Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2025, Vol. 25 (No.1), e0260012

Research article

Gamma-oryzanol, Physicochemical and Antioxidant Properties of Stabilized Rice Bran Oil from Dough and Mature Grain Stages

Boonyabhorn Duangsa[1], Porntip Rodpon[2], Chompoonuch Khongla[3], Sumalee Musika[3], Phimnipha Rattanajun[2], Patcharida Khumpumuang[3], Usana Jangkloy[2], Surawee Jampatech[3], Piyamaporn Thiamjite[2] and Apichai Sawisit[3]*

[1]Faculty of Business Administration and Information Technology, Rajamangala University of Technology Isan , Khon Kaen, Thailand
[2]Faculty of Business Administration, Rajamangala University of Technology Isan , Nakhon Ratchasima, Thailand
[3]Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand

Received: 15 September 2023, Revised: 5 March 2024, Accepted: 27 June 2024, Published: 17 October 2024

Abstract

Rice bran samples from dough and mature grain stages of rice were stabilized by heating in a hot air oven at 70ºC for 3h (or moisture <5.0%). Unstabilized and stabilized rice bran samples were subsequently pressed for oil extraction using a screw press machine. The γ-oryzanol, physicochemical, and antioxidant properties of both the unstabilized and stabilized rice bran oil (RBO) were determined. The fat content of fresh rice bran (unstabilized) from dough stage (19.19 %w.b.) was higher than that of the mature stage (13.22 %w.b.). Unstabilized RBO from mature stage (URBO-MS) had the lowest L* (35.70) and the highest a* (4.28) values. The γ-oryzanol content detected by UV-Vis spectophotometer was not significantly different among URBO-MS, stabilized RBO from the dough stage (SRBO-DS), and crude commercial RBO (CRBO). While the γ-oryzanol content of SRBO-DS detected by reversed-phase HPLC with diode array detector (DAD) was the highest, with a value of 1.00 g/100 g oil. The DPPH scavenging activity of SRBO-DS was the highest while that of CRBO was the lowest. The ABTS radical scavenging activity of unstabilized RBO from the dough stage (URBO-DS), SRBO-DS, and URBO-MS were not significantly different and were higher than that of stabilized RBO from the mature stage (SRBO-MS) and CRBO. The peroxide and free fatty acid (FFA) contents of SRBO-DS were the lowest with values of 1.19 meg/kg and 4.84% (as oleic acid), respectively. This finding suggests that stabalizing rice bran at the dough stage can increase the color values (as L* and b* values), γ-oryzanol, and DPPH scavenging activity, and decrease peroxide and FFA values of RBO. Thus, stabilized RBO from the dough stage grain may be a functional food with antioxidant activity.

Keywords: dough grain stage; rice bran oil; antioxidant activity; γ-oryzanol; RP-HPLC

*Corresponding author: E-mail: apichai.sa@rmuti.ac.th
https://doi.org/10.55003/cast.2024.260012

1. Introduction

Rice (*Oryza sativa* L.), an important food crop globally, is the main crop in Thailand. It is a good source of vitamins, minerals, dietary fiber, proteins, fats, carbohydrate, and antioxidants (Andriani et al., 2022; Preecharram et al., 2023), and is a primary part of the diet of people in Thailand and many other countries in Asia. The USDA estimates Thailand rice production was 19.5 million metric tons on a milled basis in the market year 2021-2022 (USDA Foreign Agricultural Services, 2021). Khao Dawk Mali 105 rice, known internationally as Thai jasmine rice, is the most famous exported Thai rice (Kukusamude & Kongsri, 2018). This strain is grown in the Thung Kula Rong Hai region, which includes five provinces of Thailand's northeastern region: Surin, Yasothon, Roi-Et, Sisaket, and Mahasarakham (Kukusamude & Kongsri, 2018). The Community Enterprise Banmao Career Promotion Group Limited Partnership (CEBCPGLP) has 72 members and is located at Suwannaphum district, Roi-Et province in the Thung Kula Ronghai region. Most of the members are farmers who cultivate Khao Dawk Mali 105 rice. The CEBCPGLP produces and sells Khao Dawk Mali 105 rice from both the dough and mature grains stages. Dough and mature grains of the CEBCPGLP were collected on day 20-30 and 80-90 after heading, respectively. Brown rice from the dough grain stage of the CEBCPGLP contained high nutrient values, which were 7.11% protein, 2.93% fat, 1.30% ash, 2.89 dietary fiber, and 73.70% carbohydrate. In addition, the rice had in omega 3 (α-linolenic acid) and omega 6 (linoleic acid) contents of 35.07 mg/100 g and 828.24 mg/100 g, respectively. Therefore, the polishing process of brown rice into white rice from dough stage grain could produce rice bran with high nutrient values. Anually, approximately 200,000 kg of organic Khao Dawk Mali 105 rice is produced from the CEBCPGLP. This can yield approximately 20,000 kg of rice bran per year. However, rice bran is used only as low-value animal feed. Therefore, attempts should be made to increase marketability of rice bran from the dough grains.

Rice bran is a co-product obtained from the outer layer of the brown rice kernel during the milling process and accounts for approximately 8-10% of the rice kernel. It is rich in oil content, which ranges between 8.7-25% depending on the milling process, the region of origin, and the rice variety (Lerma-García et al., 2009; Liao et al., 2020). Due to its high oil content, it can be used as a raw material for oil extraction in the rice bran oil (RBO) industry. RBO is popular because it contains many healthy components such as, γ-oryzanol, tocopherols, tocotrienols, phytosterols, and monounsaturated fatty acids (Bumrungpert et al., 2019; Endo & Aso, 2019). The γ-oryzanol in RBO could increase antioxidant activity in hyperlipidemic subjects and decrease LDL-C levels. Therefore, the consumption of RBO with γ-oryzanol may reduce cardiovascular disease risk factors (Bumrungpert et al., 2019).

However, one problem with rice bran is the presence of lipolytic enzymes (especially lipases) which hydrolyze triglycerides into free fatty acids (FFA) and cause rancidity in the RBO. After milling process, the oil is exposed to the lipases, which break down lipid triglycerides into FFA at a rate of 5-7% of the oil's weight every day (Gopinger et al., 2019). To prevent the formation of FFA and rancidity in cold-pressed RBO, rice bran is pressed within 24 h after rice polishing (Duangsi & Krongyut, 2023). However, it is not practical to separate the oil immediately after milling in small and large-scale of rice processing due to the limitations of distance and transportation of bran to the factory (Thanonkaew et al., 2012). Therefore, the stabilization process of rice bran is needed to overcome this problem and improve the quality and shelf life of rice bran as well as the RBO by decreasing peroxidase, lipases, lipoxygenase, and auto-oxidation enzymatic

activities (Lavanya et al., 2019). Recently, various stabilization processes have been applied to inhibit lipases in rice bran including heat treatments (hot air drying, steaming, toasting, roasting, and autoclaving) (Amarasinghe et al., 2009; Ilias et al., 2020; Thanonkaew et al., 2012), microwave heating (Lavanya et al., 2019), ohmic heating, and ultra-superheated stream (Moreno et al., 2021), extrusion (Guevara-Guerrero et al., 2019; Rashid et al., 2023), hot air-assisted radio frequency heating (Liao et al., 2020), and infrared radiation heating (Irakli et al., 2018). Among them, heat treatment is the most common method used to stabilize rice bran due to the fact that it is a more economical method. In addition, it is suitable for small scale industry and the CEBCPGLP. Therefore, in this work, heat treatment in the form of hot air heating was selected to stabilize rice bran before oil separation.

Generally, the two most common methods for extracting RBO from rice bran are solvent extraction and mechanical pressing. Hexane is the most widely used solvent in conventional method for commercial extraction due to its good availability, high oil extractability, and the ease of usage (Punia et al., 2021). However, the use of hexane has some drawbacks due to its toxicity, flammability, and the need for a high temperature (Balachandran et al., 2008). In addition, the use of hexane can have negative effects on oil quality such as higher contents of FFA, wax, and other undesirable products in the oil (Rigo et al., 2014). Mechanical pressing (cold pressing) is cheaper and less labor intensive than solvent extraction (Sayasoonthorn et al., 2012). Additionally, cold pressing requires no heat or chemical treatments, making it an appealing alternative to consumers who value natural and safe products (Uquiche et al., 2008). Furthermore, cold pressing is an easy process to maintain and does not require much energy. Thus, it is a suitable method for small and medium scale size RBO factories (Thanonkaew et al., 2012).

Therefore, the objectives of this work were to study the effects of stabilization (by hot air heating) of rice bran on γ-oryzanol content, peroxide value, FFA content, physicochemical, and antioxidant properties. The experimental RBO samples were derived from the dough and mature grain stages of organic jasmine rice.

2. Materials and Methods

Rice bran samples from the dough and mature grain stages of organic Jasmine rice were obtained from the Community Enterprise Banmao Career Promotion Group Limited Partnership (Suwannaphum, Roi Et, Thailand). The dough and mature grains were at days 20-30 and 80-90 after heading, respectively. Crude commercial rice bran oil (CRBO) was purchased from Roidham (Bangkok, Thailand). γ-Oryzanol was purchased from Toronto Research Chemicals Inc (Toronto, ON, Canada). 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Biochemika (Buchs, Switzerland). DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Company Ltd. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

2.1 Proximate composition analysis

The proximate compositions of the fresh rice bran samples (unstabilized) from the dough and mature stages of rice were determined according to the method of AOAC (2000).

2.2 Rice bran stabilization

In preliminary study, rice bran samples from dough and mature stage grains were stabilized by both heating in hot air oven (70°C for 3 h) or by steaming (100°C for 3 h, followed by heating in hot air oven at 60°C for 4 h). However, the steaming process produced an undesirable lowering of the oil extraction yield when compared to unstabilized and stabilized rice bran heated in a hot air oven. Therefore, in this work, only heat treatment by a hot air oven was used to stabilize rice bran. In brief, 10 kg of rice bran powder from the dough and mature grain stages were put in trays. The samples were then stabilized by heating in a hot air oven (Memmert, UF110, Germany) at 70°C for 3 h or until the moisture content was below 5.0%. Next, the samples were cooled down to room temperature, after which they were placed into polyethylene bags and kept at -20°C until the oil extraction was performed.

2.3 Rice bran oil separation by screw press

Ten kilograms of unstabilized and stabilized rice bran from the dough and mature stages were pressed for RBO separation using a screw press machine (Model FEA-200A) at Energy Friend Ltd., Part, Thailand. The screw press had a 7.5 hp motor and ran at 380 V with a screw speed of 103.18 rpm. Fine particles in the crude rice bran oil were separated by using double layers of filter cloth and then the oil was centrifuged at 8,000 rpm for 5 min. The extracted oil samples were designated as follows: URBO-DS, oil from unstabilized rice bran of dough stage grain; SRBO-DS, oil from stabilized rice bran from dough stage grain; URBO-MS, oil from unstabilized rice bran from mature stage grain; and SRBO-MS, oil from stabilized rice bran from mature stage grain . The extracted oil samples were put in glass bottle and kept at 4°C in the dark for further experiments.

2.4 Color values measurement

The color values of RBO samples (URBO-DS, SRBO-DS, URBO-MS, SRBO-MS, and CRBO) were measured in terms of CIE L*, a*, b* values using a color measurement device (Hunter Lab, Color flex 4510, USA), where L*, a*, and b* are lightness, redness, and yellowness, respectively.

2.5 γ-Oryzanol analysis

2.5.1 UV-Vis spectophotometry

The γ-oryzanol contents in RBO samples (URBO-DS, SRBO-DS, URBO-MS, SRBO-MS, CRBO) were determined according to the method described by Lilitchan et al. (2008), with slight modifications. In brief, 100 µL of RBO samples were diluted with 100 mL of isopropanol at concentration of 0.10% (v/v). The absorbance was meastured at 314 nm using a UV-Vis spectophotometer (Thermo Fisher, Scientific Genesys 10 UV scanning, USA). A stock standard γ-oryzanol (0.10 mg/mL) was prepared by mixing 10 mg of γ-oryzanol with 10 mL of isopropanol. A standard curve for γ-oryzanol was prepared in a concentration range of 0.0025-0.050 mg/mL. The γ-oryzanol content in RBO was calculated against the standard curve and expressed as mg/mL oil.

2.5.2 Reversed-phase high-performance liquid chromatography (RP-HPLC)

The γ-oryzanol contents in RBO samples were analyzed by reversed-phase HPLC with diode array detector (DAD) (Agilent 1100, HPLC system, USA). The RBO samples were filtered through a 0.45 µM syringe filter and then 20 µL of samples were injected to PFP HPLC column (4.6 x 250 mm, 5 µm). The initial mobile phase contained the mixure of 45% acetonitrile, 45% methanol, 5% isopropanol, and 5% of aqueous acetic acid (1%) which were used for the separation investigations, with a flow rate of 0.8 mL/min for 6 min. The mobile phase was changed to a linear gradient of acetonitrile: methanol: isopropanol at the ratio of 25:70:5 (v/v/v) over the next 10 min and then kept constant for 12 min before returning to the initial conditions. The γ-oryzanol were detected by the Diode array detector at 325 nm. Finally, the γ-oryzanol contents in RBO were calculated against the standard curve of pure γ-oryzanol (Azrina et al., 2008; Chen & Bergman, 2005).

2.6 Antioxidant activity assays

2.6.1 DPPH radical scavenging activity

The DPPH radical scavenging activities of RBO samples were performed according to the method described by Sanchez-Moreno et al. (1999), with some modifications. In brief, 50 µL of each diluted RBO sample at concentration of 20% (v/v) was mixed with 1950 µL of 40 mg/L DPPH in absolute ethanol. Then, the mixture was left at room temperature in the dark for 30 min. The absorbance of each mixture was read by a UV-Vis spectophotometer at 517 nm. Trolox solution at concentrations of 0.0-1.5 mM were used as standard. Abslute ethanol was used as the blank. The DPPH radical scavenging activity of the samples was calculated and expressed as mg Trolox equivalents (Eq.)/mL oil.

2.6.2 ABTS radical scavenging activity

The ABTS radical scavenging activities of RBO samples were performed according to the method described by Khongla et al. (2022a), with some modifications. Briefly, 20 µL of each diluted RBO samples at concentration of 5% (v/v) were mixed with 1980 µL of ABTS working solution (initial Abs. of 0.70±0.02). Then, each mixture was left at room temperature in the dark for 5 min. The absorbance of the mixture was read by a UV-Vis spectophotometer at 734 nm. Trolox solution at concentrations of 0.0-2.5 mM were used as standard. Abslute ethanol was used as the blank. The ABTS radical scavenging activities of the samples were calculated and expressed as mg Trolox equivalents (Eq.)/mL oil.

2.7 Peroxide value and free fatty acid (FFA) assays

The peroxide and FFA values of RBO were analyzed according to the method of AOAC (1990). The FFA content was calculated as % of oleic acid.

2.8 Statistical analyses

All experiments were performed in triplicate, except for RP-HPLC. Statistical analyses were carried out using a SPSS package. The results were analyzed using a one-way analysis

of variance (ANOVA) at $p < 0.05$. Duncan's multiple range test (DMRT) was used to determine significant mean differences at the 95% confidence level.

3. Results and Discussion

3.1 Proximate composition of rice bran

The proximate composition of the fresh rice bran from the dough and mature stage grains is presented in Table 1. The fat content of rice bran from the dough stage was 19.19±0.91%, which was higher than rice bran from mature grains, which were 13.22±0.80%. Ranathunga et al. (2023) reported that crude fat in milky stage of rice grain was the highest (3.8%, db), followed by bran from the dough (3.5%, db) and mature stages (1.3%, db). In addition, Juliano & Tuaño (2019) reported that non-starch-bound lipids and starch-bound lipids in rice grains increased until 12 days after flowering (DAF) and 20 DAF, respectively. Rice bran is the brown outer layer of rice grain and is obtained from polishing the rice down to the white starchy rice kernels. Therefore, in this study, the higher fat content in dough grain stage of rice resulted in a higher fat content in rice bran derived from the dough stage grain compared to rice bran from mature grain. Many researchers reported that rice bran had oil levels of 11.42% (Putri & Sukanta, 2012), 17.87% (Faria et al., 2012), 11.0-18.0% (Kumari et al., 2018), 16.32% (Pimpa et al., 2021), 12-22% (Devi et al., 2021), 13.92-19.81% (Chatha et al., 2011), and 19.23% (Khongla et al., 2022b). Based on our analysis of fat content, rice bran from immature rice (dough stage) appears to be a promising new source of rice bran oil. Rice bran from mature grain contained higher carbohydrate level (46.94%) than rice bran from dough stage (42.25%) (Table 1). Ranathunga et al. (2023) also stated that the carbohydrate content of rice grain increased with maturation because of a starch-filling process. Previous studies reported that rice bran had carbohydrate at levels of 46.75% (Pimpa et al., 2021), 57.39% (Faria et al., 2012), 34-62% (Kumari et al., 2018), and 46.51% (Khongla et al., 2022b). The moisture content of rice bran from mature stage (12.99%) was higher than that from the dough stage (10.30%) (Table 1). Previous studies reported that rice bran had moisture contents of 11.22% (Putri & Sukanta, 2012), 14.56% (Thanonkaew et al., 2012), 10-15% (Devi et al., 2021), 7.65% (Pimpa et al., 2021), 8.41% (Faria et al., 2012), and 12.70% (Khongla et al., 2022b). The ash, protein, and fiber content were not significantly different between rice bran from the dough and mature grain stages, which were 8.78-9.03% ash, 11.55-12.41% protein, and 6.27-7.08% fiber (Table 1). However, Ranathunga et al. (2023) reported that the protein, fat, fiber, and ash contents of rice grain decreased with maturation. It was reported that rice bran possessed 11-17% of protein, 6-14% of fiber and 8-17% of ash (Khongla et al., 2022b). Wisetkomolmat et al. (2022) reported that 11 Thai rice bran varieties contained 6.19-10.27% of ash, 4.29-6.72% of fiber, and 12.04-15.20% of protein. Faria et al. (2012) reported that rice bran contained 16.61% of protein and 8.13% of ash. Pimpa et al. (2021) reported that rice bran contained 12.65% of protein, 6.38% of ash, and 6.38% of fiber. While Khongla et al. (2022b) stated that protein and ash in rice bran were 12.63% and 8.93%, respectively. In our findings, the proximate compositions of rice bran from the dough and mature stages were within the range of previous reports as mentioned above. The chemical composition of rice bran depends on the variety, milling method, other agroclimatic conditions, and the stage of rice grain. Moreover, the proximate analysis of rice bran from the dough and mature grain in this work provides nutritional information for further applications of rice bran in foods.

Table 1. Proximate compositions of fresh (unstabilized) rice bran from the dough and mature grain stages

Proximate Composition	Content (%wet basis)	
	Dough Stage	Mature Stage
Moisture	10.30±0.15[b]	12.99±0.18[a]
Ash [ns]	8.78±0.29	9.03±0.06
Fat	19.19±0.91[a]	13.22±0.80[b]
Protein [ns]	12.41±0.47	11.55±0.30
Fiber [ns]	7.08±0.42	6.27±0.41
Carbohydrate	42.25±0.83[b]	46.94±0.25[a]

Different superscripts within a row are significantly different ($p<0.05$) ; ns is not significantly different ($p\geq0.05$).

3.2 Color values

Another important feature that determines the visual acceptability of rice bran oil is color (Thanonkaew et al., 2012). The L*, a*, and b* values indicate the lightness, redness, and yellowness of the sample, respectively. The highest L* value (41.17) was observed in crude commercial rice bran oil (CRBO), followed by oil from stabilized rice bran of the dough stage (SRBO-DS), oil from unstabilized rice bran of the dough stage (URBO-DS), oil from stabilized rice bran of the maturity stage SRBO-MS, and oil from unstabilized rice bran of the mature stage (URBO-MS) (Table 2). This indicated that CRBO had more lightness than others. The a* value of CRBO was the lowest (2.12) while URBO-MS had the highest a* value (4.28) (Table 2). It was observed that URBO-MS had darker color than the others. SRBO-DS had the highest b* value (12.00) while URBO-MS had the lowest b* value (6.24). These findings suggested that SRBO-DS had more yellowness than the others. Stabilization of rice bran increased the L* and b* values, and decreased the a* value of RBO when compared with the respective RBO from unstabilized samples. In generally, the darked color of rice bran increases with increase in heating temperature and time of stabilization because rice bran contains moderately high amounts of reducing sugars and proteins that may induce Maillard reactions during heating (Velasquez & Villarino, 2021). In our experiment, oil was separated from unstabilized and stabilized rice bran using a screw press machine at a large scale (10 kg of rice bran). Thus, Maillard reactions may occur during oil separation due to heat generated from frictional phenomena associated with the screw and barrel. Yeo & Shibamoto (1991) reported that maximum browning intensity occurred at approximately 14% moisture. Unstabilized rice bran derived from the dough and mature grain stages contained moisture contents of 10.30±0.15% and 12.99±0.18%, respectively (Table 1). These comparatively higher moisture levels might promote increased levels of Maillard reactions than stabilized rice bran (moisture content < 5.0 %) during oil separation. This may also explain the lower L* and b* but higher a* values in unstabilized RBO than that of stabilized RBO. Thanonkaew et al. (2012) reported that the lightness value of RBO derived from rice bran stabilized by hot air was comparable with unsabilized rice bran, but roasting and steaming reduced the lightness of RBO. Besides, Duangsi & Krongyut (2023) suggested that RBO from infrared-stabilized rice bran had darkened color at the beginning due to the immediate effects of IR heating on non-enzymatic oxidation leading to the formation of colored pigments. However, the colored pigments were further oxidized into colorless compounds, resulting in lightening of the color as storage progressed (Duangsi & Krongyut, 2023). They also suggested that the RBO extracted from unstabilized rice bran was lighter at the beginning but became darker during

storage due to the lipid oxidation (Duangsi & Krongyut, 2023). The oxidation of oil or fat by light, air, or heat produces free radicals which combine with oxygen to form yellow to brown secondary products (Marei et al., 2017; Rodchuajeen et al., 2016). In our experiment, unstabilized RBO might be oxidized by lipase during the oil separation with double layers of filter cloth. This was indicated by the peroxide and FFA contents in unstabilized RBO being higher than that of respective stabilized RBO (Table 2). It could also explain the darkening of RBO derived from unstabilized rice bran being greater than that of stabilized rice bran. This suggested that the stabilization of rice bran by heating at 70ºC for 3h in a hot air oven improved the lightness and yellowness of RBO.

When compared between grain stage, the RBO from dough stage had higher L* and b* values but lower a* value than mature stage (Table 3). Jiamyangyuen et al. (2017) reported that rice grain of different stages had different pigments or colors, and white rice acquired a green color at dough stage. Thus, after rice polishing, the obtained rice bran from the dough stage had a green-yellow color, while rice bran from the mature stage had a brown color. Therefore, the observation in our study could explain why RBO derived from the dough and mature grain stages were of different color.

Table 2. Peroxide values and free fatty acid of unstabilized and stabilized RBO of rice bran from the dough and mature grain stages.

Rice Bran Oil	Peroxide (meq/kg oil)	Free Fatty Acid (% as oleic acid)
URBO-DS	3.00±0.06^{c}	6.98±0.60^{a}
SRBO-DS	1.19±0.21^{e}	4.84±0.37^{b}
URBO-MS	10.49±0.18^{a}	7.42±0.64^{a}
SRBO-MS	8.86±0.09^{b}	5.12±0.18^{b}
CRBO	2.14±0.13^{d}	7.66±0.66^{a}

Different superscripts within a column indicate significant differences ($p<0.05$).

Table 3. Color values of unstabilized and stabilized RBO from rice bran from dough and mature stages of organic jasmine rice

Rice Bran Oil	Color Value		
	L*	a*	b*
URBO-DS	37.58±0.60^{c}	3.18±0.02^{c}	8.05±0.12^{c}
SRBO-DS	40.18±0.23^{b}	2.52±0.02^{d}	12.00±0.15^{a}
URBO-MS	35.70±0.31^{e}	4.28±0.01^{a}	6.24±0.18^{e}
SRBO-MS	36.38±0.24^{d}	3.94±0.02^{b}	7.04±0.10^{d}
CRBO	41.17±0.01^{a}	2.12±0.04^{e}	10.23±0.01^{b}

Different superscripts within a columen indicate significantly diffèrences ($p<0.05$).

3.3 γ-Oryzanol

γ-Oryzanol levels detected in SRBO-DS, URBO-MS, and CRBO by UV-Vis spectrophotometry were not significantly different, and were higher than URBO-DS and SRBO-MS, respectively (Table 4). Stabilization of rice bran from dough stage could improve γ-oryzanol of rice bran oil (1.67%) when compared with the respective URBO-DS

(1.40%). According to related research, γ-oryzanol may increase after stabilization using various methods, including autoclaving (121°C, 20 min), hot air (150°C, 10 min), roasting (150°C, 10 min), steaming (130°C, 2 min), microwaving (800 W, 2450 mHz, 150°C, 3 min), and steaming (130°C, 10 min) (Patil et al., 2016; Pranowo et al., 2023; Thanonkaew et al., 2012). According to Yu et al. (2020), heating may cause an increase in γ-oryzanol by improving extractability of γ-oryzanol. This could be the reason for the increase in γ-oryzanol of SRBO-DS after stabilization. However, stabilization of rice bran from mature stage decreased γ-oryzanol of SRBO-MS (1.31%) when compared with the respective URBO-MS (1.61%) (Table 4). Additionally, Pranowo et al. (2023) found that a decrease in γ-oryzanol of rice bran oil was due to a prolonged stabilization period. The main reaction that caused the degradation of γ-oryzanol may be the oxidation of the compound by the oil oxidation products generated during heat treatment (Khuwijitjaru et al., 2004). Furthermore, several variables, including the weather, rice type, growing location, extraction technique, solvent, and rice grain stage, affect the amount of γ-oryzanol in rice bran (Kim et al., 2015; Sangpradab et al., 2021). The chromatogram of γ-oryzanol in crude RBO is presented in Figure 1A-D. Three main peaks (1-3) and a small peak area (4) of γ-oryzanol were detected in RBO, with peak 2 being the highest. γ-Oryzanol is a complex mixture of esters of ferulic acid with sterols and triterpenic alcohols (Chen & Bergman, 2005). The three major components of γ-oryzanol in RBO were cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate (Chen & Bergman, 2005; Xu & Godber, 1999). Pestana et al. (2008) reported that the predominant components of γ-oryzanol in RBO were cycloartenyl ferulate, 24-methylene cycloartenyl ferulate, $\triangle^7$-campestenyl ferulate, campesteryl ferulate, and $\triangle^7$-Sitostenyl ferulate. Also, the four main components of γ-oryzanol were 24-methylene cycloartanyl ferulate, cycloartenyl ferulate, β-sitosterylferulate, and campesteryl ferulate (Lerma-García et al., 2009). The four main components of γ-oryzanol were cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campestryl ferulate, and the mixtures of β–sitosteryl ferulate and cycloartanyl ferulate (Azrina et al., 2008; Pattananandecha et al., 2019). Banchuen et al. (2010) also reported that the 1st, 2nd, 3rd and 4th main peaks of γ-oryzanol from Thail brown rice were cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate, and sitosteryl ferulate, respectively. Phan et al. (2021) reported that the 4 peaks of γ-oryzanol in rice bran oil were cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate, respectively. Based on the literature and the order of elution, the three major peaks (1-3) observed in our experiment might be cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, and campesteryl ferulate, respectively. In our study, γ-oryzanol of SRBO-MS detected by HPLC was the highest with a value of 1.00%, followed by URBO-

Table 4. γ-Oryzanol values of unstabilized and stabilized RBO from rice bran from the dough and mature grain stages as detected using UV-Vis spectrophotometer.

Rice Bran Oil	γ-Oryzanol (g/100 mL oil)
URBO-DS	1.40±0.04^{b}
SRBO-DS	1.67±0.03^{a}
URBO-MS	1.61±0.02^{a}
SRBO-MS	1.31±0.05^{c}
CRBO	1.58±0.06^{a}

Different superscripts within a column indicate significant differences ($p<0.05$).

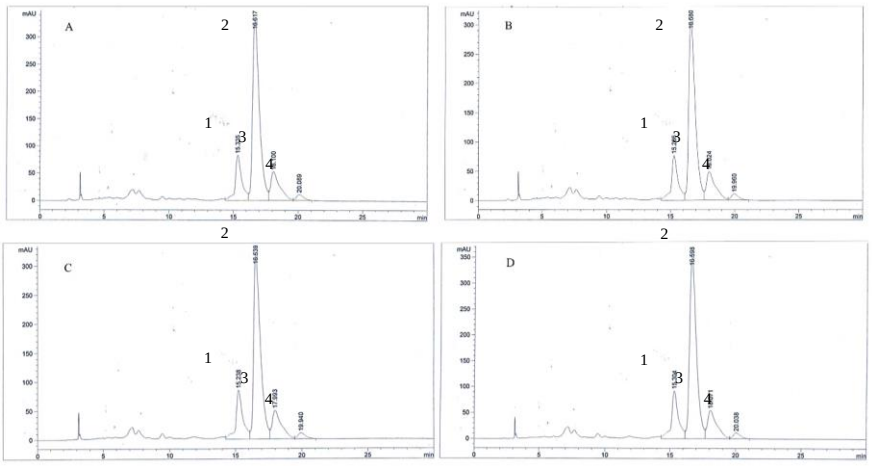

Figure 1. Reversed-phase HPLC chromatogram for γ-oryzanol determination. (A) Oil from unstabilized rice bran of dough grain stage; (B) Oil from stabilized rice bran of dough grain stage; (C) Oil from unstabilized rice bran of mature grain stage; (D) Oil from stabilized rice bran of mature grain stage.

MS (0.94%), URBO-DS (0.90%), and SRBO-MS (0.87%), respectively (Table 5). These results correlated well with the γ-oryzanol results for RBO detected by UV-Vis spectrophotometry where γ-oryzanol in SRBO-DS was the highest. The γ-oryzanol contents in RBO ranged between 0.9-2.9% (Lerma-García et al., 2009). The average γ-oryzanol content of 12 kinds of crude RBO as determined by the four methods were 1.75% by the absorptiometry, 1.29% by the existing normal-phase HPLC, 1.51% by reversed-phase HPLC, and 1.54% by novel reversed-phase HPLC method (Yoshie et al., 2009). The γ–oryzanol content detected in the crude RBO in this study was similar to those of previous reports. The differences among γ-oryzanol in crude RBO could be due to the differences in stabilization and extraction methods (cold-press or solvent extraction), mature stage of rice, cultivar, climate, genetic and environmental factors.

3.4 Antioxidant activity

Reactive oxygen species (ROS), or free radicals, can adversely affect food quality and human health. Antioxidants can reduce oxidative processes in food systems as well as the harmful effects of ROS in the human body (Gulcin, 2020). The DPPH method uses the *in vitro* antioxidant activity of reducing substances based on electron and/or proton donors to methanolic DPPH radical solution (Alam et al., 2013). The DPPH radical scavenging activity of SRBO-DS was the highest (1.73 mg Trolox eq./mL oil), followed by URBO-MS and URBO-DS, SRBO-MS, and CRBO (1.45 mg Trolox eq./mL oil), respectively (Table 6). Stabilization of rice bran from the dough stage improved the DPPH radical scavenging activity of RBO when compared with the respective URBO-DS. However, stabilization of rice bran from the mature grain decreased the DPPH radical scavenging activity of RBO when compared with the respective URBO-MS (Table 6). This result correlated with the γ-oryzanol content results. Because it contained more phenolic, flavonoid, and γ-oryzanol

Table 5. γ-Oryzanol values of unstabilized and stabilized RBO from rice bran from dough and mature grain stages detected by RP-HPLC.

Rice Bran Oil	Peak No.	Retention Time (min)	Area	Area of Components	γ-Oryzanol (%w/w)
URBO-DS	1	15.238	3,074.3	17.79	0.90
	2	16.539	11,400.7	65.96	
	3	17.993	2,403.2	13.90	
	4	19.940	406.7	2.35	
SRBO-DS	1	15.304	3,466.8	17.90	1.00
	2	16.598	12,773.1	65.94	
	3	18.071	2,687.9	13.88	
	4	20.038	442.8	2.29	
URBO-MS	1	15.335	2,955.33	16.38	0.94
	2	16.617	12,123.5	67.18	
	3	18.100	2,549.5	14.13	
	4	20.089	419.0	2.32	
SRBO-MS	1	15.275	2,960.5	17.66	0.87
	2	16.582	11,043.3	65.89	
	3	18.024	2,338.4	13.95	
	4	19.965	417.4	2.49	

than unstabilized RBO, and hot air-stabilized RBO showed higher DPPH scavenging activity (Thanonkaew et al., 2012). The DPPH radical scavenging activity of cold pressed RBO from *Oryza sativa* L. Hom Mali Gorkho showed the most potent effect with IC_{50} value of 0.08 mg/mL (Settharaksa et al., 2014). Therefore, the higher DPPH radical scavening activity of SRBO-MS could be due to its higher content of γ-oryzanol.

The ABTS assay is based on the generation of blue/green $ABTS^{\bullet+}$ that can be reduced by antioxidants and is applicable to both hydrophilic and lipophilic antioxidant systems (Floegel et al., 2011). The ABTS radical scavenging activity of unstabilized rice bran oil from the dough stage (URBO-DS) was not significantly different to that of SRBO-DS and URBO-MS, but it was higher than that of stabilized rice bran oil from the mature stage (SRBO-MS) and CRBO (Table 6). This result did not correlate with γ-oryzanol contents, with URBO-DS showing lower γ-oryzanol than SRBO-DS and URBO-MS. RBO contains phytochemical compounds, such as α-tocopherol and γ-oryzanol, which have antioxidant activities and potential health benefits (Gopala Krishna et al., 2006). Sae-ang et al. (2015) reported that the jasmine rice bran oil exhibited an $ABTS^{\bullet+}$ radical inhibition of approximately 28.77% at the level of 8 mg/mL due to the presence of γ-oryzanol and α-tocopherol in the oil. According to the literature, there were no correlations between γ-oryzanol content and antioxidant activities (DPPH and ABTS radical scavenging activities) of URBO-DS might be attributed to the different quantities of other antioxidant substances in RBO such as total phenolic compounds, flavonoids, and α-tocopherol (Gopala Krishna et al., 2006; Sae-ang et al., 2015).

Table 6. Antioxidant activity of unstabilized and stabilized RBO from rice bran from dough and mature grain stages.

Samples	Antioxidant Activity (mg Trolox eq./mL oil)	
	DPPH	ABTS
URBO-DS	1.64±0.02[b]	4.68±0.16[a]
SRBO-DS	1.73±0.03[a]	4.37±0.15[ab]
URBO-MS	1.65±0.04[b]	4.45±0.13[ab]
SRBO-MS	1.57±0.04[c]	4.14±0.36[b]
CRBO	1.45±0.04[d]	4.28±0.10[b]

Different superscripts within a column indicate significant differences ($p<0.05$).

3.5 Peroxide and free fatty acid values

The most important factor in RBO extraction is the process of stabilization of the rice bran to inactivate the enzymatic activity of lipase. Insufficient or no stabilization would increase the peroxide value (PV) and FFA content resulting in negative effect on RBO quality (Thanonkaew et al., 2012). In our experiment, PV in SRBO-DS was the lowest (1.19±0.21 meq/kg oil), followed by CRBO, URBO-DS, SRBO-MS, and URBO-MS, respectively (Table 2). The FFA levels in SRBO-DS and SRBO-MS were not significantly different but were lower than CRBO, URBO-DS, and URBO-MS. These results indicated that stabilization of the dough and mature stage rice bran by hot air heating produced lower PVs and FFAs in RBO when compared with the respective unstabilized RBO (Table 6). Our results correlated with Thanonkaew et al. (2012) who reported that hot air heating may retard the formation of FFA and PV compared to unstabilized RBO. The PV in unstabilized RBO decreased from 18.85 meq/kg oil to 12.13±0.22 meq/kg oil and FFA decreased from 5.58% to 3.51% after stabilization of bran (Thanonkaew et al., 2012). In addition, Amarasinghe et al. (2009) suggested that hot air heating may reduce the lipolytic activity of rice bran, resulting in lower FFAs compared to unstabilized rice bran. The CODEX standard suggests a maximum PV value of 15 meq/kg oil for cold pressed oils (CODEX STAN 210, 1999). According to Tan et al. (2023), RBO with too high a proportion of FFA (>10%) is not suitable for human consumption. Our results indicated that the stabilized RBO from the dough and mature stages had PVs of 1.19±0.21 and 8.86±0.09 meq/kg oil, respectively, which were within the CODEX standards. In addition, the FFAs of SRBO-DS and SRBO-MS were 4.84% and 5.12%, respectively, which were less than 10%. Although the PV in unstabilized RBO was within the CODEX standards and the FFA content less than 10%, it is still unsuitable for human consumption due to increase in PV and FFA during storage. The PV in unstabilized RBO increased rapidly from its initial value of 0.94 to 13.71 meq/kg oil during 8 weeks of storage (Duangsi & Krongyut, 2023) and 0.99 to 12.49 meq/kg during 16 weeks of storage at 25°C (Pongrat & Songsermpong, 2019). The FFA in unstabilized RBO increased rapidly from 4.37 to 28.61% during 8 weeks of storage (Duangsi & Krongyut, 2023) and from 4.42% to 53.24% during 16 weeks of storage at 25°C (Pongrat & Songsermpong, 2019). Therefore, rice bran stabilized by hot air heating may potentially be used to produce cold-pressed RBO.

When considering rice grain stage, RBO from dough stage contained lower PV than RBO from mature stage. However, FFA was not significantly different between URBO-DS and URBO-MS or SRBO-DS and SRBO-MS (Table 2). The difference in the PV of RBO derived from dough and mature stages may be due to differences in chemical compositions and lipolytic enzymes in rice bran.

4. Conclusions

Rice bran from dough grain stage was found to have more oil content than mature grain. Stabilized RBO from dough grain stage had higher lightness, yellowness, γ-oryzanol content, and DPPH radical scavenging activities but lower redness, peroxide value, and free fatty acid than RBO from unstabilized dough stage grain and RBO from stabilized mature stage grain. In addition, we were able to decrease the peroxide values and free fatty acid content of RBO by heating rice bran at 70°C for 3 h with hot air prior to RBO extraction. From our findings, stabilized RBO from dough grain stage might be an alternative functional food with antioxidant activity. For further work, the phytochemical compounds and quality of the extracted RBO stored under different condition should be studied.

5. Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152. https://doi.org/10.1016/j.jsps.2012.05.002

Amarasinghe, B. M. W. P. K., Kumarasiri, M. P. M., & Gangodavilage, N. C. (2009). Effect of method of stabilization on aqueous extraction of rice bran oil. *Food and Bioproducts Processing*, 87(2), 108-114. https://doi.org/10.1016/j.fbp.2008.08.002

Andriani, R., Subroto, T., Ishmayana, S., & Kurnia, D. (2022). Enhancement methods of antioxidant capacity in rice bran: A Review. *Foods*, 11(19). https://doi.org/10.3390/foods11192994

AOAC. (1990). *Official Methods of Analysis* (15th ed.). Washington DC: Association of Official Analytical Chemist.

AOAC. (2000). *Official Methods of Analysis* (17th ed.). Gaithersburg: The Association of Official Analytical Chemists.

Azrina, A., Maznah, I., & Azizah, A. H. (2008). Extraction and determination of oryzanol in rice bran of mixed herbarium UKMB; AZ 6807: MR 185, AZ 6808: MR 211, AZ6809: MR 29. *ASEAN Food Journal*, 15(1), 89-96.

Balachandran, C., Mayamol, P. N., Thomas, S., Sukumar, D., Sundaresan, A., & Arumughan, C. (2008). An ecofriendly approach to process rice bran for high quality rice bran oil using supercritical carbon dioxide for nutraceutical applications. *Bioresource Technology*, 99(8), 2905-2912. https://doi.org/10.1016/j.biortech. 2007.06.004

Banchuen, J., Paiboon, T., Buncha, O., Phaisan, W., & Piyarat, S. (2010). Increasing the bio-active compounds contents by optimizing the germination conditions of Southern Thai brown rice. *Songklanakarin Journal of Science and Technology*, 32(3), 219-230.

Bumrungpert, A., Chongsuwat, R., Phosat, C., & Butacnum, A. (2019). Rice bran oil containing gamma-oryzanol improves lipid profiles and antioxidant status in hyperlipidemic subjects: A randomized double-blind controlled trial. *The Journal of Alternative and Complementary Medicine*, 25(3), 353-358. https://doi.org/ 10.1089/acm.2018.0212

Chatha, S. A. S., Hussain, A. I., Zubair, M., & Khosa, M. K. (2011). Analytical characterization of rice (oryza sativa) bran and bran oil from different agro-ecological regions. *Pakistan Journal of Agricultural Sciences*, 48(3), 243-249.

Chen, M. H., & Bergman, C. J. (2005). A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. *Journal of Food Composition and Analysis*, 18(2-3), 139-151. https://doi.org/10.1016/j.jfca.2003.09.016

Codex-Stan 210. (1999). *Codex Standard for Named Vegetable Oils*. https://www.fao.org/3/y2774e/y2774e04.htm#bm4.1

Devi, R., Veliveli, V. L., & Devi, S. S. (2021). Nutritional composition of rice bran and its potentials in the development of nutraceuticals rich products. *Journal of Pharmacognosy and Phytochemistry*, 10(2), 470-473.

Duangsi, R., & Krongyut, W. (2023). Stabilization of rice bran by infrared radiation heating for increased resilience and quality of rice bran oil production. *Preventive Nutrition and Food Science*, 28(2), 189-199. https://doi.org/10.3746/pnf.2023.28.2.189

Endo, Y., & Aso, Y. (2019). Determination of γ-oryzanol in refined rice bran oil by nuclear magnetic resonance method. *Journal of Nutritional Science and Vitaminology*, 65, S72-S74. https://doi.org/10.3177/jnsv.65.S72

Faria, S. A. S. C., Bassinello, P. Z., & Penteado, M. V. C. (2012). Nutritional composition of rice bran submitted to different stabilization procedures. *Brazilian Journal of Pharmaceutical Sciences*, 48(4), 651-657. https://doi.org/10.1590/S1984-82502012000400008

Floegel, A., Kim, D. O., Chung, S.- J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7), 1043-1048. https://doi.org/10.1016/j.jfca.2011.01.008

Gopala Krishna, A. G., Hemakumar, K. H., & Khatoon, S. (2006). Study on the composition of rice bran oil and its higher free fatty acids value. *Journal of the American Oil Chemists' Society*, 83, 117-120.

Gopinger, E., Ziegler, V., da Silva Catalan, A. A., Krabbe, E. L., Elias, M. C., & Xavier, E. G. (2015). Whole rice bran stabilization using a short chain organic acid mixture. *Journal of Stored Products Research*, 61, 108-113. https://doi.org/10.1016/j.jspr.2015.01.003

Guevara-Guerrero, B., Fernández-Quintero, A., & Montero-Montero, J. C. (2019). Free fatty acids in rice bran during its storage after a treatment by twin-screw extrusion to prevent possible rapid hydrolytic rancidity of lipids. *Dyna*, 86(208), 177-181.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*, 94, 651-715. https://doi.org/10.1007/s00204-020-02689-3

Ilias, N. N., Mohd Rozalli, N. H., Vy, N. H. T., & Eng, H. Y. (2020). Rice bran of different rice varieties in Malaysia: Analysis of proximate compositions, antioxidative properties and fatty acid profile for data compilation. *Advances in Agricultural and Food Research Journal*, 1(2). https://doi.org/10.36877/aafrj.a0000164

Irakli, M., Kleisiaris, F., Mygdalia, A., & Katsantonis, D. (2018). Stabilization of rice bran and its effect on bioactive compounds content, antioxidant activity and storage stability during infrared radiation heating. *Journal of Cereal Science*, 80, 135-142. https://doi.org/10.1016/j.jcs.2018.02.005

Jiamyangyuen, S., Nuengchamnong, N., & Ngamdee, P. (2017). Bioactivity and chemical components of Thai rice in five stages of grain development. *Journal of Cereal Science*, 74, 136-144. https://doi:10.1016/j.jcs.2017.01.021

Juliano, B. O., & Tuaño, A. P. P. (2019). Gross structure and composition of the rice grain. In J. Bao (Ed.) *Rice*. (4th ed., pp. 31-35). AACC International Press. https://doi:10.1016/b978-0-12-811508-4.00002-2

Khongla, C., Chuaingan, J., Siadkhunthod, T., Somnam, P., Musika, S., & Sangsawad, P. (2022a). Physicochemical properties of rice bran hydrolysate prepared in a pilot scale process and its application in milk tablets. *Trends in Sciences*, 19(23), 2316-2316. https://doi.org/10.48048/tis.2022.2316

Khongla, C., Musika, S., Sangsawad, P., Srithamma, L., Kiatbenjakul, P., & Tanamool, V. (2022b). Antioxidant activity of jasmine rice bran hydrolysate and its application in broken rice beverages. *Suranaree Journal of Science and Technology*, 29(3), 1-10.

Khuwijitjaru, P., Taengtieng, N., & Changprasit, S. (2004). Degradation of gamma-oryzanol in rice bran oil during heating: An analysis using derivative UV-spectrophotometry. *Silpakorn University International Journal*, 4, 154-165.

Kim, N. H., Kwak, J., Baik, J. Y., Yoon, M. R., Lee, J.-S., Yoon, S. W., & Kim, I. H. (2015). Changes in lipid substances in rice during grain development. *Phytochemistry*, 116, 170-179. https://doi.org/10.1016/j.phytochem.2015.05.004

Kukusamude, C., & Kongsri, S. (2018). Elemental and isotopic profiling of Thai jasmine rice (Khao Dawk Mali 105) for discrimination of geographical origins in Thung Kula Rong Hai area, Thailand. *Food Control*, 91, 357-364. https://doi.org/10.1016/j.foodcont.2018.04.018

Kumari, N., Vinita, N. K., & Rani, P. (2018). Nutrient composition of full fat and defatted rice bran. *Asian Journal of Dairy and Food Research*, 37(1), 77-80. https://doi.org/10.18805/ajdfr.DR-1277

Lavanya, M. N., Saikiran, K. C. S., & Venkatachalapathy, N. (2019). Stabilization of rice bran milling fractions using microwave heating and its effect on storage. *Journal of Food Science and Technology*, 56(2), 889-895. https://doi.org/10.1007/s13197-018-3550-y

Lerma-García, M. J., Herrero-Martínez, J. M., Simó-Alfonso, E. F., Mendonça, C. R. B., & Ramis-Ramos, G. (2009). Composition, industrial processing, and applications of rice bran γ-oryzanol. *Food Chemistry*, 115(2), 389-404. https://doi.org/10.1016/j.foodchem.2009.01.063

Liao, M., Damayanti, W., Xu, Y., Zhao, Y., Xu, X., Zheng, Y., & Jiao, S. (2020). Hot air-assisted radio frequency heating for stabilization of rice bran: Enzyme activity, phenolic content, antioxidant activity and microstructure. *LWT- Food Science and Technology*, 131. https://doi.org/10.1016/j.lwt.2020.109754

Lilitchan, S., Tangprawat, C., Aryusuk, K., Krisnangkura, S., Chokmoh, S., & Krisnangkura, K. (2008). Partial extraction method for the rapid analysis of total lipids and γ-oryzanol contents in rice bran. *Food Chemistry*, 106(2), 752-759. https://doi.org/10.1016/j.foodchem.2007.06.052

Marei, A. M. A., Zeitoun, M. A. M., & El Hissewy, A. A. (2017). Some technical aspects affecting rice bran stability for functional components recovery. *Journal of the Advances in Agricultural Researches*, 22(1), 24-45.

Moreno, J. P. C., Ratphitagsanti, W., Suwannaporn, P., & Kamonpatana, P. (2021). Stabilization of rice bran using ohmic heating or ultra-superheated steam. *Agriculture and Natural Resources*, 55(5), 816-825. https://li01.tci-thaijo.org/index.php/anres/article/view/252990

Patil, S. S., Kar, A., & Mohapatra, D. (2016). Food and bioproducts processing stabilization of rice bran using microwave: process optimization and storage studies. *Food and Bioproducts Processing*, 99, 204-211. https://doi.org/10.1016/j.fbp.2016.05.002

Pattananandecha, T., Sirithunyalug, J., Sirithunyalug, B., Thiankhanithikun, K., Khanongnuch, C., & Saenjum, C. (2019). Bioactive compounds constituent and anti-

inflammatory activity of natural rice bran oil produced from colored and non-pigmented rice in Northern Thailand. *Journal of Pharmacy and Nutrition Sciences,* 9(4), 205-212. https://doi.org/10.29169/1927-5951.2019.09.04.2

Pestana, V. R., Zambiazi, R. C., Mendonça, C. R., Bruscatto, M. H., Lerma-García, M. J., & Ramis-Ramos, G. (2008). Quality changes and tocopherols and γ-orizanol concentrations in rice bran oil during the refining process. *Journal of the American Oil Chemists' Society*, 85(11), 1013-1019. https://doi.org/10.1007/s11746-008-1300-4

Phan, V. M., Tran, H. C., & Sombatpraiwan, S. (2021). Rice bran oil extraction with mixtures of ethanol and hexane. *Songklanakarin Journal of Science and Technology*, 43, 630-637.

Pimpa, B., Thongraung, C., & Sutthirak, P. (2021). Effect of solvents and extraction conditions on the properties of crude rice bran oil. *Walailak Journal of Science and Technology*, 18(17), 9611-9617. https://doi.org/10.48048/wjst.2021.9611

Pongrat, P., & Songsermpong, S. (2019). Stabilization of rice bran using a continuous microwave oven. *Agriculture and Natural Resources*, 53(4), 373-377. https://doi.org /10.34044/j.anres.2019.53.4.07

Pranowo, D., Savira, T. D., & Sukardi, S. (2023). Rice bran stabilization using autoclave and optimization of crude rice bran oil recovery using ultrasound-assisted extraction. *BioResources*, 18(4), 8341-8361. https://doi:10.15376/biores.18.4.8341-8361

Preecharram, S., Posoongnoen, S., Thummawongsa, T., Sripakdee, T., Tawil, S., Poomsuk, N. & Jandaruang, J. (2023). Proximate composition and antioxidant activity of Young flattened rice (Khao-Mao). *Trends in Sciences*, 20(4). https://doi.org/ 10.48048/tis.2023.6575

Punia, S., Kumar, M., Siroha, A. K., & Purewal, S. S. (2021). Rice bran oil: Emerging trends in extraction, health benefit, and its industrial application. *Rice Science*, 28(3), 217-232. https://doi.org/10.1016/j.rsci.2021.04.002

Putri, A. P., & Sukanta, E. A., (2012). Proximate analysis of rice bran as alternative comestible. In *Proceedings of the 4 th Gruber–Soedigdo Lecture Conference* (pp. 1-4). University of Groningen, Netherlands.

Ranathunga, A., Thumanu, K., Kiatponglarp, W., Siriwong, S., Wansuksri, R., & Suwannaporn, P. (2023). Image mapping of biological changes and structure-function relationship during rice grain development via Synchrotron FTIR spectroscopy. *Food Chemistry Advances*, 2, 100290. https://doi.org/10.1016/ j.focha.2023.100290

Rashid, M. T., Liu, K., Han, S., Jatoi, M. A., & Sarpong, F. (2023). Optimization of extrusion treatments, quality assessments, and kinetics degradation of enzyme activities during storage of rice bran. *Foods*, 12(6). https://doi.org/10.3390/foods12061236

Rigo, L. A., Pohlmann, A. R., Guterres, S. S., & Beck, R. C. R. (2014). Rice bran oil: Benefits to health and applications in pharmaceutical formulations. In *Proceedings of Wheat and Rice in Disease Prevention and Health* (pp. 311-322). Academic Press, Cambridge. https://doi.org/10.1016/B978-0-12-401716-0.00023-4

Rodchuajeen, K., Niamnuy, C., Charunuch, C., Soponronnarit, S., & Devahastin, S. (2016). Stabilization of rice bran via different moving-bed drying methods. *Drying Technology*, 34(15), 1854-1867. https://doi:10.1080/07373937.2016.1236345

Sae-ang, V., Tawai, C., Sukonthamut, S., Nokkoul, R., & Ruen-ngam, D. (2015). Evaluation of antioxidant activity of Khem-ngen rice bran oil by $ABTS^{\bullet+}$ assay measurement. In *Proceeding of the 27th annual meeting of the Thai society for biotechnology and international conference* (pp. 524-530). Bangkok, Thailand.

Sanchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic

constituents. *Food Research International*, 32(6), 407-412. https://doi.org/10.1016/S0963-9969(99)00097-6

Sangpradab, J., Kamonpatana, P., Suwannaporn, P., & Huang, T.-C. (2021). Ohmic heating-aided mechanical extraction of gamma-oryzanol and phytosterols in rice bran oil. *Food and Bioprocess Technology*,14(8), 1542-1554. https://doi.org/10.1007/s11947-021-02655-6

Sayasoonthorn, S., Kaewrueng, S., & Patharasathapornkul, P. (2012). Rice bran oil extraction by screw press method: Optimum operating settings, oil extraction level and press cake appearance. *Rice Science*, 19(1), 75-78. https://doi.org/10.1016/S1672-6308(12)60024-9

Settharaksa, S., Madaka, F., Charkree, K., & Charoenchai, L. (2014). The study of anti-inflammatory and antioxidant activity in cold press rice bran oil from rice in Thailand. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7), 428-431.

Tan, B. L., Norhaizan, M. E., & Chan, L. C. (2023). Rice bran: from waste to nutritious food ingredients. *Nutrients,* 15(11), 2503. https://doi.org/10.3390/nu15112503

Thanonkaew, A., Wongyai, S., McClements, D. J., & Decker, E. A. (2012). Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil (*Oryza saltiva* L.). *LWT - Food Science and Technology*, 48(2), 231-236. https://doi.org/10.1016/j.lwt.2012.03.018

Uquiche, E., Jeréz, M., & Ortíz, J. (2008). Effect of pretreatment with microwaves on mechanical extraction yield and quality of vegetable oil from Chilean hazelnuts (*Gevuina avellana* Mol). *Innovative Food Science and Emerging Technologies*, 9(4), 495-500. https://doi.org/10.1016/j.ifset.2008.05.004

USDA Foreign Agricultural Services. (2021). *Thailand Rice: Recent Dry Conditions After a Promising Start; Optimism Still Remains for this Crop Season*. https://ipad.fas.usda.gov/highlights/2021/10/Thailand/index.pdf

Velasquez, M. M., & Villarino, C. B. (2021). Comparison of microwave vacuum drying with traditional rice bran stabilization methods: impact on extracted oil quality. *Proceedings*, 70(1), 35. https://doi.org/10.3390/foods_2020-07729

Wisetkomolmat, J., Arjin, C., Satsook, A., Seel-Audom, M., Ruksiriwanich, W., Prom-U-Thai, C., & Sringarm, K. (2022). Comparative analysis of nutritional components and phytochemical attributes of selected Thai rice bran. *Frontiers in Nutrition*, 9, 833730. https://doi.org/10.3389/fnut.2022.833730

Xu, Z., & Godber, J. S. (1999). Purification and identification of components of γ-oryzanol in rice bran oil. *Journal of Agricultural and Food Chemistry,* 47(7), 2724-2728. https://doi.org/10.1021/jf981175j

Yeo, H., & Shibamoto, T. (1991). Effects of moisture content on the Maillard browning model system upon microwave irradiation. *Journal of Agricultural and Food Chemistry*, 39(10), 1860-1862, https://doi:10.1021/jf00010a035

Yoshie, A., Kanda, A., Nakamura, T., Igusa, H., & Hara, S. (2009). Comparison of gamma-oryzanol contents in crude rice bran oils from different sources by various determination methods. *Journal of Oleo Science*, 58(10), 511-518. https://doi.org/10.5650/jos.58.511

Yu, C.-W., Hu, Q.-R., Wang, H.-W., & Deng, Z. Y. (2020). Comparison of 11 rice bran stabilization methods by analyzing lipase activities. *Journal of Food Processing and Preservation*, 44(4), Article e14370. https://doi.org/10.1111/jfpp.14370