Emirates Journal of Food and Agriculture. 2015. 27(11): 849-856
doi: 10.9755/ejfa.2015.09.738
http://www.ejfa.me/

REGULAR ARTICLE

Impact of stabilization and extraction methods on chemical quality and bioactive compounds of rice bran oil

Patiwit Loypimai, Anuchita Moongngarm*, Pheeraya Chottanom

Department of Food Technology and Nutrition, Faculty of Mahasarakham University, Mahasarakham 44150, Thailand

ABSTRACT

In the production process of rice bran oil (RBO), the bran itself requires stabilization immediately after milling to protect against oil degradation from lipase. This study aimed to investigate the effects of stabilization and extraction methods on the chemical quality, bioactive content, and antioxidant activity of RBO. Steaming and ohmic heating (OHM) were used to stabilize rice bran before oil extraction using three extracting methods namely, immersion in n-hexane, Soxhlet, and an enzymatic method. The oil obtained from rice bran stabilized using OHM and extracted using the enzymes had the best chemical quality with the lowest values of FFA (1.51 ± 0.04%), highest level of γ-oryzanol (1190.1 ± 89.3 μg/g), and strongest total antioxidant activity (21.3 ± 0.32 mg AEE/g and 15.5 ± 0.24 mg BHAE/g). Results suggested that combination between OHM and enzymes is an effective process for the preparation of RBO and can be considered as an alternative extraction process.

Keywords: Enzymatic extraction; ohmic heating; rice bran oil; bioactive compound

INTRODUCTION

World rice production in 2008 was 661 million metric tons (Fabian and Ju, 2011), and approximately 8-10% of this was rice bran (Juliano and Bechtel, 1985: Loypimai et al., 2009). Rice bran consists of the outer layer (pericarp, seed coat, and aleurone layer) and germ of the grain, which is removed from the grain during the rice milling process. Our previous studies (Loypimai et al., 2009; 2015) reported that both rice bran and black rice bran contained very high bioactive components such as tocopherols, tocotrienols, γ-oryzanol and phenolic compounds, and showed antioxidant activity well known to be beneficial for human health. Rice bran is mainly used as feedstock but it has potential as a food ingredient and a production source of rice bran oil (McCaskill and Zhang, 1999). However, immediately after the milling process, rapid deterioration of fat in the bran by lipase and oxidase results in the occurrence of free fatty acid (FFA) and other volatile compounds (Zullaikah et al., 2005; Loypimai et al., 2009). After the milling process to extract the rice bran oil, inactivation of these enzymes and inhibition of lipid oxidation are necessary to prevent the deterioration of fat and valuable bioactive compounds of the bran. This procedure is known as rice bran stabilization.

Our previous studies (Loypimai et al., 2009; 2015) have reported that ohmic heating (OHM) is an effective method to stabilize tocols, γ-oryzanol, and anthocyanins in the bran. In addition, OHM increased the efficiency of solute diffusion throughout the membrane (electro-osmosis effect) resulting in a better quality product (Boussetta et al., 2009; Donsi et al., 2010). Several investigations have reported that OHM increased the extraction yields of sucrose from sugar beets (Katrokha et al., 1984), apple juice from apples (Lima and Sastry, 1999), rice bran oil and bioactive substances from rice bran (Loypimai et al., 2009; Lakkakula et al., 2004), and polyphenols from red grape pomace (Darra et al., 2013).

In general, extraction of rice bran oil has been carried out using hexane as the extraction solvent. However, hexane is flammable, volatile, toxic, and pollutes the environment (greenhouse effects and consequential health problems) (Balachandran et al., 2008; Chen and Diosady, 2003). Therefore, safety, environmental, and economical aspects are forcing the industry to use safer extraction methods or solvents such as water, bio-, or agro-solvents (Chemat et al., 2012). Enzyme-based processes to produce vegetable oils are considered as environmentally safe technology

***Corresponding author:**
Anuchita Moongngarm, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44150, Thailand, Phone: +66-43754085, Mobile: +66 819756353, E-mail: anuchitac@yahoo.co.th

Received: 12 September 2015; **Revised:** 18 October 2015; **Accepted:** 20 October 2015; **Published Online:** 20 October 2015

because there is no release of volatile organic compounds as atmospheric pollutants, and lower protein damage during extraction (Rosenthal et al., 1996). The uses of enzymes for edible oil extraction were reported previously (Rosenthal et al., 1996; Freitas et al., 1996; Hanmoungjai et al., 2001). The enzymatic process hydrolyzed and degraded the structural polysaccharides, forming the cell wall of the oils and proteins, which form the cell and lipid body membranes resulting in the release of both the oils and proteins into the aqueous system (Rosenthal et al., 1994; 1996; Hernandez et al., 2000). However, the successful use of this extraction process depended upon the main factors, enzyme/substrate mass ratio, specific enzymes, particle size of raw material, and hydrolysis time (Rosenthal et al., 1996). Moreover, previous studies (Hanmoungjai et al., 2001; Freitas et al., 1996) investigated the combinations of various enzymes in the extraction process to enhance the yields of seed oil and rice bran oil. To the best of our knowledge, stabilization techniques such as ohmic heating and steaming have not been used previously for rice bran stabilization prior to enzymatic oil extraction. The aim of this study was therefore to investigate the influence of these stabilization and extraction methods on the chemical qualities, bioactive compounds, and antioxidant activity of rice bran oil.

MATERIALS AND METHODS

Chemicals and reagents

Standard α-tocopherol was purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA). HPLC grades of methanol, acetonitrile, hexane, ethyl acetate and ethanol were purchased from BHD (Poole, UK). γ- Oryzanol standard was purchased from Tsuno Food Industrial Co., Ltd. (Wakayama, Japan). Butyratehydroxyanisole (BHA) and vitamin E were obtained from Fluka Chemical (Buchi, Switzerland). All chemicals and reagents were analytical grade.

Enzyme specification

The different types of enzymes employed in this study were purchased from the Sigma-Aldrich Chemical Co., (Singapore) including, cellulase, hemicellulase, α-amylase, and protease. The cellulase was produced from *Aspergillus niger*. The optimum conditions were pH 5.0, temperature at 37°C; with activity of 1.38 units/mg of solid (one unit will liberate 1.0 μM of glucose from cellulose in hour) (Hurst et al., 1978). The hemicellulase was manufactured from *Aspergillus niger* (maximum enzyme activity at pH 4.5 and 40°C). The concentration was 1.5 unit/mg of solid; one unit releases 1.0 μM of D-galactose per hour. The *a*-amylase (Type XII-A) was produced from *Bacillus licheniformis* with activity of 829 units/mg of protein (one unit of α-amylase activity is defined to release 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20°C. The protease was also purchased from Sigma-Aldrich Chemical Co., and produced from *Aspergillus oryzae* (0.14 units/mg of solid) and *Aspergillus oryzae* (500 units/mg of solid). In this study, we investigated by focusing on the effects of two stabilization methods and three extraction methods of rice bran oil extraction. There were therefore eighteen treatments (six treatments and three replicates) undertaken.

Rice bran and rice bran stabilization

Rice bran (*Oryza sativa* L. CV. RD6) samples were purchased from a rice milling factory in Mahasarakham Province, Thailand. The fresh bran from the milling process was immediately passed through a mesh sieve no. 20 (750 μm aperture) to remove broken pieces of rice and husks. The initial moisture content of the rice bran sample was determined using the AOAC method (AOAC, 2000). Raw rice bran samples were stabilized using two methods: the steaming, which served as the control sample, and the ohmic heating (OHM) method.

The steaming stabilization was carried out adhering to the method previously described by Juliano (1985) and Loypimai et al. (2009) with some modifications. The rice bran sample (180 g) was placed in a container before being stabilized in an autoclave (ACV-3167, IWAKI) at 105°C (cold point). When the inside temperature of the slowest heating bran reached 105°C, it was held there for 1 min. The bran was then removed from the chamber and cooled to ambient temperature. Stabilized rice bran samples were further extracted using the different extraction methods.

The moisture content of the rice bran was adjusted to 30% by adding deionized water. Then the bran was stabilized using OHM according to the method described previously by Loypimai et al. (2009). Briefly, the sample (180 g) was placed between the electrodes in a Teflon chamber. An alternating current of 50 Hz with electrical field strength of 150 V/cm was supplied. The voltage, current, and temperature were continuously measured using a data logger controller. When the temperature of the heated bran inside the chamber reached 105°C, it was held there for 1 min. Subsequent to heating, the samples were removed from the chamber and cooled to room temperature prior to the extraction processes.

Rice bran oil extraction

Three different methods were used for the extraction of rice bran oil, the immersion stirring method, the Soxhlet extraction method, and the enzymatic extraction method.

Stabilized rice bran samples were extracted using an immersion stirring method in accordance with the method

of Stanojevic et al. (2004) by immersing and stirring the rice bran in n-hexane solvent (ratio, 1:20 (w/v)) for 24 h at room temperature. The mixture slurry was separated on filter paper no.4 (Sigma-Aldrich Co., St. Louis, MO, USA), and washed three times with 10 mL of n-hexane. The oil was recovered using a rotary evaporator at 65°C and dried in a hot-air oven at 100°C for 30 min to eliminate residual n-hexane. The crude oil sample obtained was stored at -20°C until required for analysis.

The Soxhlet extraction was carried out by the method of AOAC (2000). The sample was placed into a thimble paper cone and n-hexane was used to extract the rice bran in the Soxhlet extractor for 2 h. The crude oil obtained was stored at-20°C until required for analysis.

Enzymatic extraction was performed according to Rosenthal et al. (2001) with some modifications. In the initial extraction step, the stabilized rice bran of 25g was added to distilled water at the ratio of 1:5 (rice bran: water, w/v) and the pH was adjusted to 4.75 with 0.1 N HCl (optimum pH for cellulase and hemicellulase) before the cellulase and hemicellulase were added. The mixtures were incubated at 37°C for 3 h in a shaking water bath at 80 rpm (Reshma et al., 2008). In the second step, the pH of the slurry was adjusted to 7.0 with 0.1 N NaOH and then α-amylase and protease were added, incubated at 40°C, and shaken in a water bath at 80 rpm for another 18 h. After extraction, the slurry was heated on a hot plate at 50°C for 10 min and then the crude oil portion was separated by hand and centrifuged at 7,168 g (4°C) for 20 min. The centrifuge tube was frozen at -20°C before the oil emulsion and residue layer were scraped off, and then heated up to 50°C (Dickey et al., 2008). The upper layer was collected as the crude rice bran oil. The rice bran meal was dried overnight at 85-90°C in a hot air oven. The proximate analysis was analyzed using the method of AOAC (2000). The amounts of enzyme used in experiments were 380 units of cellulose, 380 units of hemicellulase, 80 units of α-amylase, and 368 units of protease, following the previous study of Sharma et al. (2001).

Determination of chemical quality

The quality Indices of the rice bran oil samples obtained from the different extraction processes were determined by AOCS (1997). Free fatty acids (FFA) were measured by titration of the sample (1.0 g) with alkali and the FFA was calculated as oleic acid, which is the major fatty acid in rice bran oil (AOCS Cd 3a-63). The peroxide value (PV) was measured by oil titration (0.5 g) with sodium thiosulfate solution (AOCS Cd 8-53). Measurement of thiobarbituric acid (TBA) value was done by heating a 5 mL aliquot of a solution of sample (50–200 mg) in 25 mL 1-butanol with 5 mL TBA reagent at 95°C for 120 min, and reading the absorbance at 530 nm. All determinations were carried out in triplicate (AOCS, 1997).

Analysis of fatty acid composition using GC-Flame ionization detection (FID)

The fatty acids in the oil samples were esterified to fatty acid ethyl esters (FAME) using the method reported by Mondello et al. (2006) with some modifications. The 20 μL samples of rice bran oil were transesterified in a Pyrex tube using 200 μL of boron trifluoride-methanol (20% BF_3) reagent and heated at 100°C for 30 min. After cooling, 200 μL of n-hexane and 800 μL of distilled water were added to the mixture, agitated manually for a min, and then centrifuged at 8000 rpm (4°C) for 2 min before injection into the GC. The FAMEs were filtered using the Sep–Pak silica column (Alltech Associates, Inc., Deerfield, IL). The oil sample (1.0 g) was analyzed quantitatively using a Shimadzu model GC-2014 system (Shimadzu, Tokyo, Japan) fitted with a FID, eluted with H_2 at 3.0 ± 1.0 mL/min with a split ratio of 1:10. A fused silica capillary column (30 m×0.25 mm, 25 μm film thickness; Restek Co., PA, USA) was used. The injector and detector were maintained at 250°C. Nitrogen was used as a carrier gas and the temperature ran from 180°C to 230°C at 25°C/min, then to 200°C (hold 3 min) at 1.5°C/min, then to 230°C (hold 9 min) at 1.5°C/min. Fatty acid composition was identified by comparing them to the fatty acid standards (myristic, palmitic, stearic, oleic, linoleic and linolenic acids) of FAME, which were prepared and analyzed under the same condition of the sample.

Determination of bioactive compound using HPLC- Photo diode array (PDA)

The bioactive compounds in the rice bran oil namely, α-tocopherol and γ-oryzanol were determined using a HPLC–PDA apparatus following the method of Gimeno et al. (2001) with some modifications. The oil sample was dissolved in 2.0 mL of hexane and stored in a screw top vial in a freezer (-20°C) before HPLC analysis. The HPLC apparatus was a Shimadzu CL-10 with a 20 μL sample loop injector and PDA detector. The sample was injected through a security guard-column (Phenomenex 4 μm, C_{18}, 50×4.6 mm) and separated on the C_{18} column (Phenomenex 4 μm, C_{18}, 150×4.6 mm) at 45°C. The gradient elution was applied. The mobile phase solvent used was methanol: water: n-butanol (92: 4: 4, by volume) at the flow rate of 1.0 mL/min for 12 min. After that, the mobile phase was changed to methanol: water: n-butanol (92: 3: 5, by volume) with a flow rate of 1.5 mL/min within 25 min. The total gradient run time was 25 min, before returning to the initial condition. The eluent band was monitored at 292 nm for α-tocopherol and 325 nm for γ-oryzanol (Chen and Bergman, 2005; Azrina et al., 2008).

2.8 Determination of antioxidant activity

Rice bran oil extract samples were prepared according to Loypimai et al. (2009). The rice bran oil (1.0 g) was dissolved in 5 mL of mixture solvents (methanol: hexane, 3:2) by placing the mixture on a sonicator (Vibra cell, 130 W, 20 kHz) for 5 min. The extracts were used for analyzing antioxidant activities; two different assays namely lipid oxidation and total antioxidant capacity (TAC) were applied to evaluate the activity.

Lipid peroxidation assay was used to measure the lipid peroxide formed in egg yolk homogenated as lipid-rich media as described by Dasgupta and De (2004). The absorbance of supernatant was recorded at 532 nm. Inhibition of lipid peroxidation (%) was calculated as $[(1-A_e/A_c) \times 100]$; where A_c is the absorbance value of the control (without extract) and A_e is the absorbance value of the sample (the presence of oil extract). The results were calculated and expressed as inhibition concentration 50% (IC_{50}) values.

Total antioxidant capacity (TAC) assay was determined using the method documented by Dasgupta and De (2004). The extract (0.3 mL) was mixed with 3.0 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance was measured at 695 nm against a blank. The antioxidant activity is expressed as the number of equivalents of synthetic standards such as vitamin E and butylated hydroxyl anisole (BHA).

Statistical analysis

The data were analyzed using F-test (two-way ANOVA) with a statistic package program (SPSS trial version). The results were reported as mean values and standard deviations from triplicate samples of each treatment for all experiments. Two effects (stabilization methods and extraction methods) on rice bran oil quality and bioactive compound content. Therefore, eighteen raw bran samples (six treatments and three replicates) were used in a 2x3 factorial arrangement, with two stabilization methods (steaming and OHM) and three extraction methods (immersion in hexane, Soxhlet, enzymatic extraction) in a completely randomized design (CRD). Duncan Multiple Range Test was performed to determine the significant difference between treatments. Statistical significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

Chemical quality of rice bran oil

The results from the statistical analysis indicated that there was an interaction effect between the stabilization and oil extraction methods on percentage of oil recovery, as presented in Fig. 1. The stabilization of rice bran using OHM and then extracted using the Soxhlet method gave the highest oil recovery (17.11%), followed by steamed rice bran extracted with the Soxhlet method (15.10%), whereas the lowest oil recovery (7.69%) was found in the oil obtained from rice bran stabilized using the steaming method and extracted by immersion in hexane. This may be because the Soxhlet extraction is the standard method of the Association of Official Analytical Chemical (AOAC) for determination of lipid content of agricultural products. It can therefore be applied effectively to recover the whole lipid content from the extracted sample. Interestingly, the bran stabilized by OHM showed higher yield of extracted oil than that of the bran stabilized by steaming. In addition, rice bran oil recovered from the bran stabilized using OHM and extracted using enzymes and immersion in hexane had comparable oil yields of 11.78% and 11.11%, respectively (Fig. 1.). This was higher than the oil obtained from the rice bran stabilized using the steaming method, and extracted by the same methods. A similar result was observed by Hanmoungjai et al. (2001), who reported that the maximum yields of oil from rice bran obtained from enzymatic extraction were 79% of total lipids. The high oil recovery in the enzyme assisted aqueous extraction process might be due to the efficiency of rice bran stabilization using OHM for disrupting the cellular structure of the oil-bearing materials; therefore, the enzymatic oil extraction after OHM was more effective than extraction after steaming stabilization. Lakkakula et al. (2004) found that rice bran stabilization using OHM showed an increase in the total percent of lipids with a maximum of 92%, while only 53% of total lipids were recovered from the bran samples without OHM. In addition, the electric energy

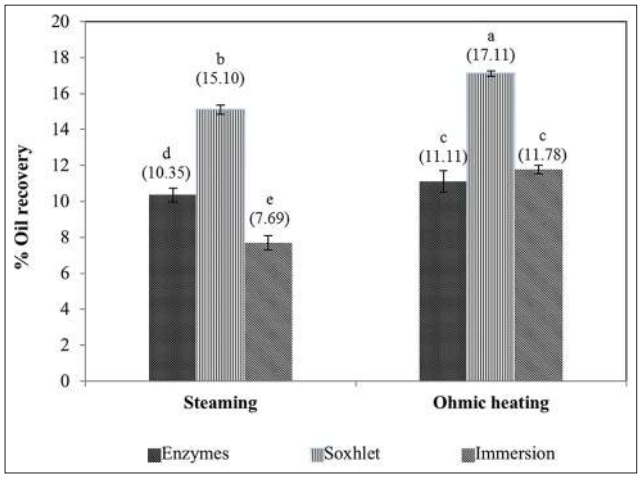

Fig 1. Oil recovery obtained from two stabilization and three extraction methods. Each observation is mean ± SD of replicate experiments (n=3). The different letters above each column indicate significant difference ($P<0.05$). The vertical bars on each column indicate the standard deviation

of OHM was responsible for the breakdown of the rice bran cell membrane (Nair et al., 2014). The heat transfer of the steam method was slow because the transfer was by conduction and convection only (Goullieux and Pain, 2005). In OHM, the heating occurs in the form of internal energy transformation (from electric to thermal) within the bran (i.e. aleurone cell and surrounding surface area) and rapidly penetrates other surrounding areas (Loypimai et al., 2015). This event may be one of the reasons for increase in oil yield of the bran stabilized using OHM and extracted using same extraction method.

On the other hand, stabilization and extraction methods had a significant effect ($P<0.05$) on the FFA content of the extracted oil (Table 1.). Among the extraction processes, the oil obtained from rice bran stabilization using OHM, or steaming and then extraction using enzymes produced the lowest level of FAA content (1.50% and 1.51%, respectively), while the highest value of FFA (3.0%) was observed in the oil obtained from the rice bran stabilized using the steaming method and extracted using hexane immersion. These results were slightly different from the results in a study by Hanmoungjai et al. (2001), who found that the FFA value in rice bran oil acquired from the enzymatic oil extraction process was significantly lower than the oil attained by n-hexane extraction.

Table 1: Effect of stabilization and extraction methods on free fatty acid (FFA) content, peroxide value, and thiobarbituric acid (TBA) value of rice bran oil

Extraction process		FFA (% as oleic acid)	Peroxide value (mM/Kg) ns	TBA value (mg MDA/ Kg oil) ns
Stabilization	Extraction			
Steaming	Immersion	3.00±0.09[a]	1.20±0.05	1.74±0.02
	Soxhlet	2.36±0.21[b]	1.21±0.08	1.72±0.01
	Enzymes	1.50±0.03[d]	1.19±0.03	1.69±0.02
Ohmic heating	Immersion	1.94±0.04[c]	1.18±0.01	1.70±0.01
	Soxhlet	1.64±0.06[c]	1.19±0.02	1.72±0.02
	Enzymes	1.51±0.04[d]	1.21±0.03	1.69±0.02

Values are means±SD of triplicate samples (n=3). Values with the same alphabet along the same columns are not significantly different ($P<0.05$). MDA: Malondialdehyde; ns: Not significantly different at $P<0.05$; FFA: Free fatty acid (%); TBA: Thiobarbituric acid

However, the concentrations of FFA in the oil obtained from all extraction processes were still suitable for human consumption, as they were less than 5% FFA, as suggested by Tao et al. (1993). These results were supported by Lakkakula et al. (2004) who showed that FFA concentration of the rice bran oil obtained from rice bran stabilized using OHM increased more slowly than the oil obtained from the bran without OHM. This may be due to the energy applied under OHM inactivating the lipase activity, causing an increase in FFA. Loypimai et al. (2009) also reported that electric field strengths of OHM between 150 and 225 V/cm and a moisture content of rice bran varying from 30% to 40% retarded the increase in FFA content and lipase activity during storage.

The oil obtained from the different extraction processes had no significant effect on the PV and TBA values (Table 1.). The PV and TBA values varied from 1.18 to 1.21 mM/ Kg and 1.9 to 1.74 mg MDA/Kg of the oil, respectively. This may be because OHM and steaming are effective methods for rice bran stabilization and inactivation of the enzymes (both lipoxygenase and lipase), which cause lipid peroxidation and destruction of unsaturated fat and lipid in the rice bran.

Fatty acid compositions

The GC-FID chromatogram of fatty acid composition of the extracted oil is shown in Fig. 2 and results are given in Table 2. It was observed that different stabilization and oil extraction methods had no influence on the fatty acid composition of the oil obtained. These results were similar to the study of Hanmoungjai et al. (2001) who reported that composition of essential fatty acids in the rice bran oil extracted by enzymes was comparable to commercial rice bran oil and solvent-extracted oil. For this study, the majority of fatty acid composition in the oil was linoleic acid (34.38% to 37.66%) and oleic acid (34.12% to 35.84%). This result was also similar to the findings of Hanmoungjai et al. (2001), who reported that oleic, linoleic, and palmitic acids were the dominant fatty acids in rice bran oil, and

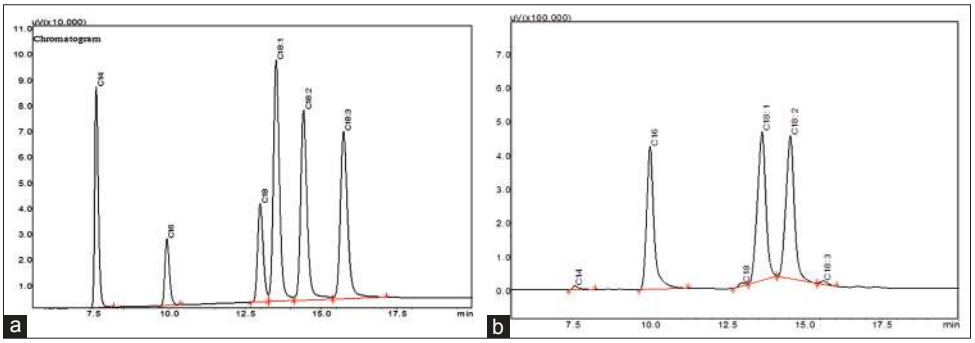

Fig 2. GC–FID chromatogram of standard fatty acid composition (a) and fatty acid composition in the oil obtained from an ohmically-stabilized rice bran and enzymatic oil extraction (b).

accounted for 95% of the total fatty acids. This may be due to OHM being an effective method for fatty acid stabilization in the bran and comparable to the steaming. This event was in agreement with the study of Gavahian et al. (2012), who mentioned that the result of GC–MS analysis did not indicate any noticeable changes of the compounds in the essential oils (*Thymus valgaris* L.) obtained by ohmic-assisted hydro-distillation in comparison with steaming hydro-distillation.

Bioactive compounds

Bioactive compounds, α-tocopherol and γ-oryzanol in the rice bran oil are illustrated in Table 3. The results indicated that there was a significant interaction ($P<0.05$) between stabilization and oil extraction methods on the concentration of α-tocopherol and γ-oryzanol. The oil obtained from rice bran stabilized using OHM and extracted by enzymes showed the highest levels of γ-oryzanol (1190.1 ± 89.3 μg/g) and α-tocopherol (42.38 ± 0.53 μg/g). Likewise, the oil recovered from rice bran stabilized using OHM and extracted with any of the other extraction methods yielded greater concentration of α-tocopherol than that obtained from steaming stabilization. This is due to electric energy applied under OHM caused electroporation of rice bran resulting in breakdown of the rice bran cell membranes which enabled the extraction using enzymes and the release of intracellular bioactive compounds into the oil. These results were supported by Loypimai et al. (2009), who pointed out that the yield of phenolic compound, α-tocopherol, and γ-oryzanol was more in ohmically-heated rice bran when compared to the steamed rice bran. Darra et al. (2013) observed that pulsed OHM pretreatment accelerated the extraction kinetics of total polyphenols from grape pomace, and induced a high degree of cell membrane damage, which increased the yield of intracellular compounds. Loypimai et al. (2015) found that application of OHM assisted solvent extraction and prepared colorant powder from black rice bran which had both high yield and high concentration of bioactive compounds.

Antioxidant activity

A significant interaction effect ($P<0.05$) on the peroxyl radical scavenging was found in the oil obtained from different oil extraction processes (Table 3.). The oil obtained from the bran stabilized using OHM or steaming methods and then extracted using enzymes had significantly lowest values of IC_{50} (42.0 ± 2.04 and 46.0 ± 2.03 mg/mL, respectively), and the strongest TAC (21.3 ± 0.32 mg AEE/g and 15.5 ± 0.24 mg BHAE/g). This may be due to an electroporation effect of OHM and enzyme digestion that digested the bran to fine particles. This process could allow for the release of antioxidative substances, such as α-tocopherol and γ-oryzanol into the oil after extraction, as the results indicated in the previous section. In addition, the successful application of enzymatic oil extraction may depend on pretreatment methods (stabilization method), enzyme specifications, and digestive conditions.

Chemical compositions of defatted rice bran (DFRB)

The extraction processes significantly affected ($P<0.05$) the chemical compositions (fat, protein, fiber, ash, and total carbohydrate) of the DFRB (Table 4). The DFRB obtained

Table 2: Effect of stabilization and extraction methods on fatty acid compositions (%) of rice bran oil

Extraction process		Myristic (C_{14}) ns	Palmitic (C_{16}) ns	Stearic (C_{18}) ns	Oleic ($C_{18:1}$) ns	Linoleic ($C_{18:2}$) ns	Linolenic ($C_{18:3}$) ns
Stabilization	Extraction						
Steaming	Immersion	2.68±0.04	21.45±0.32	0.02±0.0005	35.84±0.34	35.02±0.11	1.60±0.01
	Soxhlet	2.54±0.03	24.95±0.17	0.02±0.0001	34.48±0.26	35.03±0.42	1.69±0.02
	Enzymes	2.59±0.02	26.30±0.25	0.02±0.0001	35.17±0.29	34.38±0.53	1.56±0.01
Ohmic heating	Immersion	2.97±0.03	25.37±0.24	0.03±0.0002	34.51±0.31	34.90±0.27	2.25±0.02
	Soxhlet	2.94±0.01	24.79±0.43	0.02±0.0001	34.12±0.54	37.66±0.37	1.65±0.02
	Enzymes	3.14±0.02	25.73±0.23	0.02±0.0004	34.66±0.17	34.79±0.35	1.67±0.01

Values are means±SD of triplicate samples (n=3). ns: Not significant different at $P<0.05$

Table 3: Influence of stabilization and extraction methods on bioactive compounds and antioxidant activity of rice bran oil

Extraction process		α-tocopherol (μg/g)	γ-oryzanol (μg/g)	Peroxyl radical scavenging (mg/mL)	Total antioxidant capacity	
Stabilization	Extraction				mg VEE/g	mg BHAE/g
Steaming	Immersion	11.12±0.48^{d}	957.18±53.7^{b}	56.3±4.93^{c}	8.71±0.22^{e}	5.89±0.16^{e}
	Soxhlet	17.02±0.02^{c}	991.95±20.5^{b}	49.7±2.89^{b}	10.9±0.48^{d}	7.51±0.35^{d}
	Enzymes	24.87±0.45^{b}	1009.6±29.9^{b}	46.0±2.03^{ab}	11.1±0.46^{d}	7.62±0.33^{d}
Ohmic heating	Immersion	39.37±0.67^{a}	989.85±15.5^{b}	51.7±1.53^{b}	13.0±0.62^{c}	9.04±0.46^{c}
	Soxhlet	40.09±0.68^{a}	995.12±93.8^{b}	49.8±1.06^{b}	17.2±0.23^{b}	12.1±0.17^{b}
	Enzymes	42.38±0.53^{a}	1190.1±89.3^{a}	42.0±2.04^{a}	21.3±0.32^{a}	15.5±0.24^{a}

Values are means±SD of triplicate samples (n=3); Values with the same alphabet along the same columns are not significantly different ($P<0.05$); Peroxyl radical scavenging was expressed as IC_{50}, mg/g: Concentration of the amount of oil extract to inhibit 50% of stable free peroxyl radical; VEE: Vitamin E equivalent; BHAE: Butylatedhydroxy anisole equivalent

Table 4: Influence of stabilization and extraction methods on chemical compound of defatted rice bran

Extraction process		Chemical composition (g/100 g)				
Stabilization	Extraction	Protein	Fat	Ash	Fiber	Total carbohydrate[x]
Steaming	Immersion	8.26±0.21[a]	6.82±0.05[d]	11.13±0.07[c]	10.25±0.47[b]	55.05±5.0[b]
	Soxhlet	8.64±0.57[a]	1.24±0.03[b]	11.08±0.11[c]	10.18±0.98[b]	55.98±3.57[c]
	Enzymes	12.98±0.15[b]	7.26±0.03[d]	14.21±0.52[a]	13.01±0.39[b]	48.92±5.52[b]
Ohmic heating	Immersion	8.10±1.38[a]	4.90±0.04[c]	10.46±0.24[d]	10.19±0.24[b]	45.59±4.94[b]
	Soxhlet	8.37±1.26[a]	0.95±0.04[a]	10.22±0.72[d]	10.32±0.98[b]	55.72±6.67[a]
	Enzymes	12.74±1.13[b]	5.05±0.04[c]	12.60±0.12[b]	13.54±0.45[a]	48.49±5.54[b]

Values are means±SD of triplicate samples (dry basis, n=3). Values with the same alphabet along the same columns are not significantly different (P<0.05).
[x]calculated by a different method

from the bran stabilized by either OHM or steaming and extracted by enzymes showed significantly highest content of protein, fat, and fiber. This may be because the enzyme system used had a specific to structural component of rice bran. A similar result was observed by Hanmoungjai et al. (2001), who reported that the rice bran meal obtained by the enzymatic process was high in protein and total dietary fiber, which are valuable for use as ingredients in food products and animal feed.

CONCLUSIONS

OHM stabilization and oil extraction using the Soxhlet method yielded the highest oil recovery. However, in terms of FFA, γ-oryzanol content and total antioxidant capacity, the oil obtained from the bran stabilized using OHM and extracted using the enzymatic method presented better results than the other extraction methods. The results suggested that rice bran stabilized using OHM, and extracted using the enzymatic method had potential as functional rice bran oil. This process can also be considered as an alternative green extraction method, since no chemicals were used.

ACKNOWLEDGMENTS

Financial support provided by Mahasarakham University, Thailand and the Thailand Research Fund-Master Research Grants (TRF-MAG) is gratefully acknowledged. The authors are also grateful to Mr. Paul Alexander Dulfer, Division of Research Facilitation and Dissemination, Mahasarakham University, Thailand for his critical proofreading of this manuscript.

Author contributions

P. Loypimai participated in experiments and data-analysis and also contributed to the writing of the manuscript. A. Moongngarm designed the research plan, organized the study, participated in experiments, coordinated the data-analysis, and contributed to the writing of the manuscript. P. Chottanom participated in experiments and coordinated the data-analysis.

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