Microwave treatment of rice bran and its effect on phytochemical content and antioxidant activity

Piramon Pokkanta1,2, Jitkunya Yuenyong1, Sugunya Mahatheeranont1,3,4, Sudarat Jamyangyuen5 & Phumon Sookwong1,3,4

An alternative approach for rice bran stabilization is microwave treatment. However, the effects of the microwave treatment on the contents of bioactive compounds and antioxidant activities of the rice bran have rarely been reported in detail. In this study, microwave pretreatment (130–880 W for 0.5–5.0 min) of rice bran was proposed where the antioxidant activity, total flavonoids, and total phenolic contents were determined using UV–Vis spectrometry. Tocols, γ-oryzanols, squalene, phytosterols and phenolic compounds were quantified using high-performance liquid chromatography. The results showed an increase in the antioxidant activity (0.5 folds), total phenolic contents (1.3 folds), total flavonoid contents (0.9 folds), total tocols (2.6 folds), total γ-oryzanols (1.6 folds), and total phytosterols (1.4 folds). Phytochemicals were enhanced, especially trans-p-coumaric acid (10.3 folds) and kaempferol (8.6 folds). The microwave treatment at 440 W for 2.5 min provided the best contents of the bioactive compounds and antioxidant activity. This work revealed the microwave treatment as a potential tool for stabilizing rice bran and increasing the usability of its phytochemicals, which applies to several industries concerning the use of rice bran as an ingredient.

Recently, microwave treatment (MWT) has been introduced as an effective tool to stabilize plant seeds including sunflower, apricot kernels, and poppy seeds1,2. The stabilization process is an important procedure to deactivate lipase activity present in raw materials, thereby preventing them from the hydrolysis of triglycerides into glycerol and free fatty acids. Consequently, the latter compounds are prone to oxidation reactions, leading to unwanted characteristics during storage (hydrolytic rancidity, pH reduction, and soapy taste products)3. Several advantages of microwave stabilization of rice bran (RB), compared with other methods such as steaming, parboiling, autoclave heating, roasting, enzymatic treatment, or infrared radiations, include faster treatment time, greater convenience, better cost performance, instantaneous control as well as increasing oil yield and antioxidant activity4–6.

Bioactive compounds present in RB such as vitamin E (tocols), γ-oryzanols, phenolic compounds, and plant sterols have received increasing attention due to their therapeutic properties. Their effectiveness in the treatment of coronary heart diseases, serum hypercholesterolemia, nerve imbalance, hyperlipidemia, hyperglycemia, type I and type II diabetes mellitus, inflammatory, regulation of blood clotting, and cancer has been reported7–10. To effectively utilize the phytochemicals in the RB, stabilization processes after the rice milling has been recommended. Despite their main purpose of deactivation of lipase, stabilization processes also affect physical and chemical properties of RB11. For instance, roasting and enzymatic pretreatment on the RB reduced the yield of RB oil extracted as compared to the control4. The heat stabilizations with cooking (parboiling, steaming) led to the high loss of nutrients and antioxidants12,13. In the past, studies have shown the effects of RB stabilization on chemical changes. However, the effect of microwave stabilization has been marginally investigated, specifically in terms of bioactive compounds.

Therefore, this study aimed to investigate the influence of MWT on the antioxidant activity and contents of several bioactive compounds using spectrophotometry, and high-performance liquid chromatography (HPLC) techniques. The knowledge derived from this work would aid useful for several industries concerning the use of RB as an ingredient in industrialized processes.
### Results and discussion

**Total phenolic content, total flavonoid content and antioxidant activity.** The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA) of RB roasted in the microwave oven (MWO) are shown in Table 1. The TPC in RBs varied from 11.01 to 39.62 μg of gallic acid equivalents per g of dry weight (μg GAE/g) (Table 1) whilst the control contained 17.14 μg GAE/g. At low MW power (130 W), the oxidase and lipase could not be deactivated properly. However, the MW-induced heat was able to facilitate the oxidation and enzymatic degradation, leading to a reduction of TPCs (11.01 to 17.08 μg GAE/g). MW power of 260 and 440 W were more suitable for RB stabilization, where an increase in TPCs was observed. The highest TPC was 39.62 μg GAE/g in the bran stabilized at 440 W for 2.5 min (1.3 folds). MW power at 620 W (14.15 to 15.81 μg GAE/g) and 880 W (12.67 to 16.72 μg GAE/g) showed a reduction in the TPCs, which might be owing to the highly generated heat in the MWO destroying the phenolic compounds. The TPCs ranged from 3.88 to 11.61 μg of catechol equivalents per g of dry weight (μg CE/g) (Table 1). The control contained 6.14 μg CE/g, and the highest content was 11.61 μg CE/g in the RB treated at 440 W for 2.5 min. TPCs were increased when treated with 130 W (0.5, 1.0, and 1.5 min), 260 W (0.5 and 1.0 min), 440 W (all treatments), and 620 W (1.0 and 1.5 min). Specifically, TPCs were highest when MW power of 440 W was applied (8.24–11.61 μg CE/g), whilst MWT at 880 W caused a reduction in the TPCs compared to the control. A previous study reported a similar trend for TPCs in apricot kernels roasted in MWO. AA of the RB samples was evaluated using free radical DPPH scavenging assay, and expressed as DPPH scavenging percentage (%). AA of the control was 58.87%, and it was increased after the MWT, especially after roasting at 440 W (79.86–88.94%) and 880 W (61.81–77.03%) (Table 1). The highest value was observed in the RB treated at 440 W for 2.5 min (88.94%, increased 0.5 folds). The increase in AA after MWT agreed with a study by Wijesundera (2008), who used MWT on canola and mustard seed. The increase in AA, TPC, and TFC is involved with the capability to release phenolic compounds and other phytochemical compounds from bound structures after breaking of bonds by the MWT. Neverthless, using long exposure time and high temperature in the MWO could significantly destroy the TPC and TFC in cereals. Generally, MWT at 440 W for 2.5 min was the condition that provided the highest values of TPC, TFC, and AA of the RB. The MWT was reported to be effective in stabilizing RB and inhibiting lipase activity, therefore, obtaining desirable phytochemical properties in RB for human consumption.

### Phenolic compounds content.

Eleven phenolic compounds in the RB sample were analyzed with HPLC (Table 2), and the chromatographic elution order was gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, catechin, vanillic acid, chlorogenic acid, caffeic acid, kaempferol, epigallocatechin, trans-p-coumaric acid, and sinapic acid (Supplementary Fig. 1). It was interesting to note that the content of trans-p-coumaric acid had increased by 10.3 folds from 1.82 μg/g in the control to 20.53 μg/g after roasting at 440 W for 1.5 min. Kaempferol, a curative agent against cancer cell growth, was increased markedly by up to 9.6 folds (6.53 μg/g) after MWT at 440 W for 2.0 min. The MWT at 880 W showed a negative effect which reduced the catechin content (18.67–22.86 μg/g) from the control (23.30 μg/g). Furthermore, a reduction in gallic acid content was obtained after MWT at 130 W (3 and 5 min) and 880 W (1.5 and 2 min). The chlorogenic acid, caffeic acid, epigallocatechin, and sinapic acid in RBs showed positive changes when applied with the microwave roasting process. Protocatechuic acid and vanillic acid were the only two phenolic compounds that decreased after MWT. Similar observations were reported in a study of blue poppy seed. The combination of high power and long operation time exhibited a significant decrease in the phenolic content, which might be due to the partial burning of the RB and thermal damage from hydrothermal treatments on nutraceutical contents of RB as reported by Prateep.

### Table 1.

| Power/time | Control | 130 W | 440 W | 620 W | 880 W |
|------------|---------|-------|-------|-------|-------|
| 0.5 min    |         |       |       |       |       |
| 1 min      |         |       |       |       |       |
| 1.5 min    |         |       |       |       |       |
| 2 min      |         |       |       |       |       |
| 2.5 min    |         |       |       |       |       |
| 3 min      |         |       |       |       |       |
| 0.5 min    |         |       |       |       |       |
| 1 min      |         |       |       |       |       |
| 1.5 min    |         |       |       |       |       |
| 2 min      |         |       |       |       |       |
| 2.5 min    |         |       |       |       |       |
| 3 min      |         |       |       |       |       |
| Total phenol (μg GAE/g) | | | | | |
| 17.14 ± 0.04 & 16.64 ± 0.12 | 15.58 ± 0.17 | 12.25 ± 0.09 | 15.35 ± 0.15 & 11.01 ± 0.10 & 12.80 ± 0.11 | 17.08 ± 0.05 | 19.31 ± 0.01 & 17.24 ± 0.01 & 20.04 ± 0.01 | 25.65 ± 0.01 & 24.08 ± 0.01 & 23.84 ± 0.01 |
| Flavonoid (μg CE/g) | | | | | |
| 8.24 ± 0.01 & 8.94 ± 0.01 & 10.48 ± 0.03 | 10.02 ± 0.04 | 11.63 ± 0.04 & 6.04 ± 0.01 & 6.54 ± 0.01 & 7.75 ± 0.02 & 4.56 ± 0.03 & 4.76 ± 0.01 & 5.34 ± 0.01 & 4.15 ± 0.01 & 3.88 ± 0.01 |
| Antioxidant activity (DPPH scavenging %) | | | | | |
| 58.87 ± 0.17 & 66.09 ± 0.12 | 34.80 ± 0.25 & 45.36 ± 0.26 & 36.20 ± 0.27 | 19.61 ± 0.11 & 23.07 ± 0.18 & 36.55 ± 0.03 | 70.62 ± 0.25 & 68.78 ± 0.23 | 66.39 ± 0.02 | 62.65 ± 0.29 & 65.62 ± 0.28 |

| Power/time | Control | 130 W | 440 W | 620 W | 880 W |
|------------|---------|-------|-------|-------|-------|
| 0.5 min    |         |       |       |       |       |
| 1 min      |         |       |       |       |       |
| 1.5 min    |         |       |       |       |       |
| 2 min      |         |       |       |       |       |
| 2.5 min    |         |       |       |       |       |
| 3 min      |         |       |       |       |       |
| 0.5 min    |         |       |       |       |       |
| 1 min      |         |       |       |       |       |
| 1.5 min    |         |       |       |       |       |
| 2 min      |         |       |       |       |       |
| 2.5 min    |         |       |       |       |       |
| 3 min      |         |       |       |       |       |
| Total phenol (μg GAE/g) | | | | | |
| 19.77 ± 0.05 & 22.75 ± 0.06 | 22.95 ± 0.10 & 33.62 ± 0.12 | 16.05 ± 0.02 & 15.99 ± 0.10 & 15.46 ± 0.02 | 18.15 ± 0.01 & 15.81 ± 0.06 & 16.72 ± 0.01 & 14.27 ± 0.02 | 12.87 ± 0.07 |
| Flavonoid (μg CE/g) | | | | | |
| 8.26 ± 0.01 & 9.34 ± 0.01 | 10.48 ± 0.05 & 10.57 ± 0.08 & 11.03 ± 0.04 & 6.04 ± 0.01 & 6.54 ± 0.01 & 7.75 ± 0.02 & 4.56 ± 0.03 & 4.76 ± 0.01 & 5.34 ± 0.01 & 4.15 ± 0.01 & 3.88 ± 0.01 |
| Antioxidant activity (DPPH scavenging %) | | | | | |
| 88.71 ± 0.23 & 79.86 ± 0.06 | 82.75 ± 0.14 & 87.79 ± 0.27 & 88.94 ± 0.27 | 67.42 ± 0.02 & 62.95 ± 0.26 & 67.55 ± 0.12 | 77.03 ± 0.22 & 71.35 ± 0.27 | 61.81 ± 0.11 |
Table 2. Phenolic compounds of rice bran treated in microwave oven (µg/g). Values are means ± standard deviations (n = 3).

| Phenolic compound | Control | 130 W | 260 W |
|-------------------|---------|-------|-------|
|                   | 0.5 min | 1 min | 2 min |
|                   | 0.5 min | 1 min | 2 min |
| Gallic acid       | 25.2 ± 0.32 | 23.02 ± 0.096 | 32.67 ± 0.104 |
| vanillic acid     | 2.58 ± 0.13k | 3.39 ± 0.16k | 3.94 ± 0.19k |
| protocatechuic     | 2.10 ± 0.15k | 2.03 ± 0.19k | 2.10 ± 0.15k |
| acid              | 1.07 ± 0.15k | 1.07 ± 0.15k | 0.78 ± 0.14m |
| 4-Hydroxy-protocatechuic | 3.82 ± 0.02m | 3.62 ± 0.02m | 3.16 ± 0.02m |
| acid              | 2.15 ± 0.01k | 1.15 ± 0.01k | 4.13 ± 0.04m |
| Gallic acid       | 4.67 ± 0.04k | 4.75 ± 0.04k | 6.80 ± 0.05k |
| 4-Hydroxy-protocatechuic | 3.82 ± 0.02m | 3.62 ± 0.02m | 3.16 ± 0.02m |
| acid              | 2.15 ± 0.01k | 1.15 ± 0.01k | 4.13 ± 0.04m |
| Kaempferol        | 2.56 ± 0.06k | 2.59 ± 0.06m | 3.11 ± 0.06m |
| 4-Hydroxy-protocatechuic | 3.82 ± 0.02m | 3.62 ± 0.02m | 3.16 ± 0.02m |
| acid              | 2.15 ± 0.01k | 1.15 ± 0.01k | 4.13 ± 0.04m |

Note: Values in the same column with different superscript letters are significantly different (p < 0.05).

References:
14. Based on our results, MWT at 440 W for 1.5 min was the best MWT setting to increase the overall phenols content.
15. RB is an abundant source of tocals (α-, β-, γ-, δ-tocopherol (T), α-, β-, γ-, δ-tocotrienol (T3)), γ-oryzanols, phytosterols, and squalene. The changes in tocals content after MWT is shown in Table 3 and chromatographic results are shown in Supplementary Fig. 2 (left side). The vitamin E in the raw RB were γ-T3 (84.86 µg/g), followed by α-T (12.43 µg/g), α-T3 (8.64 µg/g), γ-T (4.29 µg/g), β-T (1.85 µg/g), δ-T (0.37 µg/g), respectively. The MWT had positive effects on the tocals content of the RB, especially at 440 W. The changes of the tocals were dependent on the exposure time and microwave power, in which the MWT at 440 W for 2.5 min obtained the highest contents of total tocals (367.09 µg/g, equivalent to 2.6-fold increase from the control of 101.95 µg/g).

The results for other functional compounds are shown in Table 4, and the chromatographic result is shown in Supplementary Fig. 2 (right side). These include γ-oryzanols, a fundamental substance with several health-beneficial effects, such as anti-oxidant activity, anticarcinogenic, and anti-diabetic.17,18 The main γ-oryzanols in the raw RB was 24-methylene cycloartanyl ferulate (24-MCFer) (716.55 µg/g), followed by cycloartenyl ferulate (CycloFer) (442.77 µg/g), campsterol ferulate (CampFer) (270.05 µg/g), and β-sitosteryl ferulate (β-SitFer) (119.94 µg/g) with the total γ-oryzanols content of control at 1549.31 µg/g. The current study showed an enhancement of phenolics after MWT. The optimum exposure power was 260 W, which the CycloFer, 24-MCFer, CampFer, and β-SitFer increased 1.3, 2.4, 0.6, and 1.4 folds than those of the control, respectively. The MWT of KDML 105 RB in this study showed a 1.6-fold increase of total γ-oryzanols while the parboiled and steamed of Sorai maunri showed 0.7 and 0.4-fold increase14. Generally, the MWT contributed to the positive changes in total tocals content.

The highest total tocals content was found in the RB treated at 440 W for 1 min (3059.56 µg/g), which increased 1.4 folds from the control (1252.01 µg/g). In most cases, microwave-treated RB showed higher levels of the tocals (β-SIT) than the raw RB (424.76 µg/g). The highest content of β-SIT was found in the RB treated...
Table 3. Tocots content (α-, β-, γ- and δ-tocopherol (T) and α-, β-, γ- and δ-tocotrienol (T3)) of rice bran treated in microwave oven (µg/g). ND non detectable. Values are means ± standard deviations (n = 3).

| Compound | Control | 130 W | 260 W | 440 W | 880 W |
|----------|---------|-------|-------|-------|-------|
|          | 0.5 min | 1 min | 1.5 min | 2 min | 0.5 min | 1 min | 1.5 min | 2 min | 0.5 min | 1 min | 1.5 min | 2 min | 0.5 min | 1 min | 1.5 min | 2 min | 0.5 min | 1 min | 1.5 min | 2 min |
| α-T     | 12.49 ± 0.23a | 6.31 ± 0.49e | 8.78 ± 0.21g | 10.38 ± 0.15e | 13.13 ± 0.20c | 9.81 ± 0.10k | 12.42 ± 0.10q | 21.60 ± 0.10q | 21.02 ± 0.12b | 10.23 ± 0.08b | 3.89 ± 0.01h | 18.58 ± 0.10p | 10.33 ± 0.12l |
| β-T     | 1.84 ± 0.15m | 1.12 ± 0.13a | 1.64 ± 0.19b | 2.04 ± 0.12c | 3.02 ± 0.05f | 2.43 ± 0.10h | 1.70 ± 0.12q | 1.80 ± 0.04j | 2.47 ± 0.33m | 2.52 ± 0.11q | 1.94 ± 0.00k | 2.32 ± 0.11q | 2.40 ± 0.22a | 2.02 ± 0.37p |
| γ-T     | 4.25 ± 0.23n | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q | 5.36 ± 0.35q |
| δ-T     | 0.14 ± 0.01p | 0.60 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p | 0.70 ± 0.01p |
| α-T3    | 6.41 ± 0.75e | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j | 9.61 ± 1.50j |
| β-T3    | ND       | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     |
| γ-T3    | 12.34 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o | 32.94 ± 0.41o |
| δ-T3    | 1.78 ± 0.01q | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r | 2.85 ± 0.01r |
| Total toc | 101.95 ± 0.87n | 109.29 ± 0.46k | 115.38 ± 0.88t | 119.21 ± 0.71i | 130.69 ± 0.52h | 127.28 ± 0.91i | 114 ± 0.53l | 63.33 ± 0.75st | 72.41 ± 0.07o | 108.36 ± 0.79lm | 183.38 ± 0.97rs | 187.5 ± 0.72q | 187.42 ± 0.64p | 75.69 ± 0.73r |

at 130 W for 5 min (1904.84 µg/g) and increased 3.5 folds from the control. The highest stigmasterol and camp- esterol (STIG + CAMP) content was found in the RB treated at 440 W for 1 min (1369.87 µg/g) which increased 0.7 folds as compared to the control (827.25 µg/g). The increase in MW power 130 W to 440 W at exposure time 0.5, 1, and 1.5 min contributed to a gradual increase in the STIG + CAMP content. However, a decrease in TOC content as compared to the control was observed for 880 W in 1.5 min and 2 min as well as 130 W for 3 min. This indicated that the high power and long exposure time in the MWT caused significant damage to the phytochemicals content.

The content of squalene (99.55 µg/g), cholecalciferol (3.01 µg/g), and phylloquinone (2.45 µg/g) in the control showed improvements after the MWT. Roasting at 440 W for 1 min obtained the highest content of squalene (303.89 µg/g), increased by 2.0 folds, and roasting at 880 W provided the highest content of cholecalciferol (14.15 µg/g, increased by 3.7 folds) and phylloquinone (11.91 µg/g, increased by 3.9 folds). The impact of exposure temperature on phylloquinone determination exhibited the same trend as the effect of time on squalene content. Pokkanta et al., (2019) reported that RBs were an abundant source of phytosterols (stigmasterol, campesterol and β-sitosterol) and squalene.

Based on our results, the changes of phytochemicals when exposed to MWT with increasing power and exposure time share the same trend. The phytochemical content in RBs after MWT proportionally increased with increasing MW power and exposure time until it reaches its highest value. Sequentially, a decrease in phytochemical content was observed for MWT at high power and long exposure time. This could be because the phytochemicals in plant cell walls, such as phenolic compounds, dissolve due to the breakage of the bonds that connect them. Solubility of the phytochemical increased as a result of its dissolution in cell tissue, increasing the released phytochemicals. The heat generated from the MWO can inactivate enzymes such as lipase, oxidase, causing deterioration of the phytochemicals. The antioxidant activity was increased partly due to the formation of the Maillard reaction products, an antioxidant in foods.

In general, MWT could improve the nutrients of food samples, however, the appropriate MW power and exposure time are required for different crop material to retain high amounts of phytochemicals. The results found different optimum conditions for the content of γ-oryzanol (260 W for 2 min), tocots (440 W for 2.5 min), phytosterols (440 W for 1 min), squalene (440 W for 2.5 min), cholecalciferol, and phylloquinone (880 W for 2 min). However, the MWT at 440 W for 2.5 min was concluded as the best overall condition, which provided the highest content of the studied bioactive compounds and antioxidant activity.
Conclusions

The study revealed that the MWT increased antioxidant activities and amounts of released bioactive compounds from the RB. The MWT was able to increase the capability of the phytochemical compounds to be released from their bound structures. The MWT required very little time, therefore, enabling the preservation of nutraceutical values and properties of the RB. The long exposure time and high power in the microwave process might cause degradation of the nutrients. The findings suggested that the MWT could be a powerful tool for the stabilization, enhancement of usability, and retainment of RB phytochemicals.

Material and methods

Plant materials. KDML 105 (the most popular aromatic rice variety in Thailand) RB sample was requested and permitted from the Suphanburi Rice Research Centre (a government office of the Rice Department, Ministry of Agriculture and Cooperative), Thailand in December 2019. The RB was sieved through 60-mesh, packed in a ziplock bag, and stored at -10 °C until the day of sample preparation.

Chemicals. Standards of phenolics, γ-oryzanol, phytosterols, squalene, cholecalciferol, phylloquinone were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Standard tocols, Folin-Ciocalteu reagent, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co., Ltd, (Darmstadt, Germany) and Eisai Food & Chemical Co., Ltd. (Tokyo, Japan). The other chemicals used were of analytical grade from RCI Labscan Co., Ltd. (Bangkok, Thailand).

Methods

The study complies with local and national guidelines.

Microwave stabilization. A MWO (R-2200F-S, cavity of 30.6×30.7×20.8 cm, Sharp, Thailand) capable of generating power of 880 W at 2450 MHz was used for the roasting experiments. A petri dish with a 100 mm diameter containing the RB sample (10 g) was placed in the middle of the rotary plate of the MW oven (i.d. 100 mm).
28 cm). The RB samples were heated with 130, 260, 440, 620, and 880 W and exposure duration of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 5.0 min.

**Spectrometry analysis of phenolics, flavonoids, and antioxidant activity in RB.** RBs (0.5 g) were extracted with 5.00 mL of 80% methanol under sonication for 1 h. The extraction solvent was chosen because it is proven to be the most effective extraction solvent for phenolics and antioxidant activity in rice. Sonication was used to maximize extraction efficacy of the targeted compounds. The resulting solution was centrifuged at 3500 rpm for 10 min, and the supernatant was filtered through a 0.45 µm nylon filter. The resulting extract was subjected to determination of phenolics, flavonoids, and antioxidant activity with a UNICO 2150-UV Spectrophotometer.

**HPLC analysis of individual phenolic, tocols, γ-oryzanos, phytosterols, squalene, cholecalciferol and phylloquinone.** Two HPLC systems were employed. The first system was applied for the analysis of the eleven phenolic compound. Phenolic compounds were extracted with the same method used in the spectrometric analysis. The system utilized a Kinetex C18 column (150 × 4.6 mm; 26 µm, Phenomenex) and a gradient elution system consisting of water/acetic acid (99:1, v/v) as mobile phase A and water/acetonitrile/acetic acid (67:32:1, v/v/v) as mobile phase B. The phenolics were detected at 275 nm. The other HPLC system was for analysis of the other functional phytochemicals (total of seventeen compounds). RBs (0.30 g) were extracted with methanol (3.00 mL) for 5 min, and the extracted RBs were then re-extracted with dichloromethane (3.00 mL) and hexane (3.00 mL), respectively. Supernatants of these three solvents were combined, evaporated, and re-dissolved with dichloromethane before analysis. The HPLC system employed a Kinetex PFP column (4.6 × 250 mm, 5 µm, Phenomenex) and a mobile phase of methanol and water. A fluorescent detector was set to detect cholecalciferol at 265 nm (0–8 min), phytosterols and squalene at 210 nm (8–18 min), and phylloquinone and γ-oryzanos at 328 nm (18–30 min).

**Statistical analysis.** Quantitative data were expressed as the mean ± standard deviation (n = 3). Statistical analysis in this study was analyzed using one-way ANOVA with RStudio version 1.2.5042. Differences are statistically significant at P < 0.05.

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Author contributions

P.P.: Conceptualization, methodology, measurement, analysis, data curation, and writing. J.Y.: measurement, and analysis. S.M.: review & editing. S.J.: review & editing. P.S.: supervision, writing—review & editing. All authors reviewed and revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to P.S.

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