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# Sustainable extraction of Amber rice bran oil (*Oryza sativa* L.): a comparative study between conventional and ultrasound-assisted extraction using ethanol as solvent

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**Abstract.** Rice bran oil (RBO) is highly valued due to its unique properties. Due to environmental obstacles such as climate variation and global warming, there has been a growing interest in green extraction approaches recently, like ultrasound-assisted extraction (UAE), which offer several advantages in terms of sustainability and environmental impact such as reduced extraction period, solvent usage, and significantly increased extracted amount compared to conventional methods. This work compared oil extraction from Iraqi Amber rice bran employing ethanol as an eco-friendly solvent and two methods, traditional ethanol soaking (SEE) and ultrasound-assisted extraction (UAEE). According to the results, the oil yield by UAEE was higher than SEE, by 19.22% and 17.98%, respectively. A minor increase in the refractive index was recorded in the oil extracted using SEE, and a slight increase in the acidity and free fatty acid values in UAEE. The  $\gamma$ -Oryzanol in the oil extracted using UAEE was higher, reaching 5.88 mg/g. Fatty acid analysis showed that the oil extracted using SEE contained mostly linoleic acid, and UAEE had the highest value of palmitoleic acid. Using ethanol as a solvent, both techniques were generally successful in extracting oil from amber rice bran, with the UAEE method outperforming SEE.

## 1. Introduction

Among the important crops is rice because it contributes to meeting food demand, 50% of the global populace consumes rice [1]. According to estimates, rice production in 2023/24 is expected to reach a record 693.5 million tons [2]. The cultivated area of rice in Iraq in the year 2021/22 reached about 384,926 dunums, with a yield of 422,463 tons [3]. Amber rice is a type of local fragrant rice variety that has been grown in Iraq for centuries. It is usually characterized by medium-sized grains that are non-sticky and tender when cooked. It has a distinctive smell when cooked through which it can be distinguished from other types of rice [4]. 70% of the rice (the endosperm) is produced during the milling process, along with byproducts like bran, husk, and germ that are typically thrown away. However, due to its rich nutritional content which includes fiber, proteins, lipids, vitamins, carbs, and antioxidant molecules rice bran can be exploited as a useful secondary product [5]. Bran of rice is the tough outer layer of rice made of pericarp and aleurone, which includes a variety of vital nutrients like phytosterols, tocotrienols, tocopherols, and oryzanols. According to a compositional study, it possesses 20–25% oil,



40–50% carbs (mostly glucose), 12–15% protein, and dietary fibres such as gum, glucan, and pectin [1]. RBO extraction is a prominent application used for rice bran. The type of rice, how the grain was treated prior to milling, the method of milling, the level of grinding, and the bran's ultimate treatment all affect its chemical makeup. Up to 25% of RBO can be found in the bran. Additionally, genotype has a major impact on the quantity of oil in bran, the distinctive profile of fatty acid, content of phenolic ( $\gamma$ -oryzanol, ferulic acid), tocopherol, and tocotrienols distinguish RBO from other edible vegetable oils [6].

One of the key parts impacting the oil's quality and amount is the extraction process utilized to extract it, the commercial method of extraction encompasses the use of solvents. Hexane is the common solvent for extraction RBO due to its low boiling point and excellent solubility, despite being highly volatile, combustible, and hazardous to both humans and the environment. Recently, A lot of work has been invested into developing alternatives to hexane, with an emphasis on ecologically sustainable extraction techniques. Among the replacement solvents used are isopropanol, ethanol, ethyl acetate, and limonene these substances can all be recovered from agricultural biomass and are bio-based solvents [7]. To reduce solvent exposure and boost the capacity of oil extraction, a recently developed extraction technique known as ultrasonic-assisted solvent extraction has been used [8]. The application of ultrasound uses the phenomenon of cavitation and tiny bubbles are produced in the solvent through a sequence of vibrations, these bubbles collapse close to the cellular membrane, allowing molecules to distribute between the solvent and the cell wall, this technique works well for extracting different biomaterials from various sources with various solvents, including organic compounds, polyphenols, and antioxidants [9]. Using ultrasound, oil of rice bran was obtained by means of ethanol and hexane at extraction ratios of 13.59 and 11.42 g/100 g, respectively. When ethanol was used to extract the oil, phenols and DPPH concentrations were higher than when hexane was used [10]. Regarding extraction using ethanol and hexane by Soxhlet, the TPC of rice bran oil was 1.65 (Gallic Acid Equivalent) and 0.80 (Gallic Acid Equivalent), respectively [11].

The principal aim of this work is the extract of Iraqi Amber rice bran oil (ARBO) utilizing a sustainable solvent (ethanol). This is the first time, as far as we know, that Iraqi ARBO has been extracted with ethanol as a solvent. Two methods of extraction will be used: the conventional maceration approach and the second method is extraction with the assistance of ultrasound. The oil's chemical and physical characteristics as well as the number of antioxidants and other active ingredients it contains, will be compared between the two approaches.

## 2. Materials and methods

### 2.1. Materials

Bran of Amber Rice was supplied from Al-Rawan Rice Husking Mill in Najaf, Iraq. Absolute ethanol as a solvent from (Honeywell) Germany, Ultrasound Cleaner device (LUC-405-IIN,350W) Korea.

### 2.2. Stabilization of rice bran

Using the procedure outlined by [12], rice bran was stabilized by heated for 10 minutes at 120°C in an oven, it was then stored at -27 °C until required.

### 2.3. Amber rice bran oil (ARBO) extraction

**2.3.1. Maceration extraction by ethanol (SEE).** The extraction process was carried out according to the approach proposed by [13] with some modifications, and the ratio of rice bran and absolute ethanol was 3:1. The ethanol was heated to 40°C, then added to the rice bran and mixed well. The process of extraction took 90 minutes at a temperature not exceeding 40°C with continuous stirring by a magnetic stirrer. After the process of extraction, the rice bran was filtered and the filtrate was segregated by centrifugation at 6000 rpm for 7 min, 25 °C, after that, a rotary evaporator with a 45 °C was used to eliminate the solvent and extract the oil. Finally, the oil was properly filtered through 0.45  $\mu$ m filter paper and stored in an airtight glass jar in the refrigerator until required.

2.3.2. *Ultrasound-assisted ethanol extraction (UAEE)*. The UAEE process was carried out according to [14] with some modifications, bran to a solvent ratio (3:1), the bran was initially soaked in ethanol for 30 minutes with continuous stirring at a temperature of 40°C, then positioned in an ultrasonic bath for 15 minutes at 40°C, and separated using centrifugation at  $\approx 4032 \times g$  for 7 min, 25°C. Then the same steps were performed in the previous extraction method. The oil yield per 100 g of bran was estimated using the specified extraction method according to the equation that follows:

$$\text{Yield of oil (\%)} = \frac{\text{mass of extracted oil}}{\text{mass of bran}} \times 100\% \quad (1)$$

#### 2.4. Characterization of RBO

2.4.1. *Physical characterization of RBO*. The examined physical characteristics of the extracted oil were density, viscosity, refractive index, and smoking point. The extracted oil's density was calculated using the method [15] using a 25 ml R.D bottle at a temperature of 20 °C. Viscosity was estimated using a glass Ostwald tube according to the mentioned method [16]. The refractive index of rice bran oil was gauged using an Abbe-type refractometer at (19.5 °C), and the smoking point was estimated according to [17].

2.4.2. *Chemical characteristics of RBO*. The chemical characteristics of the recovered oil were a value of iodine, peroxide acidity, saponification, and free fatty acids, which were estimated according to official methods. The extracted oil's iodine value was determined according to [18], the peroxide value, acidity number, free fatty acids, and saponification value according to in [19].

2.4.3. *Total fatty acids determination (TFA)*. The fatty acid content was analyzed by a Shimadzu (Japan) Gas Chromatograph (GC- FID). The sample was prepared using the approved esterification method by [20] A capillary separation column (SE-30) with a scope of 30 m and 0.25 mm diameter was used, with injection zone temperatures of 280 °C, detector 310 °C, and separation column 120-290°C at a 10 m/min of rate. The gas flow average was 100 kPa.

2.4.4. *Natural antioxidant determination*. Total antioxidant activities by DPPH methods. The free radical scavenging activity assay was used to estimate the antioxidant capacity of the oil, as per to the method explained by [21]. DPPH solution was freshly prepared, and then 0.25ml mingled with 1.75 milliliters of DPPH assay solution dissolved in methanol. The samples were incubated for half an hour in the dark, and then the absorbance of the sample was weighted at a specific wavelength of 517 nm. Free radical scavenging capability was computed by the following formula:

$$\% \text{inhibition} = \frac{(\text{Absorption of control sample} - \text{Absorption of the oil sample})}{\text{Absorbance of control sample}} \times 100 \quad (2)$$

2.4.5. *HPLC analysis of tocopherols and tocotrienols*. Tocopherol and tocotrienols were determined according to [22] with some modifications. An HPLC apparatus German (Sykam) with a fluorescent detector was used. 300  $\mu\text{L}$  of RBO was added to 2 mL quantity of n-hexane and stirred. Using a 0.22-micrometer filter, 1ml of the solution was filtered for HPLC analysis. Separation was done using column C18 (4.6 mm  $\times$  250  $\times$  5  $\mu\text{m}$ ) and as the mobile phase, a blend of ethyl acetate/n-hexane (30:70) was used at a rate of flow 1 milliliter/minute. 30 °C was the detector temperature and the solvent was monitored at 290 and 340 nm excitation wave of light and emission wavelength, respectively.

2.4.6. *HPLC assay of  $\gamma$ -oryzanol*. Gamma-oryzanol in ARBO was established according to [23] using a Sykam HPLC German apparatus. 300  $\mu\text{l}$  of RBO was added to a 2 ml volumetric vial containing n-hexane, stirred well, and then filtered 1 ml of the solution through a 0.45 micrometer filter. Separation

was performed using column C18 (4.6 mm ×250 ×5 μm) at 30 °C, the mobile phase, a mixture of water/acetonitrile (30:70) was used at a flow rate of 1 mL/minute, and a volume of injection 5 μl, 30 °C was the detector temperature and the solvent was monitored at 315 nm.

*2.4.7. Total phenolics content (TPC).* The total phenolics of the extracted oil samples were estimated based on [24] use Folin-Ciocalteu reagent. The process is summed up by placing 2 ml of oil in a 100 ml laboratory flask, then adding 3 ml of D.W. and 2.5 milliliters of Folin-Ciocalteu. The beaker was agitated for 4 minutes, then 2 ml of 5.7% sodium carbonate sol was added to it, shaken again, and left for 2 hours in darkness. The absorption level was gauged at 765 nm employing a spectrophotometer. The percentage of total phenols was calculated employing the gallic acid standard curve.

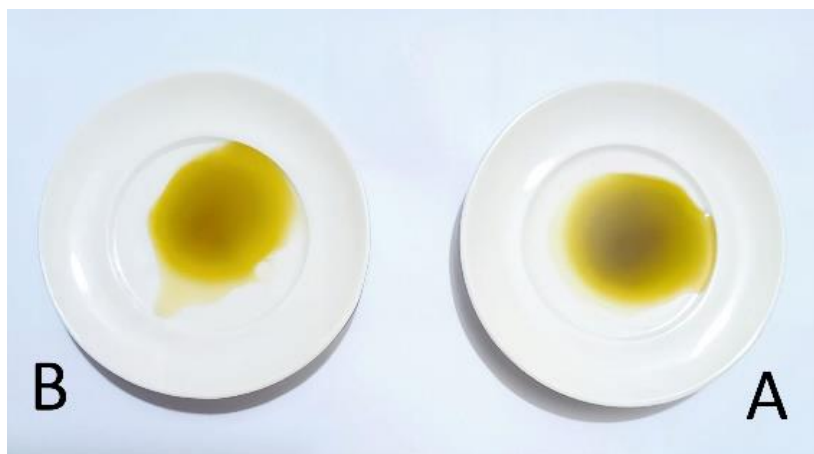
### *2.5. Data analysis*

The data was analyzed by used software SAS, and a one-way (ANOVA) was carried out. Every measurement was made three times, and the standard error (SE) was applied. A significance criterion of (P<0.05) was used to identify differences.

## **3. Results and discussions**

### *3.1. Effect of the extraction approach on yield*

ARBO was extracted with ethanol using traditional extraction (SEE) and ultrasound-assisted extraction (UAEE), which is shown in (Fig. 1). The oil production percentage ranged from 17.98% for rice bran extracted by SEE, to 19.22% for rice bran extracted by UAEE (Table 1); which was significantly more than SEE at the probability level (P<0.05). The cavitation effect induced by ultrasound enhances the extraction of oil from bran thanks to the formation of small turbulences that contribute to the dissolution of the solute and the acceleration of heat and mass transfer. Bursting bubbles on the surface produce small jets that optimize the extraction approach by enabling the solvent to enter the cell of the plant more deeply [25], which improved the extraction efficiency compared to the conventional method, as it gave a higher oil yield during a decreased extraction time than the SEE method and thus less exposure to solvent, which reduced the negative environmental impact resulting from high exposure to solvents and the large amount consumed in the conventional method. The SEE results were close to the oil content obtained by [26], who extracted the oil using ethanol maceration, which was about 16.4%. The UAEE yield is higher than what was obtained by [10], where the percentage of oil produced by ethanol was 13.59 %.



**Figure 1.** A: Oil extracted from Amber rice bran with ultrasound-assisted extraction; B: Oil extracted from bran of Amber rice using the traditional method.

**Table 1.** Physicochemical characteristics of the extracted oil from rice bran by different extraction methods.

Physicochemical parameter	solvent extraction ethanol (SEE)	Ultrasound-assisted extraction ethanol (UAEE)
Oil yield %	17.98±0.27 <sup>b</sup>	19.22±0.28 <sup>a</sup>
Density (g/cm <sup>3</sup> )	0.9089±0.003 <sup>a</sup>	0.9228±0.009 <sup>a</sup>
Viscosity (cPs)	33.41±0.52 <sup>a</sup>	31.6±0.61 <sup>a</sup>
Refractive index (RI) 19.5°C	1.4713±0.001 <sup>a</sup>	1.4646±0.001 <sup>b</sup>
Smoking point (°C)	217.67±1.8 <sup>a</sup>	216±1.15 <sup>a</sup>
Iodine value (gI <sub>2</sub> /100g)	103.84±2.21 <sup>a</sup>	101.94±1.5 <sup>a</sup>
Value of Acid (mgKOH/g)	0.29±0.009 <sup>b</sup>	0.38±0.01 <sup>a</sup>
Free fatty acids content (FFAs)%	0.086±0.06 <sup>b</sup>	0.128±0.06 <sup>a</sup>
Value of Peroxide (meq/kg)	1.19±0.003 <sup>a</sup>	1.28±0.008 <sup>a</sup>
Number of saponification (mg KOH/g)	180.5±0.8 <sup>a</sup>	182±0.6 <sup>a</sup>

### 3.2. Effect of the extraction approach on the physical analysis of ARBO

The physical properties of ARBO including density, viscosity, refractive index, and smoking point are specified in (Table 1). The density of SEE oil was 0.9089 gram/cm<sup>3</sup> and UAEE oil was (0.9228 gram/cm<sup>3</sup>). The density of the latter was slightly higher, recording that there were no considerable divergences at the likelihood level ( $P < 0.05$ ). Extraction time, temperature, and percentage of solvents used affect Significantly density, increasing extraction time and temperature results in increased oil density. our results agreed with those of [27] which were (0.92 and 0.93 g/cm<sup>3</sup>) for SEE and UAEE oil, respectively. The results for the viscosity of ARBO (Table 1) showed that it was (33.41 and 31.6 cPs) for the oil extracted by SEE and UAEE, respectively. They showed that there were no considerable divergences at the likelihood level ( $P < 0.05$ ). The vibrations produced by ultrasound provide energy that can destroy interactions between molecular bonds in compounds, causing the oil's viscosity to decrease. It can be inferred that the interactions of compounds in the bran of rice can be ruined because of the effects of heat and stirring, which consequently leads to a reduction in its viscosity [28], and this agrees with his discoveries that the viscosity of RBO decreased from 36.0 to 13.8 cPs when the temperature was elevated. Extraction from 50 to 70 °C. The refractive index of ARBO extracted by SEE was 1.4713 and that of UAEE oil was 1.4646, with considerable divergences in the two samples. This can be interpreted by the longer time of extraction in SEE, which resulted in more impurities being extracted and the refractive index changed [29], compared to the UAEE method which has a shorter extraction time and thus the effect on the oil is less. The refractive index of SEE oil agreed with what was shown [30] which was 1.4749.

### 3.3. Effect of the extraction approach on chemical analysis of ARBO

Regarding the iodine value of ARBO, it was noted that there existed no considerable divergences between the oil extracted employing SEE and the oil extracted with UAEE, and it was 103.84 and 101.94. The results differed with Shamma, who indicated that it was 91.16 and 99.22 for SEE and UAEE Oil, respectively. As noted in (Table 1) the acidity and free fatty acid values of the oil extracted from bran of rice employing SEE were reduced relative to UAEE oil, where the acidity values were 0.29 and 0.38 (mg KOH/g), respectively. As for free fatty acids, they were 0.086 and 0.128% for the oil SEE and UAEE methods extraction, sequentially. The cavitation that occurs during ultrasonic processing is the main reason for oxidation in UAEE oil, as the formation and collapse of microbubbles lead to areas of high pressure and temperature, so free radicals are created during ultrasonic waves, which might lead to an increase in free fatty acids in ultrasound-assisted extraction compared to oil extracted by the traditional method, using the exposure to ultrasound treatment is generally rapid, bran stabilization process's effectiveness in inhibiting the lipase enzyme activity increase in it compared to the traditional method is not significant [31]. Our results regarding the value of acidity and FFAs, which were low in

the extracted oil, can be explained by the bran stabilization process's effectiveness in inhibiting the lipase enzyme activity and the success of the extraction process in both methods. According to studies, when the percentage of fatty acids is high and above 5%, this means that the bran was not stable before extraction, and thus the activity of lipases led to the deterioration of the oil [30]. Regarding the peroxide value of the extracted oil, there was no significant divergences between its value by SEE and UAEE oil, which were 1.19 and 1.28 (meq /kg), respectively. As for the saponification value, the results did not record any considerable divergences between the two samples depending on the extraction method, which was 180.5 and 182 (mg KOH/g), respectively. It appears that employing ethanol as an extraction solvent gave oil good chemical properties depending on the extraction method.

### 3.4. Effect of the extraction approach on activities of antioxidants in RBO (DPPH, TPC)

The free radical scavenging ability (DPPH) test indicated that there were no considerable divergences at the likelihood level ( $P < 0.05$ ) between their ability in SEE and UAEE (Table 2), it was equal to 80.57 and 84.71%, respectively, but it can be that UAEE oil was slightly higher. As for total phenols (TPC), were in the range of 120 and 126.67 (mg GAE/100g) in SEE and UAEE, respectively. Which showed that the latter was relatively higher as well. UAE affects the ability to extract TPC as it increases the breakdown of cells, which in addition to facilitating the exit of oil, also facilitates the exit of other biologically active substances easily, and in a decreased time than the traditional method [32]. Therefore, the UAEE method was more environmentally sustainable, as with a shorter extraction time, an oil rich in antioxidants was obtained with less resource consumption.

**Table 2.** Effect of extraction approach on antioxidant of ARBO.

	solvent extraction (SEE)	Ultrasound-assisted extraction (UAEE)
DPPH %	80.57±4.6 a	84.71±3 a
Total phenolic Content (TPC) (mg GAE/100g)	120±5.8a	126.67±3.33 a

### 3.5. Effect of the extraction approach on the $\gamma$ -oryzanol, tocopherols, and tocotrienols

Table 3 shows the tocopherols, tocotrienols, and gamma-oryzanols of ARBO. The results showed that  $\alpha$ -tocotrenol and  $\alpha$ -tocopherol did not show considerable divergences depending on the extraction process, in contrast to  $\gamma$ -tocotrenol and  $\gamma$ -tocopherol, which showed considerable divergences depending on the extraction process. UAEE outperformed SEE in their quantity. As for  $\gamma$ -oryzanol, its quantity was 4.83 and 5.88 (mg/g) for the oil extracted by SEE and UAEE, respectively, which showed a considerable divergence between the two samples at the likelihood level ( $P < 0.05$ ). Although the extraction time in UAEE was shorter compared to SEE, it had a higher yield, which is since the use of ultrasound optimized the extraction conditions compared to the traditional method and increased the extraction efficiency, which in turn reduced the negative effect upon the environment.

**Table 3.** Effect of the extraction approach on  $\gamma$ -oryzanol, tocopherols, and tocotrienols.

mg/g	solvent extraction ethanol (SEE)	Ultrasound-assisted extraction ethanol (UAEE)
$\alpha$ -Tocotrenol	0.094±0.004 a	0.101±0.007 a
$\alpha$ -Tocopherol	0.130±0.007 a	0.146±0.011 a
$\gamma$ -Tocotrenol	0.309±0.012 b	0.380. ±0.007a
$\gamma$ -Tocopherol	0.176±0.009 b	0.221±0.013 a
$\gamma$ -Oryzanol	4.83±0.195 b	5.88±0.309 a

### 3.6. Effect of the extraction approach on the fatty acid content of ARBO

Table 4 illustrates the fatty acids (FAs) content present in the ARBO extracted by SEE and UAEE. The results show that the highest-value FAs in the oil extracted by SEE is linoleic acid, and in UAEE it is palmitoleic acid. There were no considerable divergences between some fatty acids based on the

extraction process, such as stearic, oleic acids, and linolenic acid, at the likelihood level ( $P < 0.05$ ). conversely, considerable divergences were observed in the extraction of linoleic fatty acid, with SEE being superior to UAEE, by 43.72 and 23.09%, respectively. The reason for the discrepancy between the percentage of fatty acids may be that ultrasound has a strong effect on fatty acids, and this effect is due to the cavitation it produces, which changes the structural and functional components of fatty acids, according to [33].

**Table 4.** Effect of the extraction approach on the fatty acid content of ARBO

Fatty Acid	Group	Solvent Extraction ethanol (SEE) %	Ultrasound-Assisted Extraction ethanol (UAEE) %
Palmitic (C16:0)	SAFA	3.26±0.89b	12.80±1.27a
Palmitoleic (C16:1)	MUFA	15.09±5.38b	33.83±1.42a
Stearic (C18:0)	SAFA	14.57±2.04a	14.65±2.31a
Oleic (C18:1)	MUFA	15.09±1.19a	10.76±1.11a
Linoleic (C18:2)	PUFA	43.72±6.54a	23.09±0.35b
Linolenic (C18:3)	PUFA	8.27±2.67a	4.87±1.49a

#### 4. Conclusion

The extraction of ARBO using an environmentally friendly approach such as green solvents (ethanol) was successful according to the results we obtained. Also, the use of effective extraction techniques such as ultrasound can increase the overall extraction efficiency and increase the oil yield obtained by green solvents such as ethanol, which in turn reduces solvent exposure, energy consumption and effort and thus reduces the environmental impact. It is also recommended to pay attention to the parameters and conditions of bran treatment during ultrasound-assisted extraction to obtain the best results from introducing this technique with green solvents.

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