compared to the unstabilized rice bran starting at 110°C and persisted until 160°C.

Thermal stabilization causes cell damage (Lima et al., 2013; Noor, 2015); therefore, the free phenolics in the cell vacuole can be more readily released due to the process. Thus, stabilized rice bran contained higher free phenolics than unstabilized rice bran. The bound phenolics content of the NCS rice bran was in the range 4,443–6,731 mg GAE/100g DW, while the bound phenolics content of the FCS rice bran was in the range 4,988–8,247 mg GAE/100g DW. The bound phenolics content of the FCS rice bran was significantly higher than for the unstabilized rice bran under all operating conditions. In contrast, the bound phenolics content of the NCS rice bran decreased in all treatments except at 160°C, where it remained unchanged.

The TPC of the FCS rice bran showed a significant increase compared to unstabilized rice bran, whereas the NCS rice bran exhibited no change in TPC compared to unstabilized bran. However, there was no significant difference between the TPC of the FCS and NCS rice bran samples at a stabilization temperature of 160°C. A high stabilization temperature appeared to be needed in the NCS treatment to achieve the same TPC amount as the FCS rice bran. The TPC was not affected by a time variation of 10 min, 30 min or 60 min.

The temperature measurements were taken at the center of the rice bran pile (data not shown). The center pile temperature of the rice bran pile took longer to achieve the set temperature in the NCS rice bran than in the FCS rice bran. There was a ±10°C difference in temperature between the NCS and FCS rice bran pile centers. The value of the temperature difference decreases as the setting temperature increases. The temperature in the FCS rice bran was more even and achieved the setting temperature more rapidly than for the NCS rice bran. This fact may explain why the FCS rice bran contained more phenolics than the NCS rice bran. The temperature determines the degree to which the rice bran cells have been damaged; the higher the temperature, the greater the cell damage (Barbosa et al., 2013; Noor, 2015). As a result, the amount of phenolics released increased.

The FCS rice bran had 1.8–7% greater free phenolics and 12–23% more bound phenolics than the NCS rice bran. Heat treatment-induced cell wall degradation and decreased the physical retention of phenolics in the cell wall. Therefore, bound phenolics can be extracted (Yilmaz et al., 2014; Saji et al., 2020). Ferulic acid and coumaric acid are the most abundant bound phenolic compounds identified in cereal crops, including rice (Călinoiu and Vodnar, 2020). Auxenfans et al. (2017) reported an increase in the TPC in wheat straw induced by the addition of ferulic acid. Ferulic acid is bound to hemicellulose via ester bonds released at a rate of 93%,

whereas ferulic acid is bound via ether bonds released at a rate of 8%. In the current study, since the bound phenolics content of the FCS rice bran was higher than that of the NSC rice bran, FCS was likely more effective than NCS at disrupting the phenolics bound in the cell wall of the rice bran.

Effect of stabilization on bioactive antioxidant activity of rice bran

The antioxidant activity of phenolics is primarily due to their redox characteristics, including their ability to serve as reducing agents, hydrogen donors and possible metal chelators (Parr and Bolwell, 2000). The antioxidant activity (AA) was assessed using its % inhibition of the free radical compound DPPH. Table 3 shows that the AA of the unstabilized rice bran extract was 64.8%, while the AA of the stabilized rice bran was in the ranges 57.9–72.1% and 58.9–72% for NCS and FCS, respectively.

The AA of the NCS rice bran was reduced as the stabilization temperature increased to 90°C. However, at a stabilization temperature of 160°C, there was a significant increase in AA compared to the unstabilized rice bran. The AA of the FCS rice bran increased, starting from 90°C, and remained significantly higher than that of the unstabilized rice bran until a stabilization temperature of 160°C. Variation in the stabilization time did not correlate directly with AA. The AA resulted in a pattern comparable to the TPC, suggesting a positive connection between TPC and AA, as discovered by other researchers (Irakli, et al., 2018; Suraiya et al., 2018; Ertürk and Meral, 2019).

The TPC and AA levels were somewhat lower in the NCS rice bran after stabilization at 70°C. However, the TPC and AA levels rose significantly when the stabilization temperature was increased to 160°C. The TPC and AA of the FCS rice bran had already increased at 70°C. Forced convection is a heat transmission mechanism in which an external force affects the movement of a fluid (Zohuri, 2017). The current study used an external source (a blower fan) to produce fluid motion. This technique is highly efficient since it allows for the effective transmission of heat from heated objects (Kosky et al., 2021). As aforementioned, the FCS rice bran contains more phenolic compounds than the NCS bran due to its more

Table 3 Effect of thermal stabilization using natural convective stabilization (NCS) and forced convective stabilization (FCS) on antioxidant activity of rice bran (mean±SD). Each data point was derived from two replicates

Treatment	Temperature (°C)	Time (min)	Antioxidant activity (% inhibition)
Unstabilized			64.8±1.3^{ef}
FCS	70	10	58.9±1.1^{ij}
		30	63.0±1.7^{fgh}
		60	63.6±0.9^{efgh}
	90	10	72.3±0.7^{b}
		30	70.9±0.7^{bcd}
		60	72.5±0.4^{b}
	110	10	70.3±0.8^{bcd}
		30	69.4±1.1^{cd}
		60	77.9±0.7^{a}
	160	10	71.9±1.9^{bc}
		30	71.9±0.9^{bc}
		60	70.5±2.0^{bcd}
NCS	70	10	66.0±2.2^{e}
		30	64.2±0.2^{efg}
		60	63.8±0.6^{efg}
	90	10	57.2±1.2^{j}
		30	61.5±0.6^{gh}
		60	61.1±0.9^{hi}
	110	10	57.9±0.9^{j}
		30	64.0±0.4^{efg}
		60	61.0±1.5^{hi}
	160	10	72.1±1.5^{b}
		30	72.0±0.2^{bc}
		60	68.9±1.3^{d}

Mean values within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different

rapid temperature rise to the setting temperature. Phenolics are bioactive substances with antioxidant properties; hence, the higher the concentration of phenolics, the greater the antioxidant activity.

Ultra-high-performance liquid chromatograms of stabilized rice bran

uHPLC with a PDA detector was used to determine the bound and free phenolics contents. The chromatograms of the free phenolic compounds for the unstabilized rice bran and the stabilized rice bran can be seen in Fig. 2A. The peak chromatogram of the stabilized bran had a larger area than that of the unstabilized rice bran, indicating a higher phenolics content. The FCS rice bran showed a peak at a retention time of 4.3 min that was not observed in the NCS or unstabilized rice bran. Further investigation to identify this peak is recommended, as this peak may be utilized as an indicator of an appropriately stabilized rice bran.

The chromatogram of the bound phenolics revealed distinctively different patterns of the unstabilized rice bran and the stabilized rice bran (Fig. 2B). The chromatograms of the NCS and FCS rice bran revealed the presence of peaks with retention times of 3.3–4.2 min, which were not observed in the chromatogram of the unstabilized rice bran. Similar to the chromatogram for the free phenolics, the peak area for the stabilized rice bran was larger than that of the unstabilized rice bran.

Peaks in the free phenolics extract chromatogram emerged in the retention time range 1.8–5 min for both stabilized and unstabilized rice bran samples. Furthermore, peaks in the bound phenolics extract were seen only up to a retention period of 4.4 min. This retention time range is proportional to the percentage of methanol eluent less than 0.2%, indicating that both the free and bound phenolic extracts of rice bran contained polar phenolic compounds.

Saji et al. (2020) examined bound phenolics in stabilized and unstabilized rice bran and found a similar pattern. There was a change in the bound phenolics content of the stabilized rice bran. For example, they discovered that heating rice bran could destroy the hexose of caffeic acid. As a result, the saccharide component of the compound is lost and the compound is transformed to its basic skeletal form, caffeic acid. They recommended further study to substantiate this assumption. Similarly, determining the phenolics in convective stabilized rice bran is a promising area for further research.

Conclusion

This study demonstrated that convective stabilization reduced lipase activity while enhancing phenolics and antioxidant activity. Convective stabilization significantly reduced the lipase activity starting at a stabilization temperature of 90°C for 10 min; however, operating at 160°C for 60 min produced the highest results in all treatments. While both FCS and NCS suppressed the lipase activity in the treated rice bran equally, FCS outperformed NCS in increasing the total phenolics and antioxidant activity levels. Therefore, FCS appears to be a viable approach for thermal stabilization to extends the shelf life and nutritional value of rice bran.

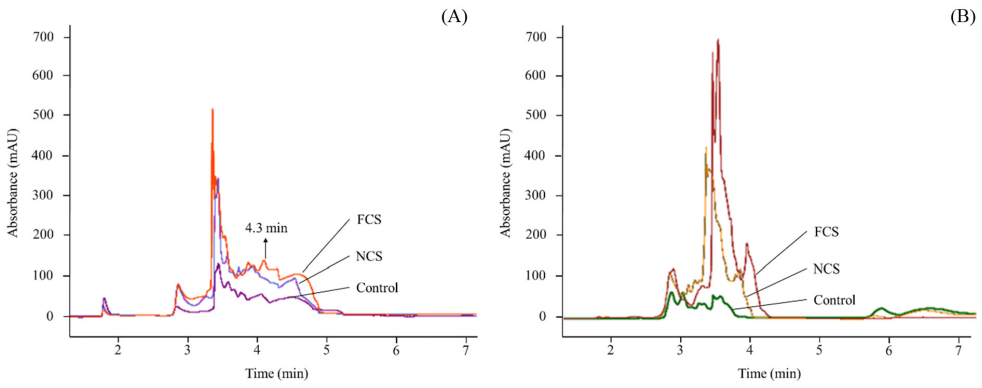

Fig. 2 Comparison of chromatograms from ultra-high-performance liquid chromatography with a photodiode array detector: (A) free phenolics; (B) bound phenolics

Conflict of Interest

The authors declare that there are no conflicts of interest.

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