Research Article

Autoclave-assisted Rice Bran Stabilization: Effect on Lipase, Antioxidants and Phytochemicals

Zafar Iqbal*[1], Noorullah[1], Zulqarnain[2], Ubairah Majid[3], Abdul Majid[1] and Saeed Ullah Khattak[4]

[1]Department of Agricultural Chemistry and Biochemistry, The University of Agriculture, Peshawar, Pakistan; [2]Directorate of Soil and Plant Nutrition, Agricultural Research Institute, Peshawar, Pakistan; [3]Directorate General Agriculture Research, Khyber Pakhtunkhwa, Pakistan; [4]Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan.

Abstract | In this study, the efficacy of autoclave heating for rice bran (RB), focusing on its effects on lipase activity, free fatty acid (FFA) content, antioxidant capacity (AC), γ-oryzanol, α-tocopherol, total flavonoid content (TFC), and total phenolic content (TPC) were evaluated. RB was autoclaved at 120 °C for 20 minutes and subsequently stored for 60 days at room temperature. Autoclave treatment effectively inactivated lipase, as evident by the limited increase in FFA content in stabilized RB (SRB), which rose from 2.88% to 3.94% during storage, well below the 5% threshold for quality deterioration. In contrast, unstabilized RB (USRB) exhibited a sharp increase in FFA content, rising from 3.13% to 14.46%. The antioxidant capacity of SRB remained relatively stable (92.53% to 86.26%) and comparable to that of USRB (94.62% to 81.67%) throughout the storage period. Phytochemical retention was significantly better in SRB. TPC in SRB declined modestly from 5.17 $mg \cdot g^{-1}$ to 4.67 $mg \cdot g^{-1}$, while in USRB, it dropped from 5.47 $mg \cdot g^{-1}$ to 3.01 $mg \cdot g^{-1}$. TFC values in SRB and USRB decreased from 4.26 to 3.91 $mg \cdot g^{-1}$ and 4.48 to 3.11 $mg \cdot g^{-1}$, respectively. Similarly, α-tocopherol and γ-oryzanol levels in SRB showed higher stability, decreasing from 4.04 to 3.03 $mg \cdot g^{-1}$ and from 4.34 to 3.62 $mg \cdot g^{-1}$, respectively. Autoclave stabilization at 120 °C for 20 minutes effectively inactivates lipase and preserves the antioxidant and phytochemical profiles.

Received | June 10, 2025; **Accepted** | February 10, 2026; **Published** | July 08, 2026
***Correspondence** | Zafar Iqbal, Department of Agricultural Chemistry & Biochemistry, University of Agriculture Peshawar; **Email:** zafar.iqbal@aup.edu.pk
Citation | Iqbal, Z., Noorullah, Zulqarnain, U. Majid, A. Majid and S.U. Khattak. 2026. Autoclave-assisted Rice Bran Stabilization: Effect on Lipase, antioxidants and phytochemicals. *Sarhad Journal of Agriculture,* 42(3): 1129-1136.
DOI | https://dx.doi.org/10.17582/journal.sja/2026/42.3.1129.1136
Keywords | Rice bran, Autoclave stabilization, Lipase inactivation, Rancidity, Nutrient retention

Introduction

Rice (*Oryza sativa*) is among the most extensively farmed crops (Loor-Bravo *et al.*, 2025). It is a primary source of calories, providing approximately 23% of the total global caloric intake, second only to wheat and maize (Ding *et al.*, 2018). Rice is rich in carbohydrates, proteins, fats, dietary fiber, vitamins and minerals (Chaudhari *et al.*, 2018).

Global rice production reached approximately 517.3 million metric tons (milled) during the year 2023/24, with Asia contributing 90%. Pakistan, yielded 9.8 million metric tons of milled rice.

Paddy rice undergoes multiple processing steps during milling, yielding byproducts (Sharma *et al.*, 2015) and amongst them RB (RB) is a nutrient-rich byproduct. It contains tocopherols, tocotrienols, oryzanols, and phytosterols (Raghav *et al.*, 2016). γ-oryzanol, a compound exclusive to RB offers significant health benefits (Sharma *et al.*, 2015). It also contains lipoic acid, which helps prevent diabetic neuropathy, Alzheimer's disease, and skin-related issues (Raghav *et al.*, 2016). RB is still underutilized, despite having a lot of nutrients, due to its rapid deterioration caused by the lipase enzyme, making it unsuitable for human consumption within hours of milling (Aher *et al.*, 2022).

Distinct stabilization techniques are employed to extend the shelf life of RB. These methods include autoclaving, microwave heating, ohmic heating, and chemical treatments. Stabilized RB (SRB) can be utilized in various food applications, providing health benefits (Manzoor *et al.*, 2023).

This study shows the use of autoclave stabilization as a method to preserve phytochemical contents in RB (RB) and, simultaneously, inactivate lipase. Unlike existing methods, the technique presents a double benefit in preserving bioactive compounds and extension of shelf stability in an effort to remove a major limitation in the use of RB.

Materials and Methods

Sample collection

Ijaz Rice Mill in Okara, Pakistan, provided fresh RB samples. The bran samples were delivered to the lab in linen bags. Bran was screened using a 60-mesh screen and refrigerated.

Moisture determination

Moisture content (%) was determined in the pre-stabilized RB sample following the AOAC (2012) method. A 1 g sample was dried in an at 105 ˚C for 4 hours, and the moisture content was measured. It was subsequently stabilized using the autoclave method described by Kim *et al.* (2014). The bran was cooled to room temperature and the oven dried to achieve a moisture content of ≤6%.

Determination of FFA value

An AOAC titration method was used to determined FFA (Guevara-Guerrero *et al.*, 2019). An n-hexane (120 mL) was added to the 20 grams of RB and stirred it at room temperature and the mixture was filtered. Combined extracts were evaporated through a rotary evaporator at 40 ˚C under decreased pressure. The residue was re-extracted using 120 mL of n-hexane. 10 mL of ethanol containing two to three drops of 1% phenolphthalein was combined with approximately 1.4 g of the extracted oil, and the mixture was titrated with 0.1 N NaOH until the pink hue disappeared. The FFA value (as oleic acid) was calculated using the formula:

$$\%\ \text{FFA (as oleic acid)} = \frac{\text{Vol NaOH} \times \text{Normality NaOH} \times 28.2}{\text{Weight of Oil}}$$

Antioxidant activity

Five grams of RB were subjected to extraction using 20 mL of methanol at ambient temperature on an electric shaker for a duration of three hours. The extract underwent filtration using Whatman No. 1 filter paper, followed by re-extraction. The combined extracts were subsequently evaporated under vacuum at 50 ˚C utilizing a rotary evaporator.

A 2 mM DPPH solution (80 mg/100 mL methanol) was prepared and stored at −20˚C in the dark. For analysis, 0.2 mL of RB extract was mixed with 4 mL of methanol and 1 mL of DPPH solution and then incubated in the dark for 30 minutes. The absorbance was measured at 527 nm using a spectrophotometer (752PC) (Chanthathamrongsiri *et al.*, 2022). The percentage inhibition of DPPH was calculated as follows:

$$\text{DPPH Scavenging \%} = \frac{\text{A0-A1}}{\text{A0}} \times 100$$

A0 = Control absorbance, A1 = Sample absorbance

Total phenolic content

The TPC was determined according to the method described (Nisa *et al.*, 2019). 20 µL of the RB extract was added to 1.58 mL of distilled water and 100 µL of FCR. The mixture was vortexed, and after sodium carbonate addition (300 µL of 20% w/v solution), it was stirred for 3 minutes, then left undisturbed for 2 hours. Absorbance was measured at 765 nm. TPC was calculated against a standard curve obtained from a diluted gallic acid stock solution prepared by sonication of 10 mg gallic acid in 100 mL of distilled water, followed by serial dilutions (0, 2, 4, 6, and 8

mg·mL^{-1}).

Total flavonoid content

1g of RB, was dissolved in 100 mL of ethanol and stirred for one hour with a magnetic stirrer. A 30 µL aliquot of the filtered extract was transferred to a 10 mL volumetric flask, followed by the addition of 5 mL of distilled water and 0.3 mL of $NaNO_3$ (1:20). After 6 minutes, 3 mL of $AlCl_3$ (1:10) and 2 mL of 1 M NaOH (prepared as 4 g/100 mL water) were introduced. Distilled water was used to bring the total volume down to 10 mL. A UV-visible spectrophotometer was used to measure absorbance at 510 nm after the solution underwent vortexing. The total flavonoid content (TFC) was determined using a calibration curve for quercetin. A stock solution of quercetin was created by dissolving 10 mg of quercetin in 100 mL of methanol, followed by serial dilutions to achieve concentrations of 0, 2, 4, 6, and 8 mg·mL^{-1} for calibration purposes.

Determination of α-tocopherol

RB (1 g) underwent extraction overnight at 28 °C and 105 rpm using 30 mL of a chloroform/petroleum ether (1:1) mixture. The solvent was evaporated to yield RB oil. 0.5 mL of oil was treated with 0.1 mL of ascorbic acid (1 g·100 mL^{-1}). 10 mL of NaOH (10 g·L^{-1}), 10 mL of ethanol, 25 mL of hexane, and 15 mL of ethyl acetate were added after a 5-minute interval. The mixture was vortexed for one minute, incubated at 105 rpm for fifteen minutes, and subsequently phase separated. The organic layer was evaporated at 40°C, and the residue was dissolved in a hexane/isopropanol mixture (4:10; 20 mL), filtered through a 0.45 µm filter in an HPLC vial (Yılmaz *et al.*, 2014). The HPLC analysis was performed under gradient conditions using a photodiode array (PDA) detector. The mobile phase consisted of hexane, isopropanol, acetic acid, and ethyl acetate (98.5:0.1:0.6:0.8% v/v). A C18 column was used with a flow rate of 1.6 mL·min^{-1} and an injection volume of 20 µL. The analysis ran for 20 minutes at 294 nm. α-Tocopherol content was quantified using a calibration curve based on tocopherol standards (0, 1, 2, 4, and 8 mg·mL^{-1}).

Determination of γ-oryzanol

RB (0.5 g) was extracted with HPLC-grade isopropanol, vortexed for 2 minutes, and centrifuged at 4500 rpm for 10 minutes. The supernatant was extracted twice, mixed, and dried. The residue was dissolved in 5 mL of isopropanol. Following filtration (0.45 µm), 20 µL was introduced into an HPLC system. An isocratic mode of HPLC was used for analysis, employing a mobile phase of acetonitrile, methanol, and isopropanol (35:55:10) on a C18 column. A PDA detector monitored γ-oryzanol at 325 nm with a 1 mL/min flow rate over 30 minutes. The content was quantified using a standard curve.

Statistical analysis

The data was statistically analyzed using a CRD design on Statistix 8.1. The experiments were performed in triplicate. Statistical significance was determined at the 5% level.

Results and Discussion

A 5 kg sample of RB of the Basmati-385 rice variety was obtained from the Ijaz Rice Mill in Okara, Pakistan. A zipped linen bag was used to convey the sample to the laboratory at the University of Agriculture, Peshawar, for additional analysis.

Moisture determination

In this study, autoclave stabilization was employed to inactivate lipase, prevent the release of fatty acids, and extend the shelf life of RB. Pre-stabilized RB is a coarse, light-brown powder with a minimum moisture content of 5%. After autoclaving at 120°C and 15 psi pressure for 20 minutes, the bran became sticky due to an increase in moisture content from 6.21% to 16.23%, resulting from the saturation of steam. The Maillard reaction and heating caused the colour to darken. Subsequently, oven drying was implemented to reduce the moisture content to ≤5%. The stabilized bran was allowed to settle to ambient temperature for one hour in preparation for additional analysis.

FFA value

The lowest initial free fatty acid (FFA) value of 2.88% was recorded in ASRB (Table 1), which was attributed to the inactivation of lipolytic enzymes. In contrast, USRB attained the highest FFA value of 14.46% by week 8, attributed to lipid decomposition. Throughout 14 to 56 days, the concentration of free fatty acids (FFA) in SRB increased from 3.17% to 3.94%, whereas in USRB it rose from 8.07% to 14.46%.

The slight rise in SRB FFA levels was attributed to non-enzymatic oxidation, also known as rancidity. ASRB maintained FFA levels below 5% after

two months, confirming the autoclave method's effectiveness in extending RB shelf life. Significant ($p < 0.05$) differences were observed among storage intervals within each treatment. While FFA values of SRB showed a modest but statistically significant rise from week 0 to week 6, the increase in USRB was much greater, confirming the autoclave's effectiveness in enzyme inactivation.

Table 1: *Free fatty acid value (FFA) of un-stabilized and autoclave stabilized rice bran at time intervals during two months' storage period*

Sample	Initially	Week 2	Week 4	Week 6	Week 8
Un-stabilized	3.24 ± 0.29 b	8.07± 0.02 d	10.14± 0.20 e	12.15± 0.04 f	14.46± 0.17 g
Stabilized	2.86 ± 0.06 a	2.97± 0.02 a	3.27± 0.21 b	3.50± 0.27 b	3.92± 0.06 c

All values are expressed as mean ± standard deviation (n=3). P≤0.05

Previous studies support these findings. Wang *et al.* (2017) reported that un-stabilized RB exceeded 10% FFA in three days, making it unsuitable for consumption. It was found that FFA levels in rough rapeseed oil reached 14% within a month, and autoclaving at 121 °C for 15 minutes was identified as an effective stabilization method. Additionally, microwave heating also showed promise. It has been demonstrated that RB stored in hot, humid conditions could see FFA levels rise 5–10% daily, reaching 70% in a month (Wiriyawattana and Suwonsichon, 2014).

Antioxidant activity of USRB and SRB

Antioxidant activity reflects a substance's ability to neutralize molecules with high oxidation/reduction potential, which can be harmful to the body. The antioxidant activity of USRB and SRB samples were initially assessed and then monitored over an eight-week storage period (Table 2).

Table 2: *Percent antioxidant activity of un-stabilized rice bran and autoclave stabilized rice bran at time intervals during two months' storage period*

Sample	Initially	Week 2	Week 4	Week 6	Week 8
Un-stabilized	94.62 ± 0.50 g	90.33 ± 0.36 e	87.60 ± 0.56 d	79.22 ± 0.38 a	81.67 ± 0.79 b
Stabilized	92.53 ± 0.52 f	90.35 ± 0.41 e	90.29 ± 0.49 e	88.34 ± 0.65 d	86.26 ± 0.46 c

All values are expressed as mean ± standard deviation (n=3). P≤0.05

Fresh USRB exhibited the highest initial antioxidant activity (94.62%), which declined to 71.64% after eight weeks of storage. In contrast, SRB showed minimal reduction in antioxidant activity, indicating that autoclave heating effectively preserved antioxidant capacity. The initial 2% decrease in SRB's antioxidant activity was likely due to heat exposure during stabilization, which inactivates lipase enzymes. Lipase-induced lipid oxidation in USRB is a primary cause of rancidity and reduced antioxidant potential (Wiriyawattana and Suwonsichon, 2014).

Table 3: *Total α-Tocopherol content of un-stabilized rice bran and autoclave stabilized rice bran at time intervals during two months' storage period, expressed as tocopherol standard (mg/g of bran)*

Sample	Initially	Week 2	Week 4	Week 6	Week 8
Un-stabilized	5.03 ± 0.03 g	4.04 ± 0.02 f	3.2 ± 0.01 c	2.94 ± 0.02 b	1.99± 0.01 a
Stabilized	4.04 ± 0.02 f	3.99 ± 0.01 f	3.58 ± 0.01 e	3.2 ± 0.08 d	3.03 ± 0.02 c

All values are expressed as mean ± standard deviation (n=3). P≤0.05

Autoclave stabilization improved the long-term stability of antioxidant compounds by inactivating lipase. It has been reported that increased temperatures and extended heating improved stabilization at 121 °C for 15 minutes (Wiriyawattana and Suwonsichon, 2014). Similar findings were reported by Rico *et al.* (2020), who found that wheat bran treated at 130 °C for 12 minutes retained antioxidant stability.

Studies on black, red, and brown varieties of RB have revealed that black rice bran (RB) has the highest antioxidant activity (Ghasemzadeh *et al.*, 2018). It has also been reported in a study that red RB oil demonstrated 7% higher antioxidant activity compared to white RB oil (Bopitiya and Madhujith, 2015).

Total phenolic content

The impact of stabilization on total phenolic content (TPC) was assessed by analyzing RB both before and after treatment.

Table 3 presents TPC data for SRB and USRB. Initially, USRB exhibited the highest TPC (5.47 $mg \cdot g^{-1}$), which declined significantly to 3.01 $mg \cdot g^{-1}$ after storage due to enzymatic oxidation. The stabilized sample (SRB) showed only a 6% reduction in TPC, likely due to heat exposure during autoclaving, but remained stable throughout storage. This indicates

that autoclaving effectively preserves phenolic content while preventing degradation.

Wiriyawattana and Suwonsichon (2014) reported that TPC decreases with increasing autoclave temperature and time but remains relatively stable at 105 °C for 5 minutes. Similar results were observed by Howlader *et al.* (2019), who compared autoclave, roasting, steaming, and microwave stabilization. Autoclaving resulted in a 10% reduction in TPC, aligning with our findings.

It has been reported that Extraction methods also influence TPC, as Sukrasno *et al.* (2017) observed TPC variations in different RB extracts, with ethanolic extracts of black rice showing the highest content. Srisawat *et al.* (2010) reported that water-extracted TPC from Sangyod red rice was three times higher than that from Dawk Mali 105 white rice.

Phenolics, characterized by hydroxyl groups on aromatic rings, contribute to plant defense and offer human health benefits, including protection against diseases (Bhuyan and Basu, 2017). TPC plays a crucial role in RB's antioxidant properties and disease prevention (Webber *et al.*, 2014).

Total flavonoid content (TFC)

Flavonoids are vital polyphenols found in plants, contributing to antioxidant activity and offering health benefits such as antiviral, antibacterial, and antioxidant properties. They also regulate gene expression and modulate enzymatic activity in humans (Irina and Mohamed, 2012). Structurally, flavonoids consist of a 15-carbon skeleton with two phenyl rings and a heterocyclic ring. They scavenge free radicals by stabilizing reactive oxygen species, primarily through the reactivity of their hydroxyl (–OH) groups.

The total flavonoid content (TFC) of rice bran (RB) was measured using a quercetin standard curve (Table 4). Initially, unstabilized RB (USRB) had a higher TFC (4.48 mg·g^{-1}) compared to autoclave-stabilized RB (SRB). However, by the end of the eight-week storage period, TFC in USRB decreased significantly to 3.11 mg·g^{-1} due to lipase-induced rancidity, which promotes oxidation and the breakdown of free fatty acids (FFAs). This process generates free radicals, lipid hydroperoxides, and ketones, reducing TFC and compromising nutritional quality (Wiriyawattana and Suwonsichon, 2014).

Table 4: *Total γ-oryzanol content of un-stabilized rice bran and autoclave stabilized rice bran at time intervals during two months' storage period, expressed as oryzanol standard (mg/g of bran).*

Sample	Initially	Week 2	Week 4	Week 6	Week 8
Un-stabilized	6.35 ± 0.02 h	5.28 ± 0.03 g	4.23 ± 0.05 e	3.62 ± 0.04 b	2.61± 0.02 a
Stabilized	4.34 ± 0.03 f	4.22 ± 0.02 e	3.99 ± 0.02 d	3.8 ± 0.02 c	3.62 ± 0.02 b

All values are expressed as mean ± standard deviation (n=3). P≤0.05

Previous studies support these findings. El-Gammal (2017) reported a gradual decrease in TFC in thermally stabilized RB, attributing it to high-temperature exposure. Similarly, Parvez *et al.* (2019) observed a reduction in TFC in cowpea after autoclaving, consistent with the inactivation of lipase. Yadav *et al.* (2018) noted that autoclave heating and boiling caused TFC loss in cowpea seeds due to cell wall rupture and leaching of water-soluble polyphenols.

Research on pigmented RB varieties revealed differences in total folate content (TFC). Srisawat *et al.* (2010) found higher content of flavonoids in red rice compared to white rice. Ghasemzadeh *et al.* (2018) found that black RB exhibited the highest levels of phytochemicals and antioxidant activity, with red and brown RB following, underscoring its potential for functional food applications.

α-tocopherol content

The α-tocopherol content in RB was quantified using HPLC. The analysis revealed that USRB initially contained 5.03 mg·g^{-1} of α-tocopherol, which decreased significantly to 1.99 mg·g^{-1} after eight weeks of storage. Lipase activity hydrolyzes fats into free fatty acids (FFAs), reducing bioactive compounds like α-tocopherol. In contrast, SRB showed a slight decrease in α-tocopherol content, attributed to heat exposure during stabilization, which can degrade heat-sensitive tocopherols (Kim *et al.*, 2014). Despite this, SRB retained adequate α-tocopherol levels.

Studies by Yu *et al.* (2020) support these findings, highlighting that excessive heat can decompose tocopherols, but autoclave treatment at optimal conditions minimizes such losses.

γ-oryzanol content

The initial γ-oryzanol content in USRB was 6.35 mg·g^{-}

[1], which declined significantly to 2.61 mg·g^{-1} over eight weeks. In contrast, SRB showed a minimal decrease, from 4.34 to 3.62 mg·g^{-1}, indicating that autoclave stabilization effectively preserved γ-oryzanol. The initial reduction in SRB was attributed to heat exposure during stabilization, which may degrade γ-oryzanol (Howlader *et al*., 2019).

Research by Yu *et al*. (2020) confirmed that temperatures below 120 °C have minimal impact on γ-oryzanol, while higher temperatures cause degradation. Similarly, Srisawat *et al*. (2010) observed that the stability of γ-oryzanol in RBO decreases significantly at temperatures exceeding 120 °C.

Autoclave stabilization effectively preserves α-tocopherol and γ-oryzanol in RB by inactivating lipase and minimizing oxidative degradation. While heat exposure during stabilization may cause slight reductions in these compounds.

Conclusions and Recommendations

The study found that autoclaving RB for 20 minutes at 120 °C stabilized it by deactivating lipase, preventing an excessive buildup of free fatty acids (FFA). Throughout the 60-day storage period, this stabilization technique kept FFA levels below the threshold for quality deterioration. Autoclaved RB (SRB) preserved the antioxidant potential and phytochemical constituents. Future research on other alternative stabilization methods, such as irradiation or enzymatic treatments, to enhance the preservation of bioactive compounds in RB are recommended.

Novelty Statement

The results may lead to improved processing methods that enhance the health-promoting properties of RB and extend its shelf life, making it a valuable and sustainable ingredient for various applications.

Author's Contribution

Zafar Iqbal: Designed the study and supervised it
Noorullah: Experimented and collected the results
Zulqarnain, Ubaira Majid and Abdul Majid: Analyzed and interpreted the results
Saeed Ullah Khattak: Performed the statistical analysis

Generative AI or AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors declare no conflicts of interest.

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