

## Research article

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# Effects of Ultrasound Assisted Extraction on Efficiency and Quality of Rice Bran Oil

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### Abstract

#### Keywords

rice bran oil;  
fatty acids;  
soxhlet extraction;  
the pressurized liquid  
extraction;  
ultrasound assisted  
solvent extraction

Effects of ultrasound-assisted extraction (UAE) conditions viz., ultrasound power and treatment time on extraction efficiency and quality of oil from rice bran were studied. The sonication power and irradiation time varied between 6.11, 12.23, and 18.35 W/cm<sup>2</sup>; and 5, 15, and 25 min, respectively. The recovery, physicochemical and phytochemical properties of UAE oil were measured and compared with conventional soxhlet extraction (SE) and pressurized liquid extraction methods (PLE). The results showed that increasing the ultrasonic irradiation time and power led to greater oil extractability. However, the oil quality and ultrasonic intensity were inversely correlated. Specifically, higher power intensity (18.35 W/cm<sup>2</sup>) and longer irradiation time (15-25 min) impaired the unsaturated fatty acids (UFAs) and phytochemical compositions of the extracted oil. The maximum oil extractability obtained by PLE and UAE (12.23 W/cm<sup>2</sup> and 15 min) were 16.77 mg/g rice bran and 16.59 mg/g rice bran, respectively, and higher than the SE process (14.96 mg/g rice bran). No significant differences were shown among PLE and UAE (12.23 W/cm<sup>2</sup> and 15 min) oils for the physicochemical and phytochemical properties. Similar to the PLE and SE oils, oleic acid was the dominant fatty acid in the UAEs oils, followed by linolenic and palmitic acids. Scanning electron microscopy (SEM) results showed that the UAE process effectively damaged the cell walls of rice bran, resulting in improved oil yield. Thus, UAE is a promising method for extracting oil from rice bran.

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## 1. Introduction

Rice bran, a by-product of rice processing, comprises 8-11% of the grain weight and contains 15-23% lipids [1, 2]. Rice bran oil is a rich source of unsaturated fatty acids (UFAs), including oleic, linoleic, and linolenic acid. UFAs could help in the prevention of atherosclerosis and cardiovascular diseases [2]. Rice bran oil also contains high in tocopherols, tocotrienols, gamma-oryzanol, and B vitamins [3]. These bioactive compounds possess strong antioxidant capacities against free radicals [3-5]. Previous studies have proven that gamma-oryzanol, tocopherols, and tocotrienols are beneficial for decreasing plasma cholesterol [4], platelet aggregation [2], and cholesterol absorption [5]. Therefore, rice bran oil has found many applications in food, pharmaceutical, and cosmetic products [6].

The suitable extraction method is required to obtain rice bran oil with high oil extractability and quality. Conventionally, soxhlet extraction is the most popular method for rice bran oil because it is simple and easy to operate, and gives higher oil extractability [7, 8]. However, the soxhlet extraction requires a long extraction time and low capacity [7-9]. In recent years, ultrasound-assisted extraction and supercritical fluid extraction, newly invented extraction systems, have been developed for the extraction of oils from rice bran. They are high extraction efficiency, low solvent consumption, and short extraction time as well. The disadvantages of the pressurized liquid extraction done at higher temperature and pressure, resulted in the degradation of bioactive compounds [10, 11]. By contrast, in ultrasound-assisted solvent extraction, the temperature can be maintained at lower levels during sonication, thus thermal degradation could be avoided [12, 13].

Hexane, a non-polar organic solvent, is commonly used for oil extractions, but it is flammable and toxic. Meanwhile, isopropanol has higher operational safety, relatively low cost, and low toxicity than hexane [7, 14, 15]. To improve the oil extractability and its quality as well as reducing the cost and toxicity, the present study explored the possibility of extracting oil from rice bran (assisted by ultrasound) by using isopropanol as solvent extraction. In addition, the efficiency of the ultrasound-assisted extraction method was compared with the soxhlet extraction (SE) and pressurized liquid extraction (PLE) methods. The findings are expected to provide the most suitable extraction method for the rapid and efficient extraction of oils from rice bran.

## 2. Materials and Methods

### 2.1 Materials and reagents

The jasmine rice bran was provided from Van Phu Hung factory in Ba Ria Vung Tau province, Vietnam. The rice bran was dried at 70°C for 2 h until the moisture content was lower than 8%. Afterwards, it was ground in a mill and screened by a 60-mesh sieve (0.25mm) trainer. The powder samples were vacuum-packed and stored in a laboratory at 4°C until analysis [8].

Methanol (99.9%), n-hexane (99.8%), ethanol (99.8%), NaOH anhydrous pellets (98%), isopropanol (99.9%), hanus reagent, ammonium molybdate (99.5%), and ammonium metavanadate (99.5%) were obtained from Sigma-Aldrich (St. Louis, MO). Fatty acid methyl ester (Supercool 37 component FAME Mix) was purchased from Merck KGaA, Darmstadt, Germany.

## 2.2 Methods

### 2.2.1 Conventional soxhlet extraction (SE)

Conventional soxhlet extraction (SE) was performed to extract rice bran oil according to Phan *et al.* [8] with some modifications. Rice bran powder (50 g) was extracted by a Soxhlet extractor with 300 mL of isopropanol at 90°C for 7 h. The extract was centrifuged at 8,000 rpm using a centrifuge (Model TGL-16G, Shanghai, China). The solvent was then evaporated with a rotary vacuum evaporator (R-300, BÜCHI Labortechnik AG, Germany) at 55°C, and weighed. The oil extractability was calculated as the ratio between the weight of extracted oil and the weight of initial rice bran (equation 1):

$$\text{Oil extractability (\%)} = \text{Weight of extracted oil} \times 100 / \text{initial weight of sample} \quad (1)$$

### 2.2.2 Pressurized liquid extraction (PLE)

All samples were extracted using a pressurized liquid extraction system (FMS Inc., Watertown, MA, USA). Samples (1.5 g rice bran) were loaded into the extraction stainless steel vessel (11 cc vessel) to form a bed of solids. The system was maintained at 30°C for 15 min for stabilization prior to the extraction. The isopropanol was pumped at the constant flow rate of 0.2 mL/min. The extraction condition was set up with the constant extraction temperature and pressure (80°C and 10,000 kPa, respectively). The extraction program was set up as: the heating rate of 5 min, extraction (static) time of 25 min, and the total solvent volume of 20 mL based on the preliminary investigation showing a suitable extraction condition.

### 2.2.3 Ultrasound-assisted solvent extraction (UAE)

The influences of ultrasonic power intensity and irradiation time on the oil extractability and quality were studied. In order to perform ultrasound-assisted extraction, 50 g rice bran powder was mixed with 300 mL isopropanol in a 500 mL beaker. The UAE experiments were conducted using a 40 kHz and 750 W ultrasonic processor (VCX750; Sonic & Materials, Newtown, USA). The power intensity of ultrasound and time were varied from 6.11 to 18.35 W/cm<sup>2</sup> and 5 to 25 min with pulse durations of 5 s on/10 s off, and the temperature was maintained at 25±2°C using a circulating cooling bath. After sonication, the crude rice bran oil was collected by a rotary vacuum evaporator at 55°C. Then, the mixture was separated by centrifugation at 8,000 rpm using a centrifuge (Model TGL-16G, Shanghai, China). The extracted rice bran oil was collected, and the oil extractability was calculated using equation (1).

### 2.2.4 Determination of tocopherol content in rice bran extract

The tocopherol content ( $\alpha$ -,  $\beta$ -,  $\gamma$ -) of rice bran oils was evaluated by reversed-phased HPLC (Agilent 1200 series equipped with Hypersil ODS column (250×4.0 mm, 5.0  $\mu$ m, Phenomenex, USA)) using a modification of the method of Urvaka *et al.* [16]. The solvent mixture of acetonitrile and methanol was used in the mobile phase under a gradient condition. The gradient solvent was set as follows: methanol (5%v/v) and acetonitrile (95%v/v) for 5 min, methanol (100%v/v) and acetonitrile (0%v/v) for 25 min. A UV/VIS detector (DAD) with wavelengths of 290 and 330 nm was equipped for the sample detection. The overall flow rate was set at 1.0 mL/min and the injection volume was 20  $\mu$ L.

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

The fatty acid of rice bran oil was analyzed by GC-FID following the method of Phan *et al.* [8] with slight modifications. Twenty mg rice bran oil was totally dissolved in 1 mL 0.5M KOH and heated to 90°C for 15 min. The mixture was neutralized by adding 1 mL 0.1M HCl, and 5 mL BF<sub>3</sub>-methanol was then added. The solution was vigorously shaken for 5 min and heated in a water bath maintaining at 90°C for 30 min. After heating, the samples were subsequently cooled in cooling water to room temperature. The methylated oil was extracted with n-hexane solvent, and the solvent was removed by nitrogen gas. The methylated oil was analyzed by GC-FID. The temperature protocol was set up as follows: the injection temperature of 150°C, and the increase rate of oven temperature from 45°C to 250°C (at a rate of 40°C/min). The ion source temperature was maintained at 230°C. The fatty acids were identified by comparing the retention of oil components against the standards.

### 2.2.6 Scanning electron microscopy (SEM) analysis

A scanning electron microscope (S-3500N, Hitachi SEM) was used to determine the microstructure of materials. In order to investigate the effects of different UAE conditions on rice bran morphology, the samples were gold-sputtered to ensure sufficient electron refraction, and the morphological characteristics of different rice bran samples were determined.

### 2.2.7 Other analysis

The physiochemical properties of rice bran oils (peroxide value, acid value, and iodine value) were determined according to the AOCS official methods [17]. The color of crude rice bran oil was determined by Lovibond Tintometer PFXI-880L (Tintometer Ltd, USA). The results were expressed as 5×red+1×yellow Lovibond units.

The quantitative analysis of the gamma-oryzanol in rice bran oil was determined using UV-Vis-NIR Spectroscopy (Shimazu, UV-2600, Japan) according to the Joshi *et al.* method [18].

### 2.2.8 Statistical analysis

All the statistical analyses were conducted in triplicates, and the experimental results were expressed as mean±standard deviation (SD). Data were analyzed using the Stagraphic Centurion XV program (Statsoft Inc., Umeå, Sweden). The one-way analysis of variance (ANOVA) with a 95% confidence level was used to evaluate the differences between the results.

## 3. Results and Discussion

### 3.1 Effect of ultrasound assisted extraction of rice bran oil

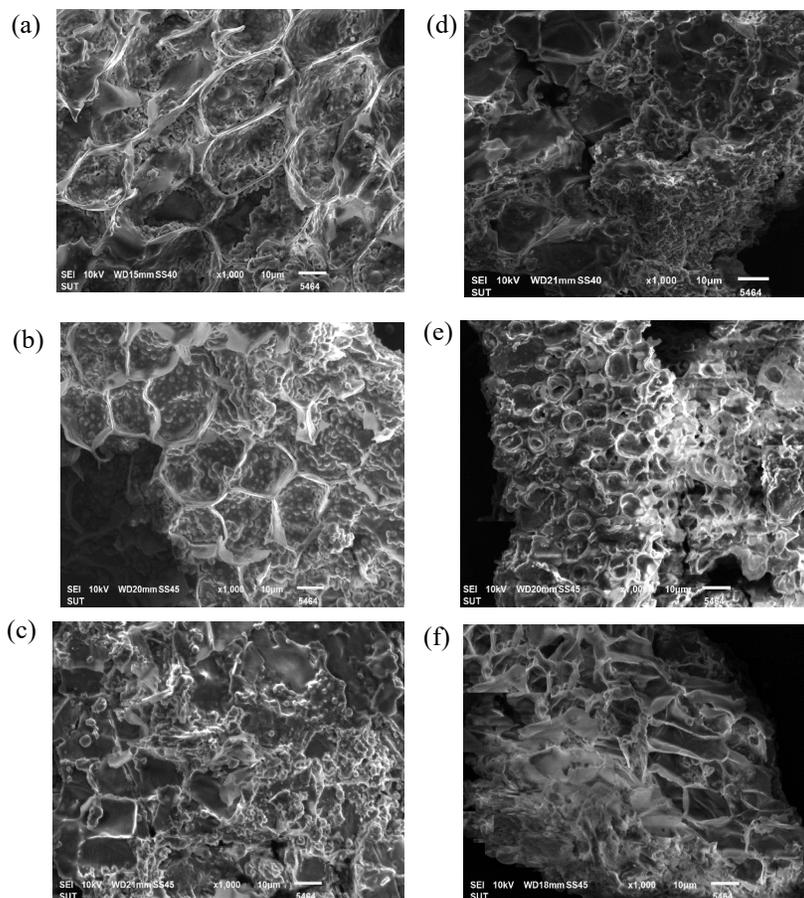
Table 1 illustrates effects of different extraction methods (SE, PLE, and UAE) on rice bran oil extractability. The PLE process achieved the highest oil extractability (16.77 g/100 rice bran), and higher than 14.96 g/100 g rice bran in the SE method. Under UAE, the oil extractability fluctuated from 14.41 to 16.59 g/100 g rice bran. The oil extractability increased first (from 14.41 to 16.59 g/100 g rice bran) and, then decreased (from 16.59 to 14.90 g/100 g rice bran) with the increase of

**Table 1.** Extraction yield and physicochemical characteristics of rice bran oil under PLE, SE and UAE methods

Extraction method	Conditions	Oil extractability (g oil/g rice bran)	Peroxide value (meqO <sub>2</sub> /kg oil)	Acid value (mgKOH/g oil)	Iodine value (g I <sub>2</sub> /100g oil)
PLE	-	16.77 <sup>a</sup> ±0.54	0.95 <sup>a</sup> ±0.02	3.49 <sup>a</sup> ±0.14	110.31 <sup>a</sup> ±2.15
SE	-	14.96 <sup>c</sup> ±0.53	1.09 <sup>ab</sup> ±0.03	3.99 <sup>c</sup> ±0.15	99.34 <sup>c</sup> ±2.25
	6.11W/cm <sup>2</sup> ,5min	14.41 <sup>d</sup> ±0.50	0.96 <sup>a</sup> ±0.03	3.56 <sup>a</sup> ±0.13	110.21 <sup>a</sup> ±2.15
	12.23W/cm <sup>2</sup> ,5 min	14.99 <sup>c</sup> ±0.50	0.97 <sup>a</sup> ±0.01	3.56 <sup>a</sup> ±0.15	110.25 <sup>a</sup> ±2.16
	18.35W/cm <sup>2</sup> ,5 min	15.55 <sup>b</sup> ±0.49	0.97 <sup>a</sup> ±0.02	3.58 <sup>a</sup> ±0.11	109.21 <sup>a</sup> ±2.17
	6.11W/cm <sup>2</sup> ,15min	14.95 <sup>c</sup> ±0.56	0.96 <sup>a</sup> ±0.02	3.58 <sup>a</sup> ±0.16	110.00 <sup>a</sup> ±2.18
UAE	12.23W/cm <sup>2</sup> ,15min	16.59 <sup>a</sup> ±0.47	0.98 <sup>a</sup> ±0.04	3.58 <sup>a</sup> ±0.17	109.58 <sup>a</sup> ±2.19
	18.35W/cm <sup>2</sup> ,15min	16.06 <sup>ab</sup> ±0.56	1.08 <sup>ab</sup> ±0.05	3.72 <sup>b</sup> ±0.10	107.21 <sup>b</sup> ±2.20
	6.11W/cm <sup>2</sup> ,25min	15.59 <sup>b</sup> ±0.62	0.99 <sup>a</sup> ±0.03	3.57 <sup>a</sup> ±0.14	109.45 <sup>a</sup> ±2.21
	12.23W/cm <sup>2</sup> ,25min	16.10 <sup>ab</sup> ±0.45	1.11 <sup>ab</sup> ±0.03	3.84 <sup>b</sup> ±0.17	105.43 <sup>b</sup> ±2.22
	18.35W/cm <sup>2</sup> ,25min	14.90 <sup>c</sup> ±0.48	1.25 <sup>b</sup> ±0.02	4.09 <sup>c</sup> ±0.11	105.10 <sup>b</sup> ±2.23

Means with different capital letters in the same column indicate statistically significant differences among treatments ( $p < 0.05$ ). Values are expressed as the mean±SD.

power intensity (6.11 to 18.35 W/cm<sup>2</sup>) and extended sonication time (5 to 25 min). Under the UAE process, the maximal oil extractability (16.59 g/100 g rice bran) was observed at the power intensity of 12.23 W/cm<sup>2</sup> and sonication time of 15 min. This result was higher than that of the SE process (14.96 mg/g rice bran). The efficient extraction of rice bran oil by UAE could be due to the cavitation forces, which enhances destruction of the cellular matrix, thereby improving the release of oils as observed through scanning electron microscopy (SEM). Figure 1 shows a set of SEM images of rice bran at magnification factor of 1000×: (a) native rice bran, (b) SE extracted rice bran, (c) UAE at 6.11 W/cm<sup>2</sup>, 5 min; (d) UAE at 12.23 W/cm<sup>2</sup>, 15 min, (e) UAE at 18.35 W/cm<sup>2</sup>, 25 min, and (f) PLE extracted rice bran. In Figure 1A, it can be seen that the native rice bran had smooth surface without pores and fissure, making it less ideal for rice bran oil extraction. In Figure 1B, some cracks on the SE rice bran surface due to solvent penetration, resulted in dragged lipids in the solvent extraction. Meanwhile, UAE and PLE pretreated rice bran exhibited the numerous micropores fissures and rough surface (Figure 1C-1E). These changes were more obvious for the UAE and PLE extraction than the sample obtained using the SE method. The results are consistent with Trentini *et al.* [19]; Zhang *et al.* [20], who reported that the PLE and UAE processes disrupted the cell walls of the plant, thus improving oil extractability compared with the SE process. However, the oil extractability exhibited a slight decrease when the power intensity exceeded 18.35 W/cm<sup>2</sup> and the prolonged extraction of 25 min. The decrease of oil extractability could be explained that the increase in ultrasonic power and the prolonged time caused the solvent volatile. The less solvent resulted in the decrease of the contact area of the solvent and rice bran powder, so the oil extractability was influenced. In addition, the decrease of oil extractability could also be due to the free radicals, which lead to the degradation of oils. Its finding is similar with Hashtjin and Abbasi [21]; Phan *et al.* [8], who reported that the application of high ultrasonic power and extended irradiation time reduced the oil extractability.



**Figure 1.** SEM images ( $\times 1,000$ ) of: (a) native rice bran, (b) SE extracted rice bran, (c) UAE at  $6.11 \text{ W/cm}^2$ , 5 min; (d) UAE at  $12.23 \text{ W/cm}^2$  for 15 min, (e) UAE at  $18.35 \text{ W/cm}^2$  for 25 min; (f) the pressurized liquid extraction (PLE)

### 3.2 Physicochemical properties of extracted rice bran oil

The peroxide and acid values were used to indicate the oxidation and rancidity of oils. The lower peroxide and acid values suggested lower deterioration and longer shelf life [8, 22]. As seen in Table 1, the UAE ( $6.11\text{-}12.23 \text{ W/cm}^2$ ; 5-15min) and PLE oils showed a significantly lower peroxide value ( $0.95\text{-}0.98 \text{ meqO}_2/\text{kg oil}$ ) and acid value ( $3.49\text{-}3.58 \text{ mg KOH/g}$ ) compared to SE oil ( $1.09\pm 0.03 \text{ meq O}_2/\text{kg oil}$  and  $3.99 \text{ mg KOH/g}$ ), indicating that the oil obtained with the UAE and PLE methods exhibited a better quality with less rancidity. However, both the acid and peroxide values of UAE oils significantly increased with an increase in ultrasound intensity ( $12.23\text{-}18.35 \text{ W/cm}^2$ ) and irradiation time (15-25 min). The results could be attributed to hydrolysis and oxidation reactions of unsaturated fatty acids under high ultrasound irradiation and high oxygen. The obtained results agreed with those reported by Phan *et al.* [8] and Chemat *et al.* [22], who documented that ultrasound waves release free radicals, leading to an increase in the acid value and peroxide value of the extracted oils. According to the CODEX Standard [23], for edible rice bran oils, the peroxide and

acid values should be below 10 meq O<sub>2</sub>/kg oil, and 0.5 mg KOH/g oil, respectively. However, the acid values of PLE, SE, and UAE (all conditions) were higher than that of CODEX standard for edible rice bran oil. Therefore, the refining of PLE, SE, and UAE oils is necessary to reduce the acid value to comply with the current CODEX standard.

The iodine value is often used to measure the degree of unsaturation of fatty acids in oils and fats [1, 24]. The higher the iodine values, the more unsaturated fatty acids are present in oils or fats. In Table 1, the iodine value of the extracted oils was significantly affected by different extraction methods (SE, PLE, and UAE). The iodine values of PLE oils (110.31 g I<sub>2</sub>/100 g oil) and UAE oils (105.43-110.25 g I<sub>2</sub>/100 g oil) were significantly higher than that of the SE oil (99.34 g I<sub>2</sub>/100 g oil). The reason for the higher iodine content could be the relatively low extraction temperature and short extraction time of UAE and PLE. Such extraction prevented the oxidation and decomposition of unsaturated fatty acids. In Table 1, it can be seen that an increase in ultrasonic intensity (18.35 W/cm<sup>2</sup>; 15-25 min) slightly reduced the iodine value of oils. This could be due to high power intensity and extended irradiation time induced peroxide radicals to form and accelerated degradation and oxidation of poly-unsaturated fatty acids. Similar results were also reported for rice bran oils pretreated with high ultrasonic intensity [8].

Table 2 showed the changes in Red (R), and Yellow (Y) values of the extracted oil during the extraction process. The color of the crude rice bran oil extracted by PLE and SE increased in both red and yellow units. These values were comparable to the UAE oils obtained from high power intensity (12.23-18.35 W/cm<sup>2</sup>) and extended sonication time (15-25 min). Meanwhile, low ultrasonic intensity significantly lowered the color of the oil. The results could be attributed to the fact that the extraction at elevated ultrasonic power as well as a longer extraction period facilitated the mass transfer of impurities from the sample matrix to the extracted oil. Samaram *et al.* [24] reported that the longer irradiation time at high temperatures accelerated the degradation of chlorophylls and phospholipids, causing oil to become dark-colored. Our result is consistent with Phan *et al.* [8], who reported that the oils obtained by high ultrasonic intensity had a significantly dark color than low ultrasonic intensity. However, the total color values of this study were lower than 20, which indicated good color quality [10].

**Table 2.** The color of crude rice bran oil extracted by PLE, SE and UAE

Extraction method	Conditions	Red (R)	Yellow (Y)	5R+Y
PLE	-	1.73 <sup>b</sup> ±0.02	5.50 <sup>cd</sup> ±0.06	14.15 <sup>b</sup> ±0.04
SE	-	1.72 <sup>b</sup> ±0.01	5.50 <sup>cd</sup> ±0.05	14.10 <sup>b</sup> ±0.04
	6.11W/cm <sup>2</sup> ,5min	1.55 <sup>a</sup> ±0.02	5.02 <sup>a</sup> ±0.01	12.52 <sup>a</sup> ±0.05
	12.23W/cm <sup>2</sup> ,5 min	1.59 <sup>a</sup> ±0.02	5.20 <sup>b</sup> ±0.01	13.15 <sup>a</sup> ±0.05
	18.35W/cm <sup>2</sup> ,5 min	1.84 <sup>c</sup> ±0.03	5.22 <sup>b</sup> ±0.01	14.42 <sup>b</sup> ±0.05
UAE	6.11W/cm <sup>2</sup> ,15min	1.72 <sup>b</sup> ±0.01	5.03 <sup>a</sup> ±0.03	13.63 <sup>ab</sup> ±0.03
	12.23W/cm <sup>2</sup> ,15min	1.71 <sup>b</sup> ±0.02	5.21 <sup>b</sup> ±0.02	13.76 <sup>ab</sup> ±0.05
	18.35W/cm <sup>2</sup> ,15min	1.80 <sup>c</sup> ±0.02	6.00 <sup>d</sup> ±0.03	15.00 <sup>c</sup> ±0.03
	6.11W/cm <sup>2</sup> ,25min	1.70 <sup>b</sup> ±0.02	5.33 <sup>c</sup> ±0.04	13.83 <sup>ab</sup> ±0.04
	12.23W/cm <sup>2</sup> ,25min	1.79 <sup>bc</sup> ±0.02	5.50 <sup>cd</sup> ±0.05	14.45 <sup>b</sup> ±0.04
	18.35W/cm <sup>2</sup> ,25min	1.85 <sup>c</sup> ±0.02	6.50 <sup>d</sup> ±0.06	15.75 <sup>c</sup> ±0.05

Different letters in the same column indicate statistical differences between treatments ( $p < 0.05$ ) ( $p < 0.05$ ).

### 3.3 Fatty acid compositions of oil

Table 3 shows the fatty acid compounds of the extracted oil by PLE, SE, and UAE methods. Six fatty acids were determined including three saturated fatty acids (myristic, palmitic, stearic acids), one mono-unsaturated fatty acid (oleic acid), and two poly-unsaturated fatty acids (linoleic and  $\alpha$ -linolenic acids). As shown in Table 3, it can be seen that the oil products obtained from PLE and low UAE processes (6.11-12.23 W/cm<sup>2</sup>; 5-15 min) were rich in unsaturated fatty acids (75.70-75.77%), and the higher content of unsaturated fatty acids could cause a higher iodine value. Additionally, it was found that the unsaturated fatty acid of SE oil was slightly lower than the PLE and low UAE processes. The high extraction temperature and long extraction time of SE might induce the oxidation and decomposition of unsaturated fatty acids in the SE oil.

Our results also showed that the high UAE process (18.35 W/cm<sup>2</sup> and 25 min) significantly reduced the unsaturated fatty acid compositions of rice bran oils. Under high UAE process (18.35 W/cm<sup>2</sup> and 25 min), the UFAs of UAE oils decreased by 7.25-7.29%, compared with PLE and UAE (6.11-12.23 W/cm<sup>2</sup>; 5-15 min). Thus, the results indicated that high power intensity and extended sonication time impaired the unsaturated fatty acids. The findings are consistent with Stanisavljević *et al.* [9] and Phan *et al.* [8], who reported that high-intensity ultrasonic treatment had a strong effect on unsaturated fatty acids. Law [25] documented that mono- and poly-unsaturated fatty acids in rice bran oil could reduce the risk of heart attack associated with cholesterol. Thus, the high content of unsaturated fatty acids in the extracted rice bran oil makes it become a desirable oil for eating.

### 3.4 Phytochemical properties of extracted rice bran oil

Tocopherol and gamma-oryzanol play an important role in the quality of extracted oil. These components can convert free radicals and lipid radicals into a more stable product. In Table 4, it was found that the tocopherol and gamma-oryzanol contents fluctuated between 612.59-694.22 mg/kg and 0.90-1.05%, respectively, suggesting the effect of different SE, PLE, and UAE processes on the tocopherol and gamma-oryzanol contents.

In this study, the oils obtained by PLE had the highest total tocopherol and gamma-oryzanol contents (694.22 mg/kg oil and 1.05 mg/100g oil). For the UAE oils, the yields of tocopherol and gamma-oryzanol increased under low to moderate ultrasonic intensity (6.11-12.23 W/cm<sup>2</sup> and 5-15 min). However, both tocopherol and gamma-oryzanol contents decreased with the enhancement of the ultrasound power (from 12.23 W/g to 18.35 W/cm<sup>2</sup>) and extension of sonication time (from 15 to 25 min). This was attributed to ultrasonic-induced cavitation that influenced the tocopherol and gamma-oryzanol contents. According to Hashemi *et al.* [26], high ultrasound power and duration generated high pressure, resulting in higher temperatures which affected the content and stability of tocopherol contents of the extracted oil.

According to our results, the major isomer of tocopherol in all the PLE, SE, and UAE extracts was an  $\alpha$ -tocopherol, followed by  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols, respectively. Under the UAE process, the content of these compounds changed depending on the ultrasonic intensity. The  $\alpha$ - and  $\beta$ -tocopherol yields increased first from 536.14 to 557.03 mg/kg and 105.56 to 120.55 mg/kg, and then decreased from 557.03 to 500.13 mg/kg and 120.55 to 89.31 mg/kg, respectively. Meanwhile,  $\gamma$ - and  $\delta$ -tocopherols remained stable in a range of 11.90 to 12.19 mg/kg and 0.90 to 1.02 mg/kg as increasing the ultrasonic power intensity from 6.11 to 18.35 W/cm<sup>2</sup> and irradiation time from 5 to 25 min. The findings are in agreement with the results of Moradi *et al.* [27], who found that the application of ultrasound in the extraction of oil affected the tocopherol yields. The content of  $\alpha$ -tocopherol was highest in rice bran oil, but it was easily degraded in ultrasonic treatment. Bruscatto *et al.* [28] experimented with the degradation of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - tocopherols in the refining of rice bran oil. The experiment resulted in the degradation of these compounds depending on their chemical

**Table 3.** Fatty acid composition (%) of the rice bran oils obtained by PLE, SE, and UAE using isopropanol as a solvent extraction

Extraction method	Condition	Myristic acid	Palmitic acid	Stearic acid	Saturated fatty acids	Oleic acid	Linoleic acid	$\alpha$ -linolenic acid	Unsaturated fatty acids
		(C14:0)	(C16:0)	(C18:0)	(SFAs)	(C18:1)	(C18:2)	(C18:3)	(UFAs)
PLE	-	0.26 <sup>c</sup> ±0.05	22.46 <sup>c</sup> ±0.60	1.55 <sup>b</sup> ±0.14	24.27 <sup>c</sup> ±0.35	41.50 <sup>a</sup> ±1.00	32.69 <sup>a</sup> ±0.90	1.54 <sup>a</sup> ±0.11	75.73 <sup>a</sup> ±0.11
SE	-	0.29 <sup>c</sup> ±0.04	25.45 <sup>b</sup> ±0.58	1.94 <sup>a</sup> ±0.15	27.68 <sup>b</sup> ±0.26	41.49 <sup>a</sup> ±1.19	32.67 <sup>a</sup> ±0.93	1.56 <sup>a</sup> ±0.10	72.32 <sup>ab</sup> ±0.11
	6.11W/cm <sup>2</sup> ,5min	0.28 <sup>c</sup> ±0.04	22.44 <sup>c</sup> ±0.58	1.57 <sup>b</sup> ±0.15	24.29 <sup>c</sup> ±0.36	41.49 <sup>a</sup> ±2.01	32.67 <sup>a</sup> ±0.91	1.55 <sup>a</sup> ±0.10	75.71 <sup>a</sup> ±0.11
	12.23W/cm <sup>2</sup> ,5 min	0.29 <sup>c</sup> ±0.06	22.42 <sup>c</sup> ±0.59	1.59 <sup>b</sup> ±0.15	24.30 <sup>c</sup> ±0.29	41.47 <sup>a</sup> ±2.00	32.68 <sup>a</sup> ±0.93	1.55 <sup>a</sup> ±0.10	75.70 <sup>a</sup> ±0.11
	18.35W/cm <sup>2</sup> ,5 min	0.29 <sup>c</sup> ±0.05	24.50 <sup>bc</sup> ±0.49	1.90 <sup>a</sup> ±0.11	26.69 <sup>b</sup> ±0.34	41.40 <sup>a</sup> ±1.01	30.35 <sup>b</sup> ±1.01	1.56 <sup>a</sup> ±0.12	73.31 <sup>ab</sup> ±0.11
	6.11W/cm <sup>2</sup> ,15min	0.28 <sup>c</sup> ±0.09	22.40 <sup>c</sup> ±0.21	1.55 <sup>b</sup> ±0.10	24.23 <sup>c</sup> ±0.25	41.38 <sup>a</sup> ±2.01	32.70 <sup>a</sup> ±1.01	1.69 <sup>a</sup> ±0.16	75.77 <sup>a</sup> ±0.11
UAE	12.23W/cm <sup>2</sup> ,15min	0.29 <sup>c</sup> ±0.08	22.37 <sup>c</sup> ±0.45	1.60 <sup>b</sup> ±0.13	24.26 <sup>c</sup> ±0.35	41.35 <sup>a</sup> ±1.81	32.69 <sup>a</sup> ±0.94	1.70 <sup>a</sup> ±0.13	75.74 <sup>a</sup> ±0.11
	18.35W/cm <sup>2</sup> ,15min	0.49 <sup>b</sup> ±0.19	25.55 <sup>b</sup> ±1.89	1.92 <sup>a</sup> ±0.17	27.96 <sup>b</sup> ±0.29	40.05 <sup>ab</sup> ±1.67	30.65 <sup>b</sup> ±0.96	1.34 <sup>ab</sup> ±0.13	72.04 <sup>ab</sup> ±0.11
	6.11W/cm <sup>2</sup> ,25min	0.25 <sup>c</sup> ±0.09	24.41 <sup>bc</sup> ±0.51	1.59 <sup>b</sup> ±0.21	26.25 <sup>b</sup> ±0.35	40.50 <sup>a</sup> ±1.06	31.65 <sup>a</sup> ±0.99	1.60 <sup>a</sup> ±0.12	73.75 <sup>ab</sup> ±0.11
	12.23W/cm <sup>2</sup> ,25min	0.49 <sup>b</sup> ±0.18	26.84 <sup>a</sup> ±0.35	1.82 <sup>a</sup> ±0.10	29.02 <sup>a</sup> ±0.26	39.34 <sup>b</sup> ±1.49	30.74 <sup>b</sup> ±1.05	1.04 <sup>b</sup> ±0.21	70.98 <sup>b</sup> ±0.11
	18.35W/cm <sup>2</sup> ,25min	0.66 <sup>a</sup> ±0.19	27.70 <sup>a</sup> ±0.39	1.85 <sup>a</sup> ±0.10	29.76 <sup>a</sup> ±0.35	39.04 <sup>b</sup> ±1.81	30.09 <sup>b</sup> ±1.04	1.11 <sup>b</sup> ±0.17	70.24 <sup>b</sup> ±0.11

Different letters in the same column indicate statistically significant differences between treatments ( $p < 0.05$ ). The values are mean $\pm$ SD of duplicate runs.

**Table 4.** Gamma-oryzanol and the total tocopherol concentrations extracted by PLE, SE and UAE methods

Extraction method	Conditions	Gamma-oryzanol (mg/100 g oil)	Individual tocopherols (mg/kg)				Total tocopherol content (mg/kg)
			$\alpha$	$\beta$	$\gamma$	$\delta$	
PLE	-	1.05 <sup>a</sup> ±0.02	559.43 <sup>a</sup> ±2.05	120.49 <sup>a</sup> ±2.00	12.20±0.05	1.05±0.02	694.22 <sup>a</sup>
SE	-	1.02 <sup>a</sup> ±0.01	548.56 <sup>ab</sup> ±2.15	120.50 <sup>a</sup> ±2.01	12.19±0.05	1.05±0.02	691.30 <sup>a</sup>
	6.11W/cm <sup>2</sup> ,5min	0.99 <sup>a</sup> ±0.02	536.14 <sup>b</sup> ±1.95	105.56 <sup>c</sup> ±1.95	11.90±0.05	0.90±0.02	654.50 <sup>b</sup>
	12.23W/cm <sup>2</sup> ,5 min	1.01 <sup>a</sup> ±0.03	556.98 <sup>a</sup> ±1.95	120.19 <sup>a</sup> ±1.95	12.05±0.05	1.09±0.01	690.31 <sup>a</sup>
	18.35W/cm <sup>2</sup> ,5 min	1.02 <sup>a</sup> ±0.01	550.12 <sup>a</sup> ±2.11	114.15 <sup>b</sup> ±1.90	11.91±0.05	1.05±0.01	677.23 <sup>ab</sup>
	6.11W/cm <sup>2</sup> ,15min	1.02 <sup>a</sup> ±0.02	554.50 <sup>a</sup> ±2.02	118.58 <sup>b</sup> ±2.09	10.10±0.05	0.95±0.02	684.13 <sup>ab</sup>
UAE	12.23W/cm <sup>2</sup> ,15min	1.05 <sup>a</sup> ±0.01	557.03 <sup>a</sup> ±2.05	120.55 <sup>a</sup> ±2.01	12.09±0.05	1.05±0.03	690.72 <sup>a</sup>
	18.35W/cm <sup>2</sup> ,15min	0.94 <sup>b</sup> ±0.03	539.41 <sup>b</sup> ±2.03	100.13 <sup>c</sup> ±2.01	12.03±0.05	0.97±0.02	652.54 <sup>b</sup>
	6.11W/cm <sup>2</sup> ,25min	1.01 <sup>a</sup> ±0.02	554.15 <sup>a</sup> ±1.95	112.54 <sup>b</sup> ±1.89	12.00±0.05	0.99±0.01	679.98 <sup>ab</sup>
	12.23W/cm <sup>2</sup> ,25min	1.03 <sup>a</sup> ±0.02	554.09 <sup>a</sup> ±1.95	109.85 <sup>a</sup> ±1.95	11.99±0.05	0.99±0.02	676.92 <sup>ab</sup>
	18.35W/cm <sup>2</sup> ,25min	0.90 <sup>b</sup> ±0.01	500.13 <sup>c</sup> ±1.95	89.31 <sup>d</sup> ±2.00	12.14±0.05	1.01±0.05	612.59 <sup>c</sup>

Different letters in the same column indicate statistically significant differences between treatments ( $p < 0.05$ ). The values are mean±SD of duplicate runs.

structure. These authors also concluded that  $\alpha$ -tocopherol is destroyed more rapidly than  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols under high-temperature processes (100-140°C). In general, high ultrasonic intensity causes a decrease in the total tocopherol content.

As shown in Table 4, it can be seen that an increase in ultrasonic intensity leads to a decrease in gamma-oryzanol content. In Table 3, the UAE (6.11-12.23 W/cm<sup>2</sup>; 5-15 min) oils had the highest gamma-oryzanol content (0.99-1.05%) and an insignificant difference compared with the PLE and SE methods. After 15 min, elevated ultrasonic power intensity from 12.23 to 18.35 W/cm<sup>2</sup> significantly decreased gamma-oryzanol content. The result could be explained that during ultrasound-assisted extraction, the bubbles were generated and then collapsed. The collapse of bubbles created extremely high local temperature and pressure gradients, resulting in gamma-oryzanol degradation [8, 30]. The findings are consistent with Phan *et al.* [8], who reported that high ultrasonic power and extended irradiation time accelerated the degradation of gamma-oryzanol.

#### 4. Conclusions

In this study, the effects of different UAE extraction conditions on rice bran oil extractability and quality were investigated and compared with the SE and PLE methods. Higher ultrasonic power (18.35 W/cm<sup>2</sup>) and longer sonication time (15-25 min) impaired oil quality (i.e. peroxide and acid values, unsaturated fatty acids, tocopherol, and gamma-oryzanol). The optimal conditions for UAE were found to be ultrasonic power of 12.23 W/cm<sup>2</sup> and irradiation time of 15 min. At these conditions, the extractability of rice bran oils was improved, and insignificant differences in comparison with the PLE method. This result was higher than that of the SE process. Meanwhile, the physiochemical and phytochemical properties of oils obtained in UAE (12.23 W/cm<sup>2</sup> for 15 min) were similar to the PLE method. The SEM results provided excellent evidence of the effectiveness of UAE on rice bran morphology. Ultrasound-assisted solvent extraction induced structural changes, fissures, and cavities in the rice bran. The GC-FID results showed that oleic, linoleic, and palmitic acids were the dominant fatty acids in rice bran oil. This study demonstrated that UAE technology is the better alternative to SE and PLE processes regarding low solvent consumption and short-time extraction at a laboratory scale.

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