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Original Article

Rice bran oil extraction with mixtures of ethanol and hexane

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Abstract

The main objective of this study was to improve the extractability and quality of rice bran oil using different mixtures of ethanol (Eth) and hexane (He). Eth/He ratios of 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0 (%v/v) were used for the extraction. The ultrasound extraction processes were experimented at 2.5 W/g, 30 °C for 15 min. The results indicated that the highest oil extractability was found in the 60:40 (%v/v) Eth/He mixture. Experimental results also showed that the mixture of 60:40 (%v/v) Eth/He had significant effect on the physiochemical properties and phytochemical content of crude rice bran oil (p<0.05). Especially, the crude rice bran oil was low in peroxide value (3.03 meqO_2/kg oil) and acid value (2.01 mg KOH/g oil), high in iodine value (105.00 mg I_2/100g oil), and lighter in color. Further analysis revealed that oleic, linoleic, and palmitic acids were the dominant fatty acids in the Eth/He extracted oils.

Keywords: lipid extraction, crude rice bran oil, antioxidant activities, physiochemical properties

1. Introduction

Rice bran is a by-product from rice milling, and is considered as waste (Srikaeo, 2014; Tao, Rao, & Liuzzo, 1993). Rice bran comprises of 12-22% of oil, which contains high unsaturated fatty acids and bioactive phytochemicals (phenolic acids, flavonoids, gamma-oryzanol, tocopherols, and sterols) (Grosso, Valentão, Ferreres, & Andrade, 2015; Pengkumsri *et al.*, 2015). These bioactive compounds have high capacity of antioxidants, serve to eliminate free radicals, thereby preventing the reaction of free radicals and biomolecules causing damages to the body. Previous studies have proven that rice bran oil helps lower cardiovascular risk and platelet aggregation (Grosso *et al.*, 2015), and decrease plasma cholesterol (Grosso *et al.*, 2015; Wilson, Nicolosi, Woolfrey, & Kritchevsky, 2007). Therefore, rice bran can be considered as a potential source of lipids, vitamins, minerals, and some phytochemicals with health-enhancing values.

Conventional extraction methods (cold maceration, reflux, and soxhlet techniques) have been used for many decades although these methods are very time-consuming and require a relatively large amount of highly solvents (Mingyai, Kettawan, Srikaeo, & Singanusong, 2017; Phan, Junyusen, & Liplap, 2018; Stanisavljević *et al.*, 2009;). In recent years, the ultrasound assisted solvent extraction, a newly invented extraction system, has been introduced to improve the oil extractability and reduce solvent usage (Daud *et al.*, 2018; Karmakar, Rajor, Kundu, & Kumar, 2018). In ultrasound assisted extraction, mechanical cavitation is induced, resulting in the cell disruption and thus allows more thorough solvent extraction of the cell contents enhancing the recovery of oil extractability and nutraceuticals (Phan *et al.*, 2018).

Solvent extraction methods are based on the affinity between a solute and a solvent where many different types of interactions are needed to increase the effectiveness of the extraction process. In oil extraction, polar organic solvents (i.e., ethanol and water) disrupt hydrogen bonds between polar

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lipids, whereas non-polar organic solvents (i.e., hexane, petroleum ether, and ethyl ether) break down hydrophobic interactions between solvent and neutral lipids and some active compounds (Escorsim *et al.*, 2018). The most common organic solvent for vegetable oil extraction is hexane, which presents a high selectivity for non-polar compounds, but is flammable and toxic. Meanwhile, ethanol is a relatively non-toxic and more environmentally friendly chemical than hexane (Baümler, Carrín, & Carelli, 2016). In addition, ethanol can also be produced from a wide variety of biological materials using simple technology, thus its is relatively low cost (Ferreira-Dias, Valente, & Abreu, 2003).

Therefore, in order to improve the oil extractability and quality as well as reduce the cost and toxicity, this study explored the possibility of extracting oil from rice bran (assisted by ultrasound) by using a mixture of hexane and ethanol.

2. Materials and Methods

2.1. Materials

Rice bran (RB) of jasmine varieties was provided by *Korat Yongsanguan Rice mill* Co., Ltd (Thailand), and dried at 100 ℃ for 15 minutes. Then it was milled and separated by a 60-mesh sieve (0.25mm) trainer. The final moisture content was 6-8%, and thereafter rice bran was vacuum-packed and placed in a chiller at 4 ℃ for further analysis (Hamm *et al.*, 2013).

Absolute methanol (Mallinckrodt), acetonitrile, *n*-hexane, chloroform value (98.5%) and absolute ethanol value (99.5%) were purchased from Merck. 2,2'- diphenyl-1-picrylhydrazyl (DPPH), ABTS (2,2′-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid), 2,4,6-tris(2-pyridyl)-s-triazine, and Folin–Ciocalteu solution were obtained from Sigma-Aldrich. Catechin (98.0%), gallic acid (97.0%) (Sigma-Aldrich Co., St. Louis, MO), and gamma-oryzanol (98.0%) (Oryza Oil & Fat Chemical Co., Ltd, Japan) were used for quantification.

2.2. Methods

2.2.1. Extraction procedure

The experiments were performed in a laboratory-scale unit. The experiment was carried out by using 40 KHz ultrasound device (VCX750 Vibracell; Sonic & Materials, Inc., Newtown, CT, USA) with a 3.0 mm flat tip probe. 20 g rice bran was extracted by a mixture of ethanol (Eth) and hexane (Hex) ratios (0:100, 20:80, 40:60, 60:40, 80:20, and 100:0 (%v/v)). The solvent to solid ratio was 10:1, and these mixtures were sonicated at 2.5W/g, 30 ℃ for 15 min based on preliminary investigation showing a suitable extraction condition. The extracts were centrifuged for 10 min at 5000 rpm and filtered. The solvents were then evaporated under vacuum condition with a pressure of 600 mbar at 40 ℃ and the extracts were dried until the constant weight achieved using a mild nitrogen gas. The crude rice bran oil extractability was calculated by dividing the weight of the extracted oil and the weight of the initial rice bran.

2.2.2. Determination of total phenolic content (TPC)

The total phenolic content (TPC) was measured following the method of Singleton, Orthofer, & Lamuela-Raventós (1998) with minor modifications. Briefly, 50 µl of CRBO was dissolved in 150 µl methanol, and the solution was then mixed with 500 µl of Folin-Ciocaltue reagent and 800 µl of 10% sodium carbonate. The mixture was diluted to 4 mL with deionized water (DI) and shaken well for 2 min. After incubation at the ambient temperature for 60 min, the absorbance was taken at 765 nm by using a UV-spectrophotometer/NIR (Shimazu, UV-2600, Japan). The TPC was calculated as gallic acid equivalents per g of rice bran oils (g GAE/100 g oil).

2.2.3. Determination of gamma-oryzanol content in rice bran extract

Gamma oryzanol was analyzed by-using reversed phasedHPLC according to the method reported by Sakunpak, Suksaeree, Pathompak, Charoonratana, & Sermkaew (2014). A 200 mg sample was weighed and diluted into 5 ml of methanol. After the centrifugation at 9,000 rpm for 10 min, the solvent was transferred to 1.5 ml vials for further analysis. The phase-reversed HPLC consisted of an Agilent 1200 series HPLC (Agilent Technologies, Inc., CA, USA) equipped with a Poroshell 120 EC-C18 column (3.0 mm×150 mm, 2.7 µm), and a diode-array UV/VIS detector. The UV detector and the column temperature were operated at 325 nm and 25 ℃. The (B) and acetonitrile (A) mobile phase was methanol.The flow min for total running time of 15 min, /rate was kept at 1 mL and the gradient program was as follows: 100:0% (v/v), 50:50% (v/v), and 40:60% (v/v) A and B for 5 min each. The injection volume was 20 µl. To construct the calibration curves, the standard solutions of gamma-oryzanol (3-200 µg/ml) were used. The gamma-oryzanol concentrations were identified by comparing the retention times and the peak area values with the standards.

2.2.4. Determination of α-tocopherol content in rice bran extract

The tocopherol contents (α-, β-, γ-) of the rice bran extract were evaluated by phase-reversed HPLC (Agilent 1200 series equipped with Hypersil ODS column (250×4.0mm, 5.0µm, (Phenomenex, USA)). The solvent mixture of acetonitrile and methanol was used as in the mobile phase under a gradient condition. The gradient program was set as follow: methanol (5%v/v) and acetonitrile (95%v/v) for 3 min, and methanol (100%v/v) and acetonitrile (0%v/v) for 30 min. A UV/VIS detector (DAD) with wavelengths of 290 and 330 nm was equipped for the sample detection, and the flow rate was set at 1.0 mL/min. The injection volume was 20 µl. A standard curve for each tocopherol in the range of 0.01-10mg/ml was prepared. The correlation coefficients (R^2) and relative standard deviations (RSD) of the peak areas against the tocopherol standard concentrations for each compound were calculated after three replicates of each tocopherol standard solution were injected. The tocopherol concentrations were identified by comparing the retention times and the peak area values with the standard.

2.2.5. Gas chromatography with flame-ionization detection (GC-FID) analysis

The fatty acid (FA) compositions were determined by GC-FID (Agilent 7890C axis detector, England), following the method described by Stanisavljević *et al.* (2009). In the analysis, a 20 mg rice bran oil sample was first weighed into a test tube and dissolved in 1 mL 0.5 M KOH. The solution was heated in a water bath at 90 °C for 30 min, and then neutralized with 0.6 M HCl before adding 3.0 mL BF_3 in methanol. The mixture was re-heated at 90 °C for another 15 min in a water bath. The methylated oil was then extracted with n-hexane, and then the solvent was removed by nitrogen gas prior to the FA analysis.

The GC-FID conditions operated at the initial temperature of 50 °C for 2 min, then increased to 250 °C at a rate of 40 °C/min with the total run time of 75 min. The FAs were identified by the GC–FID mass fragmentation pattern and spectral, and compared against the standards.

2.2.6. Physiochemical characteristics analysis

The American Oil Chemists' Society (AOCS) official methods (1997) were used: Cd 8b-90 for peroxide value (PV) and Ca 5a-40 for acid value (AV) and ,Cd 1d-92 for iodine value (IV). The color of CRBO was determined by Lovibond Tintometer PFXI-880L (Tintometer Ltd, USA) with glass cells of one inch. Results were expressed as 5×red+1×yellow Lovibond units.

2.3. Statistical analysis

All the statistical analyses were carried out in triplicate, and the experimental results were expressed as mean±SD. The statistical analysis was performed using Stagraphic Centrution XV (Statsoft Inc., Umeå, Sweden). One-way analysis of variance (ANOVA) with 95% confidence level was carried out to determine differences among group means.

3. Results and Discussion

3.1. Oil extractability

Table 1 illustrates the effect of the Eth/He mixture variables on the crude rice bran oil using the soxhlet extraction, where the Eth/He ratios were varied between 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0 (%v/v). The results in Table 1 indicate that the extractability of oil from rice bran depended significantly on the different Eth concentrations in the Eth/He mixtures. The highest oil extractability was achieved with Eth concentration in a range of 60 and 80 %v/v (16.04-16.05%), slightly lower than 16.7 g oil/100 g rice bran in Gunawan, Vali, & Ju (2006). Also, these results showed that the Eth/He mixtures provided higher oil extractability compared to the individual solvents (Eth or He) ($p<0.05$). This could be explained that the moderation of the polarity of the Eth/He mixtures can decrease the difference in the phase boundary between the surface tensions, improve the separation phase, and produce the higher yield. The present findings are similar to the results obtained by Escorsim *et al.* (2018), who reported that the combination of Eth and He favors both enthalpic and entropic interactions that decrease the Gibbs free energy of the system and facilitate the transport of lipids through the cell wall.

Table1. RBO yield and physicochemical properties of the rice bran oils under different extraction solvents

Eth/He ratio (%v/v)	Yield (g/100g rice bran) [1]	Iodine value (mg I_2/100g oil)[2]	Acid value (AV) (mg KOH/g oil)[3]	Peroxide value (PV) (meqO_2/kg oil)[4]
0:100	11.42^{c}±0.09	100.15^{ab}±1.03	2.00^{a}±0.10	3.04±0.06
20:80	13.44^{b}±0.90	101.51^{ab}±1.02	2.00^{a}±0.05	3.04±0.04
40:60	16.05^{a}±0.80	105.16^{a}±1.14	2.01^{a}±0.04	3.03±0.09
60:40	16.04^{a}±1.00	105.00^{a}±1.03	2.01^{a}±0.02	3.03±0.10
80:20	16.04^{a}±1.00	106.00^{a}±1.03	2.13^{b}±0.02	3.05±0.10
100:0	13.59^{b}±1.00	105.09^{a}±1.12	2.19^{b}±0.07	3.05±0.09

[1,2,3,4,5]Different letters denote statistically significant differences between treatments ($p<0.05$). The values are the mean of three replications ± standard deviation.

Thus, the Eth/He mixtures contained 60 or 80 (%v/v) Eth were suitable for improving oil extractability from rice bran. Besides, the mixed solvent (Eth and He) was also considerably less explosive and costly than pure He.

3.2. Physiochemical characteristics

Table 1 shows the physiochemical properties of crude rice bran oil extracted with the different Eth/He ratios. As shown in Table 1, the acid value ranged from 2.00 to 2.19 mg KOH/g oil, depending on the Eth concentrations in the Eth/He mixtures. The highest acid value was 2.19 mg KOH/g oil under 100 %v/v Eth. The acid value of the oil extracted by 80 %v/v and 100 %v/v Eth in the Eth/He mixtures was slightly higher than that of oil samples extracted with 0 to 60% (v/v) ($p<0.05$). The finding is similar to Kuk, Tetlow & Dowd (2005), who reported that the acid value of the oil samples increased with the increased solvent polarity. This is in agreement with previous studies which suggest that polar solvents tend to extract higher amounts of free fatty acids (Efthymiopoulos *et al.*, 2018; Al-Hamamre, Foerster, Hartmann, Kröger, & Kaltschmitt, 2012).

On the contrary, variation in Eth concentration had no effect on peroxide value (Table 2). The peroxide value was used as an indicator of deterioration of oils or fats (Chemat *et al.*, 2004). Peroxide formation indicates lipid degradation by oxidation. According to CODEX Alimentarius Commission (2015), peroxide values of fresh oil should be less than 10 meqO_2/kg oil. The peroxide value of the crude rice bran oil in this study was found between 3.03-3.05 meqO_2/kg oil, which was suggested lower deterioration and longer shelf life.

Iodine value is a parameter that represents the degree of unsaturated fatty acids or the average number of double bonds of fatty acids. There is a quality test for edible oils. The higher the iodine value, the higher the degree of unsaturation. The iodine value found in this work was from 100.15 to 106.00 mg I_2/100g oil. As shown in Table 1, the iodine values obtained from the high ethanol concentrations in the Eth/He mixtures were slightly higher than those from the low ethanol concentrations ($p<0.05$). The results could be explained that the ethanol concentrations affected the selectivity of specific extracting solvents for certain

Table 2. Color of crude rice bran oil extracts from different extraction solvents.

Eth/He ratio (%v/v)	Read (R)[1]	Yellow (Y)[2]	Blue (B)[3]	Neutral (N)[4]	5R+Y[5]
0:100	1.99^{c}±0.01	8.52^{b}±0.01	0.00	0.00	18.47^{c}±0.05
20:80	1.95^{c}±0.01	8.50^{b}±0.01	0.00	0.00	18.25^{c}±0.05
40:60	1.90^{c}±0.01	8.02^{b}±0.01	0.00	0.00	17.52^{c}±0.05
60:40	1.67^{a}±0.02	5.50^{a}±0.03	0.00	0.00	13.85^{b}±0.06
80:20	1.00^{a}±0.02	5.52^{a}±0.02	0.00	0.00	10.52^{a}±0.05
100:0	1.01^{a}±0.01	5.50^{a}±0.03	0.00	0.00	10.52^{a}±0.03

[1,2,3,4,5] Different letters denote statistically significant differences between treatments *(*$p<0.05$*)*. The values are the mean of three replications ± standard deviation.

unsaturated fatty acids. The obtained results agreed with those reported by Hanmoungjai, Pyle, & Niranjan (2001), Oluremi, Solomon, & Saheed (2013), who documented that the iodine value of crude rice bran oil was 95–115 g I_2/100 g oil. It means crude rice bran oil is rich in unsaturated fatty acids.

For the color of the crude rice bran oil, the results were shown in Table 2. The color of the crude rice bran oil extracted by 0, 20, 40, and 60 (%v/v) Eth in the Eth/He mixtures increased in both red and yellow units, whereas higher ethanol concentrations in the Eth/He mixtures (80 and 100 %v/v) significantly lowered the color of oil ($p < 0.05$). The results could be attributed to the high polarity and viscosity of ethanol, which might facilitate the mass transfer of the pigments and other impurities from the sample matrix to the extracted oil. Our result is similar to Arnold & Choudhury, (1962), who reported that the Eth oil extracted from rice bran had a significantly dark color than the hexane oil. However, the total color value of this study was lower than 20, which indicates a good color quality (Mingyai *et al.*, 2017).

3.3. Phytochemicals content

Table 3 lists the contents of gamma-oryzanol and tocopherols in the rice bran oil samples obtained by various Eth concentrations in the Eth/He mixtures. As presented in Table 3, the major isomer in all the samples was α-tocopherol, followed by β-, γ-, and δ-tocopherols, respectively. According to our results, α- and β-tocopherol yields increased from 55.60 to 510 ppm and 15.05 to 120 ppm, respectively, whereas δ- and γ-tocopherol decreased from 10.10 to 2.00 ppm and 1.10 to 0.90 ppm as increasing the Eth concentrations in the solvent mixtures from 0 to 100 (%v/v). The reason is that the low polarity of δ- and γ-tocopherol affected their solubility in Eth. This result is consistent with Tir, Dutta, & Ahmed (2012), who reported the δ- and γ-tocopherols were less soluble in Eth than α- and β-tocopherols. However, in this study β-tocopherol was not detectable in the extracts obtained in the extract without Eth. According to our results, the highest amount of tocopherols (α-, β-, δ-, and γ-) were obtained when extracting the rice bran oil using 60:40 (v/v) Eth/He.

In this study, the solubility of gamma-oryzanol varies in different solvents. It was found that 0–60% (v/v) Eth in the Eth/He mixtures has a greater effectiveness in extracting gamma-oryzanol compared to 80 and 100 (%v/v) Eth. When the Eth concentration in the Eth/He mixture increased from 60 to 100 (%v/v), the gamma-oryzanol content significantly decreased from 1.82 to 0.89 mg/100 g oil. This could be attributed to the low solubility of gamma-oryzanol in alcohol (Antonio, Eduardo, Mariana, & Marcelo, 2011; Narayan, Barhate, & Raghavarao, 2006). Chromatograms of gamma-oryzanol fractions showed that the mass spectral profile of gamma-oryzanol in the crude rice bran oil sample extracted with the Eth concentration at 0 and 100 %v/v consists of four peaks (Figure 1). The four major components are campesteryl ferulate (retention time (RT)=7.860 min), 24-methylene cycloartanyl ferulate (RT=7.152 min), β-sitosteryl ferulate (RT=9.098 min), and cycloartenyl ferulate (RT=6.322 min), respectively.

The total phenolic content (TPC) of the extracted oil was expressed as gallic acid equivalent (GAE). The phenolic compounds in rice bran contribute to the antioxidant properties of crude rice bran oil. The TPC values of the extracts range from 3.40 mg GAE/g to 4.79 mg GAE/g for 0 to 100 %v/v Eth concentration. The TPC of the extract using the Eth concentration from 40 to 100 (%v/v) is significantly higher than that of 0 and 20 (%v/v) ($p<0.01$). Similar observation for TFC was found in the oil obtained with different extraction solvents. The highest TFC was obtained with the Eth concentration at 60 %v/v, followed by 80 %v/v and 100 %v/v in the Eth/He mixture. This could be attributable to the possible complex formation of phenolic and flavonoid compounds in rice bran that are more soluble in Eth and Eth/He mixture than He. Furthermore, high Eth concentration increased in opening the cell walls to allow more thorough solvent extraction reported by SEM results (Figure 2), and in enhancing the solubilization of TPC, TFC, unsaponifiable and other polar compounds. The SEM results

Table 3. Phytochemicals and antioxidant activity of crude rice bran oil extracts from different extraction solvents

Eth/He ratio (%v/v)	TPC (mgGAE/mL oil)[1]	TFC (mg catechin/ mL oil)[2]	Tocopherol (ppm)[3]				Gamma-oryzanol (mg/ 100 mL oil)[4]	DPPH (mg TEAC/ mL oil)[5]	ABTS (mgTEAC/ mL oil)[6]
			α-	β-	γ-	δ			
0:100	3.40^{d}±0.21	0.65^{d}±0.01	55.60^{d}±1.09	ND	10.10^{a}±0.09	1.08^{a}±0.00	1.83^{a}±0.04	13.77^{d}±1.05	5.38^{c}±0.15
20:80	4.41^{c}±0.25	1.05^{c}±0.02	201^{c}±1.90	15.05^{c}±1.10	10.08^{a}±0.07	1.10^{a}±0.00	1.83^{a}±0.04	20.77^{c}±1.05	9.38^{ab}±0.15
40:60	4.68^{ab}±0.21	1.84^{c}±0.02	230^{b}±1.89	95.00^{b}±1.05	9.15^{ab}±0.07	1.08^{a}±0.00	1.83^{a}±0.05	23.78^{a}±1.04	10.25^{a}±0.15
60:40	4.79^{a}±0.12	1.94^{a}±0.05	504^{a}±1.78	110.01^{a}±1.90	8.81^{ab}±0.05	1.09^{a}±0.00	1.82^{a}±0.05	23.87^{a}±1.00	10.32^{a}±0.17
80:20	4.77^{a}±0.15	1.93^{a}±0.02	510^{a}±1.91	120.00^{a}±1.01	3.89^{c}±0.05	0.91^{b}±0.00	0.99^{b}±0.02	23.46^{ab}±0.52	9.79^{ab}±0.19
100:0	4.71^{ab}±0.12	1.90^{ab}±0.05	509^{a}±1.78	120.01^{a}±1.79	2.00^{d}±0.06	1.00^{ab}±0.00	0.89^{b}±0.05	23.47^{ab}±1.00	9.83^{ab}±0.17

[1,2,3,4,5,6] Different letters denote statistically significant differences between treatments *(*$p<0.05$*)*. The values are the mean of three replications ± standard deviation.

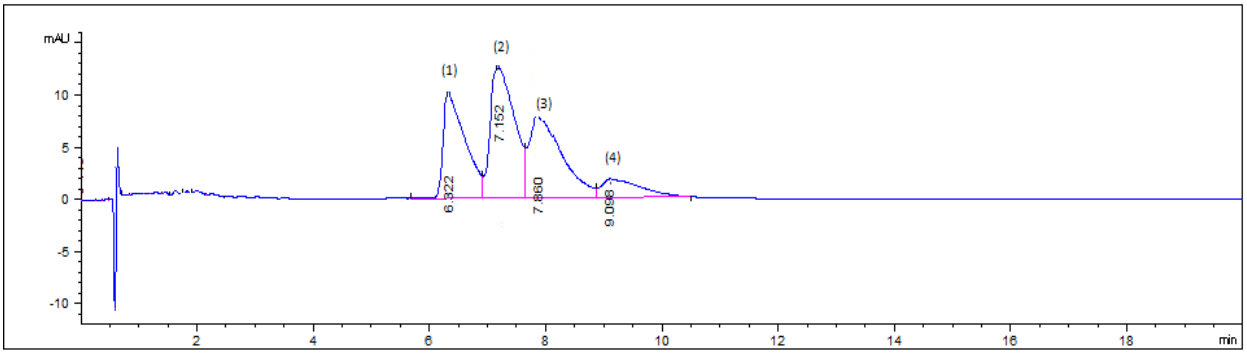

Figure 1. Typical chromatograms for rice bran oil gamma-oryzanol: denotes (1) cycloartenylferulate, (2) 24-methylenecy cloartanyl ferulate, (3) campesterylferulate, and (4) *β*-sitosteryl ferulate.

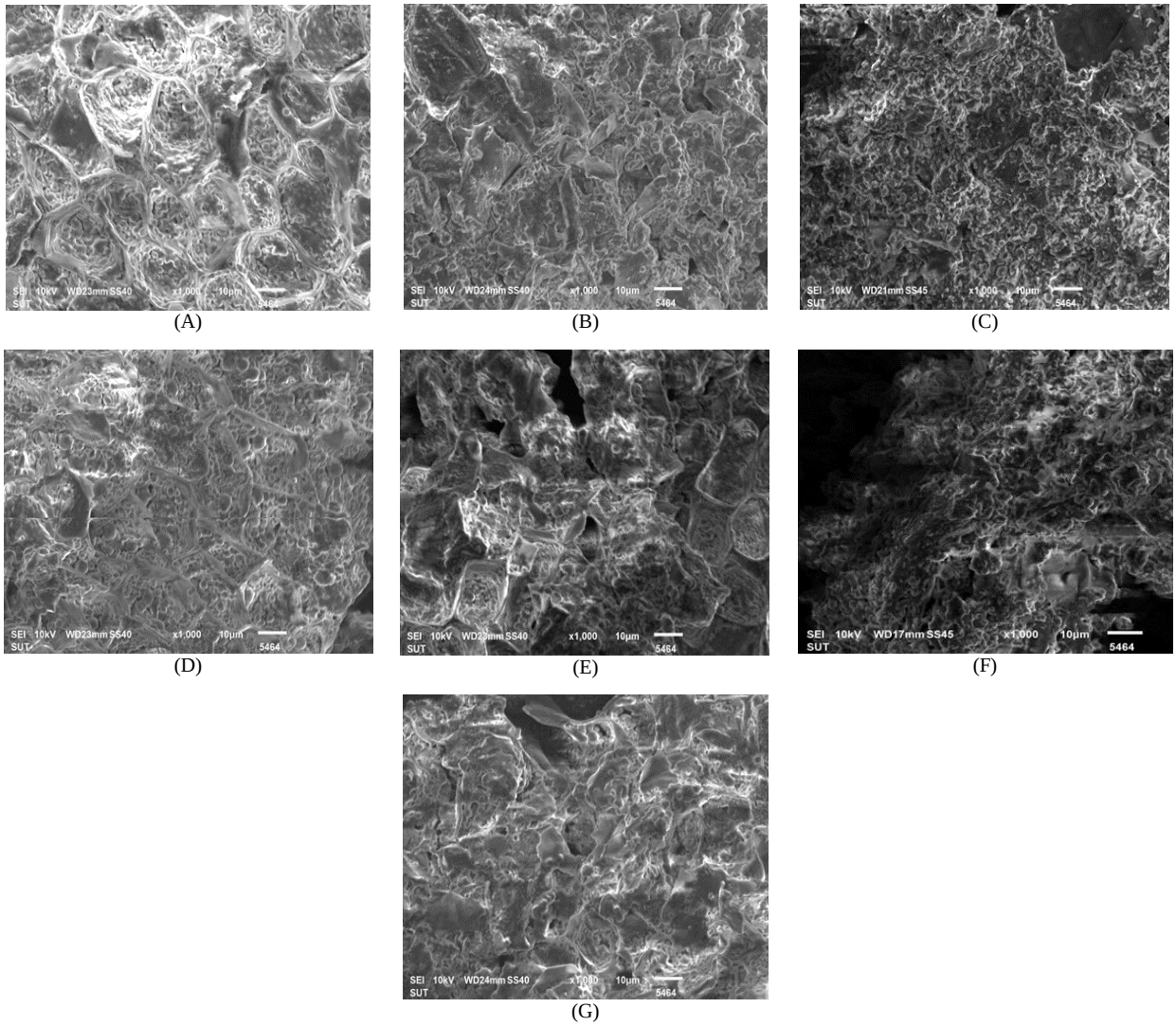

Figure 2. SEM images (×1,000) of: (A) native rice bran, (B) 0:100% (v/v) Eth/He, (C) 20:80% (v/v) Eth/He, (D) 40:60% (v/v) Eth/He, (E) 60:40% (v/v) Eth/He, (F) 80:20% (v/v) Eth/He, (G) 100:0% (v/v) Eth/He

represent the effect of different solvent extractions on rice bran tissues using the same ultrasound assisted extraction condition. Figure 2A shows the native rice bran has a regular and smooth surface. In Figure 2B-G, the micro-fractures and cracks had on the rice bran surface, especially with the Eth concentration in range of 60-80%v/v in the mixture (Figure E

and F). The ultrasound assisted solvent extraction expanded the cellular structures, and then the solvent penetrated and partially dragged the lipids, phenolic and flavonoid compounds, and gamma-oryzanol into the solvent extraction. Phenolic, flavonoid and gamma-oryzanol may contribute directly to anti-oxidative properties of rice bran oil.

3.4. Antioxidant capacity of rice bran oil

The scavenging activity of $DPPH^{\bullet+}$ and $ABTS^{\bullet+}$ has been widely used to determine the free radical-scavenging activity. $DPPH^{\bullet+}$ and $ABTS^{\bullet+}$ are stable radicals. They are dissolved in Eth and their colors are changed at the absorption at 517 and 765 nm, respectively. From the ANOVA results, the antioxidant activities of rice bran oil were significantly affected ($p<0.05$) by the types of solvent. The maximum DPPH and ABTS values of the rice bran oil were obtained by using the 40-60%v/v ethanol concentration. The lowest scavenging activities (DPPH and ABTS) were observed in the He extracted oil with 13.77 mg TEAC/mL oil and 5.38 mg TEAC/mL oil. The results were consistent with the data found by Daud *et al.* (2018), who reported that the different extraction solvents results in different levels of scavenging activities. Thus, this result indicates that rice bran oil is an electron donor and can terminate radical chain reactions.

3.5. Gas chromatography analysis for fatty acid composition

Table 4 tabulates fatty acid composition of the extracted oil by different extraction solvents. Regardless of the extraction solvents, oleic (41.12–42.18%), palmitic (15.51–17.94%), and linoleic (27.67–29.67%) were the major fatty acids of crude rice bran oil. The unsaturated fatty acids of the oil extracted by the mixed solvent with the Eth concentration from 60 to 100 %v/v (72.71-76.09%) were slightly higher than the 20-40 %v/v Eth (71.72-73.49%) and He (71.72%). This variation could be attributed to the selectivity of specific extracting solvents for certain fatty acids (Carvalho, Barros, Conceição, & Sousa, 2012; Tan, Gun Hean, Hamzah, & Ghazali, 2017). As reported by Péres *et al.* (2006), the interaction between unsaturated fatty acids with a polar solvent (e.g., ethanol and methanol) is stronger than a nonpolar solvent (e.g., hexane). From the results, the abundance of unsaturated fatty acids in crude rice bran oil is considered as nutritional and health oil. According to Law (2000), rice bran oil rich in unsaturated fatty acids has been reported to reduce the risk of heart attack associated with cholesterol. Thus, rice bran oil has potential applications in food, pharmaceuticals, and drug productions.

4. Conclusions

This study illustrated the possibility and advantages of replacing He with Eth. As shown in this work, the mixture of Eth and He proved to be efficient solvent for oil extraction and nutraceutical compounds from rice bran. The results showed that the highest extraction yield was achieved with the Eth concentration in a range of 40-80 (%v/v). Specifically, the mixture of Eth/He with 60% Eth improved not only oil extractability but also oil quality (i.e., lower oxidative stability, and higher tocopherols, gamma-oryzanol, and unsaturated fatty acids). The Eth/He mixture with 60% Eth also induced structural changes and fissures in the rice bran, enhancing the oil extractability and antioxidant compounds. Besides, Eth is less flammable than hexane. It also exhibits a lower toxicity and is commonly available. Thus, a 60%v/v Eth concentration in the solvent mixture was adopted to optimize the ultrasound assisted solvent extraction for extracting rice bran oil. However, the acid value of the crude rice bran oil was still higher than that of the CODEX standard for edible oil. Thus, the refining of rice bran oil is necessary to reduce the acid values to meet the CODEX standard.

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Table 4. Fatty acid composition (%) of crude rice bran oils extracted by different extraction solvents.

Eth/He ratio (%v/v)	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	α-linolenic acid
	(C14:0)	(C16:0)	(C18:0)	(C18:1)	(C18:2)	(C18:3)
0:100	5.78^{a}±0.04	17.94^{a}±0.98	3.57^{b}±0.35	41.49^{b}±2.19	27.67^{b}±1.03	3.55^{b}±0.10
20:80	5.78^{a}±0.04	17.94^{a}±0.98	3.57^{b}±0.35	41.46^{b}±2.19	27.70^{b}±1.03	3.55^{b}±0.10
40:60	5.98^{a}±0.04	16.63^{b}±1.49	3.90^{a}±0.91	41.12^{b}±1.01	28.35^{ab}±0.74	4.02^{ab}±0.12
60:40	4.40^{c}±0.09	15.51^{d}±1.21	3.60^{b}±0.10	42.18^{a}±2.09	29.67^{a}±1.35	4.64^{a}±0.21
80:20	4.79^{b}±0.18	15.77^{c}±1.35	3.40^{c}±0.10	42.04^{a}±1.81	28.09^{a}±0.94	4.11^{ab}±0.17
100:0	4.78^{b}±0.19	15.70^{c}±1.89	3.53^{bc}±0.17	42.00^{a}±1.67	29.05^{a}±1.36	4.04^{ab}±0.53

Different letters in each column denote statistically significant differences between treatments ($p<0.05$). The values are mean of three replications±SD.

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