

RESEARCH ARTICLE

Effects of different stabilization conditions and extraction methods (Soxhlet and ultrasonic-assisted) on quality of rice bran oil

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ABSTRACT

High rice production produces a high amount of waste, especially rice bran (~10%), suitable as cooking oil. This study was performed to investigate the rice bran dried at different temperatures and times for stabilization treatment and the oil extracted using Soxhlet and Ultrasonic-assisted extraction (UAE). The rice bran produced the highest oil yield (31.9%) and β -carotene (7.82%) than control after stabilized at 50 °C for 1 h. Unstabilized rice bran (control) and stabilized rice bran (50 °C for 1 h) were used to compare the changes in viscosity, oxidative stability and fatty acids composition of the extracted oil using Soxhlet and UAE. The rice bran oil's viscosity obtained by stabilization and different extraction methods were decreased compared to the non-stabilized treatment. Meanwhile, stabilized rice bran oil extracted by the Soxhlet method produced higher oxidative stability than the control for both extraction methods due to the low amount of unsaturated fatty acids. Therefore, the Soxhlet extraction method had more stable rice bran oil than the UAE process, and selected parameters (50 °C for 1 h) for stabilization treatment are the most suitable for reducing rice bran oil oxidation.

Keywords: Rice bran oil; Stabilization; Soxhlet; Ultrasonic-assisted extraction; Oxidative stability

INTRODUCTION

Rice (*Oryza sativa*) is the number one food crop globally and always be a control item for trade and distribution to maintain the food supply. According to BERNAS, the local white rice is mainly produced from local rice varieties like MR211, MR219, MR220 and MR232. Paddy provides approximately 73.5% of white rice, 3.5% of broken rice, 15% of husk and 8% rice bran during milling (Pandey and Shrivastava, 2018). Rice bran can be obtained at the external layer of rice grain and known as a by-product removed from starchy endosperm during the milling process. It is widely used in industries as food ingredients, oil sources and animal feedstock. Previous research has proven that rice bran extraction using Soxhlet produces 18.9% of oil (Oliveira et al., 2011). Rice bran oil is a healthy edible oil that contains many nutrients, including protein, vitamin B complex, vitamin E (tocopherol and tocotrienol), vitamin K and γ -oryzanol. It also reduces human cholesterol level,

anti-oxidation, anti-carcinogenic and anti-allergic (Khoei and Chekin, 2016).

Rice bran is separated during milling; thus, it may contribute to hydrolysis and oxidation reaction since it is rich in lipids. The free fatty acids were formed by hydrolysis of lipid from rice bran, leading to a hydrolytic rancidity process due to the unstable rice bran (Amarasinghe et al., 2009). Hence, the drying process has been chosen as a stabilization treatment to inactivate the lipolytic enzymes and protect rice bran from hydrolysis and oxidation reaction. The drying process is selected due to its simple technique, inexpensive equipment, non-chemical and others. Various stabilization methods such as dry or moist heating, microwave heating, ohmic heating, extrusion, gamma irradiation, refrigeration and acidification have been conducted (Kim et al., 2014). Although numerous studies have been conducted on the various stabilization methods, limited research has been reported on the locally produced rice bran from Malaysia.

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Various methods are developed to extract rice bran oil with higher yield and superior quality. Previous studies show that methods applied include supercritical fluid extraction, microwave-assisted extraction, cold press method, aqueous extraction and others (Khoei and Chekin, 2016). All methods are suitable to extract oil from their origin with advantages as well as limitations. The traditional method generally used by researchers was conventional Soxhlet extraction. Soxhlet extraction is an inexpensive and straightforward method with repeatability properties in the distillation process (Sukri, 2012). The method was carried out using organic solvents such as hexane, petroleum ether and others under reflux. Even though hexane has excellent stability, lower corrosiveness, lower residue in oil content and better sensory properties, hexane was not chosen as solvent due to its hazardous and toxic nature that lead to environmental pollution (Javed et al., 2014). Thus, petroleum ether was selected as a solvent in Soxhlet extraction due to its ability to produce higher oil yield than other solvents.

Ultrasonic-assisted extraction (UAE) is an alternative method to conventional extraction techniques (Khoei and Chekin, 2016). Many researchers mentioned that extraction time and temperature influence the oil yield to enhance the internal diffusivity by solvent penetration (Sukri, 2012; Selvamuthukumar and Shi, 2017; Khoei and Chekin, 2016). In a preliminary study, the best extraction condition with higher oil yield production was found at 30 °C for 50 min. Ethanol is an environmentally friendly solvent and has higher polarity making it suitable as a solvent for UAE. Apart from that, the high boiling point of ethanol also affects the process of extraction, which may promote high oil production (Toda et al., 2016). The mechanical and cavitations in the UAE system may lead to the breakdown of cell wall structure, particle size reduction, and mass transfer across the cell membrane. These changes have resulted in higher oil yields for a short extraction time (Falleh et al., 2012).

Therefore, this study was conducted to select the rice bran treated with different drying temperatures (50, 80 & 105 °C) and times (1, 2 & 18 h) to produce high oil yield and β -carotene. Later, the changes in viscosity, oxidative stability and fatty acids composition of extracted oil using Soxhlet and Ultrasonic-assisted extraction (UAE) were compared.

MATERIALS AND METHODS

Materials

Ethanol (95%) v/v was used as a solvent for ultrasonic-assisted extraction (UAE) was purchased from Fisher Scientific (Selangor, Malaysia), whereas petroleum ether

used as a solvent for Soxhlet extraction was provided by Fisher Chemical (Loughborough, UK). Chemicals like acetic acid, chloroform and isooctane were purchased from Fisher Chemical (Loughborough, UK). Wij's solution, sodium lauryl sulphate, extra pure sodium chloride, sodium methoxide were obtained from ACROS Organics (USA). Potassium iodide, soluble starch, sodium thiosulphate and *p*-anisidine reagent were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulphate purchased from QReC (Asia) Sdn Bhd (Malaysia). The β -carotene standard was obtained from Sigma-Aldrich (St. Luis, USA).

Sample collection and preparation

Rice bran was collected from the rice milling factory at Bernas Tanjung Karang, Selangor. It was mixtures of different varieties such as CL2, MR219, MR220 and other paddy hybrids at more than 10% of milling degree. The fresh rice bran was stabilized using the dry oven (UNPlus, Memmert, Germany) at different temperatures (50, 80 & 105 °C) and times (1, 2 & 18 h). The stabilized rice bran powder was packed in an aluminum foil zip lock, self-sealing bags. The samples were stored in the chest freezer (HCF-N42, Hesstar, Malaysia) at temperature -20 °C before extraction. Rice bran without drying has been used as a control. The samples used in this work were selected based on the preliminary study:

- The particle size of rice bran less than 355 μm produced higher oil production than 355 μm and 425 μm .
- Stabilization process was done at different drying temperatures (50, 80 & 105 °C) and different times (1, 2 & 18 h). Drying condition to stabilize the rice bran at 50 °C for 1 h was chosen due to higher oil production and higher β -carotene than others.
- Two different types of solvents; petroleum ether and ethanol were used for Soxhlet and UAE extraction. It was found that petroleum ether produced a higher oil yield than ethanol. However, ultrasound power in the UAE system helps ethanol to produce high oil yield. Therefore, petroleum ether was selected as a solvent for Soxhlet extraction, while ethanol was selected for UAE.

Soxhlet extraction

The round bottom flask was placed into the oven for 30 min, and the dry weight was measured. About 200 mL of petroleum ether was poured into the round-bottomed flask. 5 g of test samples were put into the thimble and insert in a Soxhlet extractor. Then, setting the whole apparatus where the Soxhlet extractor connects to a 250 mL flask containing 200 mL of petroleum ether. The extraction was conducted at the boiling temperature of petroleum ether for 8 h. After extraction, the mixed sample with solvent

was in the rotary evaporator to remove the petroleum ether solvent to determine the yield (Danlami et al., 2015).

$$\text{Percentage yield (\%)} = \frac{\text{mass of oil (g)}}{\text{mass of rice bran powder (g)}} \times 100\%$$

Ultrasonic-assisted extraction

Approximately 20 g of samples were weighed and placed into an amber Schott bottle that contains 200 mL of 95% ethanol. The extraction was performed by using an ultrasonic cleaner bath (DC150H, Delta, Taiwan; dimension: 300 × 160 × 150 mm; frequency: 40 kHz and ultrasonic power of 150 W). The extraction was operated at a temperature of 30 °C for 50 min. Cold water was added during the extraction to control the temperature within ± 0.5 °C by the calibrated thermometer. After extraction, the mixed sample with solvent was filtered using filter paper (Whatman No. 1). The filtered solution (oil + solvents) were collected in a 250 mL conical flask. Then, the solution was placed in the rotary evaporator to remove the ethanol solvent to determine the oil yield (Krishnan et al., 2015).

$$\text{Oil recovery (\%)} = \frac{\text{maximum oil yield extracted}}{\text{total bran oil content}} \times 100$$

Determination of β-carotene

Sample from different drying temperatures was used to determine the β-carotene content in oil extracted using Soxhlet and UAE. The β-carotene was identified by UV-extraction using the direct spectrophotometer method following the procedures by Munasinghe and Wansapala (2015).

Determination of rheology

Rheology was studied to measure the flow of oil materials as a function of shear or load rate, time and spatial orientation. Viscosity is the measure of a fluid's resistance to flow. The rheological measurement was carried out using RheoStress following the procedures by Hasan et al. (2010).

Oxidative stability test

The oxidative stability test was done on the selected oil samples extracted using Soxhlet and UAE, which produced high oil yield and β-carotene. The analysis has been carried out as follows:

Iodine value

Iodine value was usually used to identify the degree of unsaturation in oil (Mingyai et al., 2017). The iodine value was determined according to AOCS official methods Cd1c-85 (AOCS, 2017).

$$\text{Iodine value (g I}_2\text{ / 100 g oil)} = \frac{(B - T) \times M \times 12.69}{W}$$

where B = blank titrate of 0.25 M sodium thiosulphate; T = sample titrate of 0.25 M sodium thiosulphate; M = molarity of sodium thiosulphate; W = weight in grams of the sample of oil.

Peroxide value

The peroxide value of rice bran oil was determined using AOCS Cd 8-53 (AOCS, 1998).

$$\text{Peroxide value (meq O}_2\text{ / kg oil)} = \frac{(S - B) \times N \times 1000}{\text{mass of sample}}$$

where S = sample titration volume; B = blank titration volume; N = normality of sodium thiosulphate solution.

Anisidine value

Anisidine value (AV) refers to secondary oxidation of the oil. The AV of rice bran oil was determined using AOCS official method Cd 18-90 (AOCS, 2017).

$$\text{Anisidine value} = \frac{df(1.2A_s - A_b)}{m}$$

where df is the dilution factor of the vegetable oil tested (mL solution/mL vegetable oil), 1.2 is the dilution factor of the oil solution used in the post-reaction (mL total solution/ mL vegetable oil) A_s = absorbance of the reacted solution; A_b = absorbance of the fat solution; m = mass, in g of test portion.

Total oxidation value (TOTOX Value)

Total oxidation value was the sum of primary oxidation and secondary oxidation of the oil. The TOTOX value was calculated using the equation as below:

$$\text{TOTOX} = (2 \times \text{PV}) + \text{AV}$$

Determination of free fatty acid

Rice bran oils obtained by different extraction methods were collected to determine the free fatty acid (FFA) content. The standard titration method was used to determine the FFA of rice bran oil. The rice bran oil (1 g) was dissolved in 100 mL of ethanol and diethyl ether mixtures (1:1) and titrated 0.01 N of potassium hydroxide and ethanol solution. The FFA of rice bran oil was measured as oleic acid equivalent, stated as a total lipids percentage (Kim et al., 2014).

Determination of fatty acid composition

The fatty acid composition of rice bran oils was identified using gas chromatography (GC6890, Hewlett

Packard, California) following the method proposed by Krishnan et al. (2015) with slight modification. Before injection, fatty acid methyl ester (FAME) was prepared by the sodium methoxide method and stored at -20 °C before analysis. The GC operating conditions were as follow: Carrier gas (He) flow rate at 1 mL min⁻¹; GC system (GC 6890, Hewlett Packard, California) equipped with 30 mm x 0.25 µm x 0.25 µm of high performance crosslinked 5% phenylmethyl siloxane capillary column (Hewlett Packard, HP-5); the split ratio at 10:1; the initial oven and detector temperature at 100 °C for 2 min, then increased to 230 °C at the rate of 5 °C/min in ramp 1, finally increased without holding time to 260 °C at the rate of 5 °C/min in ramp 2. About 1 µL of the solution was manually injected into the front inlet, and it took 35 min for the system to run after injection. The chromatogram was loaded into Chemstation software and was revealed on PC. About 1 µL of standard FAME was injected under the same GC operating condition set. The peak identities were determined by comparing the retention time of the sample with the standard FAME.

$$\text{Area percentage of each FAME (\%)} = \frac{\text{peak area of a particular FAME}}{\text{Total area of FAME}} \times 100$$

Statistical analysis

Each experiment was performed in triplicate. All the data were analyzed using one-way and two-way ANOVA in Minitab software. All the value was expressed as a mean standard deviation to identify the significant differences between the samples at a confidence interval of 95%.

RESULTS AND DISCUSSION

Selection of stabilization temperature and time

Table 1 shows the selection of drying conditions to stabilize rice bran before oil extraction. There were three different temperatures (50, 80 and 105 °C) and times (1, 2 and 18 h) to stabilize the rice bran. The stabilization conditions have been selected according to the highest oil yield produced. As shown in Table 1, there was a significant difference ($p < 0.05$) between all stabilization conditions. The stabilized rice bran at 50 °C for 1 h showed the highest oil yield for both extraction methods (Fig 1). Rice bran also

contains cell wall polysaccharides that easily breakage when the heat is released to release the oil. However, enzymes in the cell wall can be denatured at high temperatures caused by low oil production (Sinica et al., 2015). Therefore, low drying temperature and short time (50 °C, 1 h) were suitable to stabilize the rice bran to produce high oil yield and selected for further analysis to compare with the control sample. Also, the non-heating of the stabilization process could help maintain the nutritional quality of rice bran oil (Bagchi et al., 2014).

β-Carotene content of rice bran oil

Analysis of β-carotene was done to determine pigment content in the rice bran oil. Rice bran oil with higher β-carotene content was selected for further study. As shown in Table 2, β-carotene for oil of unstabilized (control) and stabilized rice bran has a significant difference ($p < 0.05$) for both extraction methods due to the effect of different heat treatments. Temperature affects the stability of β-carotene in which higher heat treatment may reduce the β-carotene content (Munasinghe and Wansapala, 2015). In addition, the exposure of rice bran to heat, light, enzymes, or catalysis by metals can cause the conversion of energy which contributes to oxidation (Selvamuthukumar and Shi, 2017). Unsaturated fatty acids can accelerate rapid photooxidative rancidity upon exposure to artificial light or daylight (Selvamuthukumar and Shi, 2017). Therefore, the stabilization temperature cannot be too high because it can cause oxidative rancidity to the rice bran.

Based on Table 2, it shows that oil extracted using Soxhlet gives higher β-carotene compared to UAE. The UAE system's cavitation effect produces a high amount of energy caused by compression and expansion cycles of

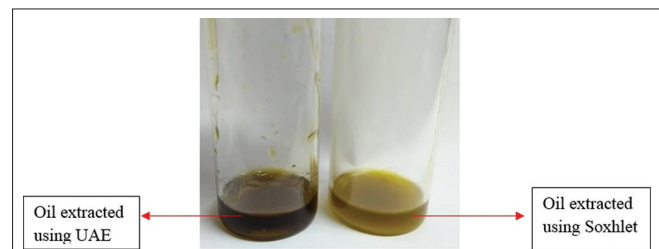


Fig 1. Rice bran oil extracted using Soxhlet and UAE.

Table 1: Different stabilization conditions on the yield of rice bran oil extracted using Soxhlet and UAE

Stabilization temperature (°C)	Oil yield (%)					
	Soxhlet (h)			UAE (h)		
	1	2	18	1	2	18
50	31.90 ± 0.58 ^{Aa}	26.08 ± 0.62 ^{Ab}	23.42 ± 0.57 ^{Ac}	20.52 ± 0.42 ^{Aa}	14.48 ± 0.26 ^{Ac}	18.69 ± 0.48 ^{Ab}
80	24.87 ± 0.97 ^{Ba}	22.20 ± 0.32 ^{Bb}	20.91 ± 0.29 ^{Bc}	18.71 ± 0.57 ^{Ba}	15.82 ± 0.88 ^{Bc}	16.15 ± 0.58 ^{Bb}
105	25.10 ± 0.88 ^{Ca}	23.97 ± 0.33 ^{Cb}	20.18 ± 0.95 ^{Cc}	15.87 ± 0.79 ^{Ca}	16.19 ± 0.26 ^{Cc}	15.26 ± 0.21 ^{Cb}

Values with different letters in the same row (a-c) are significantly different at $p < 0.05$ for each extraction method

Values with different capital letters in the column (A-C) are significantly different at $p < 0.05$ for each extraction method.

ultrasonic waves (Selvamuthukumaran and Shi, 2017). The cavitations may create bubbles where bubbles are heated where conversion of energy occurs in the UAE system. Therefore, it might cause a reduction in β -carotene content due to the presence of extra heat. In UAE, the control sample that did not undergo stabilization has higher β -carotene ($3.33 \pm 0.27 \mu\text{g}/100\text{g}$) than samples that undergo stabilization due to the non-exposable heat before extraction.

Viscosity of rice bran oil

Viscosity is the resistance of a liquid to flow and analyzed based on the relationship between shear stress and shear rate. Based on Table 3, the viscosity of oil of unstabilized rice bran was higher than oil of stabilized rice bran for both extraction methods. Oil extracted using UAE was significantly different ($p < 0.05$) for stabilized rice bran which is less viscous than oil of unstabilized rice bran. The presence of heat could help destroy rice bran walls (Phongthai et al., 2017). The UAE system's vibration provides the energy that can ruin the interactions of intermolecular bonding between the compounds cause low viscosity of the oil. It can be concluded that the interaction of compounds in rice bran can be destroyed in the presence of heat and agitation, thus lower its viscosity (Makarev et al., 2016).

Oxidative stability of rice bran oil

Oxidative stability of rice bran oil obtained by different extraction methods were examined through the determination of free fatty acid (FFA), iodine value (IV), peroxide value (PV), anisidine value (AV) and Totox value as tabulated in Table 4.

In general, the FFA, IV, PV, AV, and Totox value of rice bran oil obtained by conducting UAE was significantly

Table 2: β -Carotene content of control and stabilized rice bran oil at different stabilization temperature

Stabilization temperature ($^{\circ}\text{C}$)	β -carotene ($\mu\text{g}/100\text{g}$)	
	Soxhlet	UAE
50	7.82 ± 0.40^a	2.51 ± 0.00^a
80	4.18 ± 0.22^b	1.09 ± 0.13^c
105	3.39 ± 0.45^b	1.51 ± 0.09^b

Values within a column with the same letter signify no significant difference ($p > 0.05$) as indicated by Tukey's test.

Table 3: The viscosity of rice bran oil extracted using different extraction methods from selected stabilization condition

Method of extractions	Stabilization temperature ($^{\circ}\text{C}$)	Viscosity (Pa.s)	k (Pa.s n)	n	R^2
Soxhlet	Control	0.0741	2.8988	0.2039	0.9851
	50	0.0723	0.5702	0.5515	0.9641
UAE	Control	0.0655	6.5548	0.2003	0.9505
	50	0.0261	0.1497	0.6204	0.9815

K is consistency index; n is flow behaviour; R^2 is regression coefficient.

($p < 0.05$) higher than Soxhlet. The IV of rice bran oil extracted using different extraction methods showed a significant difference at $p < 0.05$. The IV indicates the degree of unsaturated fatty acids in the oil. The range of IV in crude and refined rice bran oil should be within 85-105 g $\text{I}_2/100\text{g}$ of oil (Ramachandran, 2001; Oluremi et al., 2013); thus, rice bran oil extracted from both methods fell within those ranges. Moreover, IV of stabilized rice bran oil at 50 $^{\circ}\text{C}$ was lower than those of unstabilized rice bran (control). It indicates that heat destroyed double bonds, decreasing the unsaturated fatty acids (Ngassapa et al., 2012). Unstabilized rice bran oil was prone to oxidation compared to oil stabilized rice bran oil due to the high amounts of unsaturated fatty acids (Sadoudi and Ali, 2012).

Peroxides are the major primary reaction products of lipid oxidation. The result shows that the PV of unstabilized rice bran oil was significantly ($p < 0.05$) higher than stabilized rice bran oil for both extraction methods. Higher PV means that oil contains high amounts of peroxides molecules produced during lipid oxidation (Ishak et al., 2020). The peroxide molecules are unstable and can be further decomposed into carbonyls and other oxidation products when the high temperature was applied. Every oil production by the government and industries should have a PV of lower than 2 meq O_2/kg oil (Laillou et al., 2012). Based on this study, the high PV produced in rice bran oil from both extraction methods was caused by increased peroxides due to the sample being stored before analysis (Laillou et al., 2012). Thus, the PV is not generally used to measure rice bran oil deterioration.

Anisidine value measures the secondary oxidation products such as aldehydes. Based on Table 4, the oil of stabilized rice bran has significantly ($p < 0.05$) lower AV than the unstabilized rice bran oil for both extraction methods. It indicates that the thermal heat process is the most common technique to stabilize the rice bran oil during storage (Lavanya et al., 2019). Moreover, PV and AV can be calculated to identify the total oxidation (Totox value) in fats and oils. The increase of Totox value in unstabilized rice bran oil for both extraction methods indicates a high amount of polyunsaturated fatty acids.

Other than that, free fatty acid (FFA) has been determined to measure the free fatty acids formed during the decomposition of oil. Based on Table 4, FFA content in unstabilized rice bran oil was significantly ($p < 0.05$) higher than stabilized rice bran oil. An increase in FFA content was believed due to enzymatic lipase activity in the rice bran. The stabilization process is essential before extraction to inactivate the lipase enzymes and reduced the FFA content in oil extracted (Amarasinghe et al., 2008).

Table 4: Oxidative stability of rice bran oils from different extraction methods and from selected stabilization condition

Oxidative Stability Test	Soxhlet		UAE	
	Control	50 °C	Control	50 °C
Iodine value (g I ₂ /100 g oil)	93.88 ± 0.03 ^{Ba}	91.16 ± 0.60 ^{Bb}	104.86 ± 0.07 ^{Aa}	99.22 ± 0.34 ^{Ab}
Peroxide value (meq O ₂ /kg oil)	11.79 ± 0.25 ^{Ba}	10.39 ± 0.75 ^{Bb}	15.41 ± 0.81 ^{Aa}	13.34 ± 0.66 ^{Ab}
Anisidine value	4.13 ± 0.08 ^{Ba}	3.14 ± 0.09 ^{Bb}	6.47 ± 0.55 ^{Aa}	4.56 ± 0.23 ^{Ab}
Free fatty acid (%)	7.84 ± 0.37 ^{Ba}	7.00 ± 0.01 ^{Bb}	8.43 ± 0.02 ^{Aa}	7.72 ± 0.18 ^{Ab}
*Totox value	27.71 ± 0.00	23.92 ± 0.00	37.29 ± 0.00	31.24 ± 0.00

* Totox value (Total oxidation value) = 2PV+AV

Values with different letters in the same row are significantly different at $p < 0.05$ for each extraction method.

Table 5: Fatty acid composition of oils of unstabilized and stabilized rice bran extracted using Soxhlet and UAE

Fatty Acid (%)	Soxhlet		UAE	
	Control	50 °C	Control	50 °C
Lauric (C12:0)	1.66 ± 0.05 ^a	0.71 ± 0.08 ^a	0.41 ± 0.49 ^a	0.40 ± 0.41 ^a
Myristic (C14:0)	2.26 ± 0.59 ^a	0.93 ± 0.19 ^a	0.99 ± 0.53 ^a	0.80 ± 0.26 ^a
Palmitic (C16:0)	13.85 ± 0.40 ^a	11.62 ± 0.59 ^b	8.25 ± 0.33 ^c	7.62 ± 0.35 ^c
Stearic (C18:0)	2.13 ± 0.61 ^a	1.61 ± 0.10 ^a	1.33 ± 0.00 ^a	1.15 ± 0.00 ^a
Oleic (C18:1)	44.41 ± 0.87 ^a	42.53 ± 0.51 ^{ab}	46.88 ± 0.31 ^{bc}	46.43 ± 0.35 ^c
Linoleic (C18:2)	38.71 ± 0.51 ^a	36.11 ± 0.28 ^a	41.31 ± 0.69 ^{ab}	41.13 ± 0.94 ^b
Alpha-linolenic (C18:3)	1.56 ± 0.02 ^a	1.45 ± 0.04 ^a	1.66 ± 0.02 ^{ab}	1.64 ± 0.06 ^b
Saturated	19.90 ± 1.65	14.87 ± 0.96	10.98 ± 1.35	9.97 ± 1.02
Monounsaturated	44.41 ± 0.87	42.53 ± 0.51	46.88 ± 0.31	46.43 ± 0.35
Polyunsaturated	40.27 ± 0.53	37.56 ± 0.32	42.97 ± 0.71	42.77 ± 1.00

Values within a row with the same letter signify no significant difference ($p > 0.05$) as indicated by Tukey's test for each extraction method.

FFA content can be reduced once it is saponified with a base during extraction (Amarasinghe et al., 2008). Thus, the oil extracted was free or low in FFA.

Fatty acid compositions of rice bran oil

Gas-chromatography coupled with mass spectrometry was conducted to identify and measure the composition of fatty acids present in rice bran oil extracted using Soxhlet and UAE. Seven compounds exist in rice bran oil, including saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). Table 5 shows that the stabilized rice bran oil has a significantly ($p < 0.05$) lower percentage of fatty acid compounds than oil of unstabilized rice bran for both extraction methods. Lipase enzyme gives faster reaction with rice bran lipids after the bran layers are removed from the endosperm (Zúñiga-Díaz et al., 2017). Thus, proper stabilization is required straight after the milling process to allow an appreciable amount of unsaturated fatty acids, limiting hydrolysis and oxidation reactions. It has shown in Table 5 that the stabilization process at 50 °C for 1 h can help in reducing lipase activity by inactivating the lipase enzyme.

Based on Table 5, Soxhlet extraction produced more stable oil than UAE due to oil extracted using Soxhlet containing a higher percentage of SFAs and a lower percentage of MUFAs and PUFAs compared to UAE. The higher MUFAs and PUFAs values might be prone to

oxidation due to the availability of double bond to reaction compared to saturated fatty acids, which are more stable to oxidation (Selani et al., 2016). Essential fatty acids such as linoleic acid and linolenic acid were required in oil for body maintenance, physiological functions, and growth (Oluremi et al., 2013). According to Ishak et al. (2021), oil high in PUFA especially linoleic acid should be kept in room temperature by proper packaging to prevent lipid oxidation occurred during storage. Therefore, rice bran oil provides health benefits because of linoleic acid, which gives the second-highest value for both extraction methods.

CONCLUSIONS

The study consists of two different extraction methods, which were Soxhlet and Ultrasonic-Assisted extraction (UAE). Petroleum ether and ethanol were employed as solvents in both methods. The rice bran is readily oxidized by lipase and lipoxygenase after being separated in the milling process. Therefore, the stabilization process needed to inactivate the enzyme and prolong the shelf life of the oil. Drying condition at 50 °C for 1 h has proved the most suitable stabilization method to produce the highest yield and β -carotene of rice bran oil. A low temperature of the stabilization process can also maintain nutritional quality. Moreover, exposure to heat treatment during stabilization and extraction also affect the viscosity of rice bran oil. The viscosity of rice bran oil

obtained by Soxhlet was higher than in the UAE due to the stability of antioxidants in the oil. Other than that, stabilized rice bran produces a better quality of oil than unstabilized rice bran. As shown by the overall oxidation state of rice bran oil, the Soxhlet method has been proven to produce superior oil quality due to a lower amount of oxidative stability. It contains a smaller percentage of MUFAs and PUFAs composition compared to oil extracted using UAE.

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Authors' contributions

Norhayati Hussain: Supervision, Inspection, Idea Contribution, Funding acquisition, Writing.
Izzreen Ishak: Inspection, Writing, Article submission.
Baizura Aya Putri Agus: Inspection, Writing.
Nor Fatin Atikah Samah: Project administration, Inspection, Writing

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