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## Quality parameters of ultrasound assisted aqueous enzymatic extraction of rice bran oil

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**Abstract**

The quality parameters like free fatty acids, peroxide value, colour and viscosity of conventional (Hexane) and ultrasound assisted aqueous enzymatic extraction of rice bran oil were examined. It was found that the ultrasound extracted oil had lower contents of free fatty acids, colouring matters and had higher contents of peroxide value, viscosity than conventional (Hexane) extracted oil. Hence, in conclusion, ultrasound assisted aqueous enzymatic extraction has the potential to replace the conventional solvent extraction.

**Keywords:** Ultrasound extraction, free fatty acid, quality parameters of rice bran oil, peroxide value

**Introduction**

Rice is the seed of the grass species *Oryza sativa*. As a cereal grain, it is the most widely consumed staple food for a large part of the World's human population, especially in Asia. It is the agricultural commodity with third highest worldwide production of 741.5 million tonnes (FAOSTAT, 2017) [4]. Rice bran is a by-product of rice milling process (the conversion of brown rice to white rice). It has a high nutritive value. Besides proteins, Rice bran is an excellent source of vitamins B and E. It also contains small amounts of anti-oxidants, which are considered to low cholesterol in humans. Rice bran contains 12-22% oil. Rice bran oil is known for its high smoke point of 232 °C and mild flavour, making it suitable for high-temperature cooking methods. It is popular as a cooking oil in several Asian countries, including, Bangladesh, Japan, India and China (Orthoefer, 2005) [11]. Rice bran oil consists of a peculiar component called oryzanol which extensively helps in increasing good cholesterol and lowering down the bad cholesterol and has protective effect against Thyroid, cancer.

Rice bran oil extraction methods include mainly mechanical press method, solvent (Hexane) extraction, enzymatic extraction and supercritical CO<sub>2</sub> extraction method (Wang *et al.*, 2008; Zhang *et al.*, 2015) [12, 16]. Traditionally, solvent (hexane) extraction has been applied for vegetable oil extractions in the food industry. The main drawbacks of this extraction method are health concerns and increased environmental regulations due to the toxicity of hexane. In addition, the extracted oils can also be of low quality in terms of unwanted free fatty acids, unsaponifiable matter.

In recent years, ultrasound-assisted aqueous enzymatic extraction (UAEE) has become an effective method for edible oils and fats (Lin *et al.*, 2008; Wu *et al.*, 2011; Zhang *et al.*, 2009; Chen *et al.*, 2010; Huang *et al.*, 2013) [9, 14, 15, 3, 7]. UAEE is an inexpensive, simple and efficient alternative to conventional extraction techniques. Mechanism of UAEE is attributed to mechanical and cavitation efficacies which can result in disruption of cell wall and enhanced mass transfer across cell membrane and eliminates the problems associated with the use of organic solvents and possibly improves the oil quality (Hossain *et al.*, 2012; Wang *et al.*, 2013) [13, 6].

Thus, the main aim of the present work is to examine the quality parameters of conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil.

**Materials and Methods****Materials**

Fresh full fat rice bran, type BPT 5204 used for experiments was obtained from the local rice mills of Bapatla, Guntur dist, Andhra Pradesh.

Fresh rice bran was sieved through 710 µm aperture sieve to remove broken grains, hull fragments, paddy kernels and foreign materials. Enzymes, Cellulase from *Aspergillus sp.*, having an activity  $\geq 1000$  U/g,  $\alpha$ -Amylase from *Aspergillus Oryzae*,  $\geq 800$  U/g and Protease from *Aspergillus Oryzae*,  $\geq 1000$  U/g were purchased from Sigma-Aldrich Co.

## Methods

### Soxhlet Extraction

For hexane extraction, 8 g of rice bran (moisture 11.80%) was placed in a thimble followed by extraction with hexane in a Soxhlet apparatus (SOC PLUS, SCS 06 AS DLS) for 6 h. The oil obtained was dried in a hot air oven at 100 °C for 30 min to eliminate residual hexane (Hanmoungjai *et al.*, 2000) [5].

### Ultrasound-Assisted Enzymatic Extraction

The rice bran was stabilised at 110° C for 20 min in order to inactivate lipase enzyme. The combination of enzyme as cellulase, amylase and proteases of concentration 270 U, 216 U and 135 U respectively was added. The mixture was incubated using an incubator (Bacteriological incubator, GMP model) at a specific temperature of 37° C for 4 h. The incubated rice bran was commixed with distilled water at a ratio of 1:6 (w/v) and pH was adjusted to 7.0 with 0.1 N NaOH. Then, the sample was treated with ultrasound processor (probe sonicator, D P 120) contained a probe of 12 mm diameter, frequency 20 kHz and ultrasound power of 120 W. The samples were subjected to treatments with treatment time of 15, 30, 45 and 60 min with pulsation of 60 s on and 5-20 s off. The treated sample was centrifuged at 8000 rpm for 20 min to separate supernatant. The water was removed from the supernatant by evaporation. Subsequently, the weight of oil was calculated.

### Quality Analysis

The quality parameters like free fatty acids, peroxide value, viscosity and colour of rice bran oil was determined as follows.

#### Free Fatty Acid

Free fatty acid value was determined by directly titrating the oil in an alcoholic medium against standard potassium hydroxide solution. Five grams of oil was dissolved in 50 mL of neutral solvent (25 mL hexane+25 mL 95% ethanol+1% of phenolphthalein solution) in a 250 mL conical flask. Then, about 2-3 drops of phenolphthalein was added to the solution. The sample was titrated against 0.1N potassium hydroxide (KOH) solution shaking vigorously until pink colour was obtained (Krishnan *et al.*, 2015).

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre value} \times N \times 56.1}{\text{weight of the sample}}$$

Where,

Titre value = mL of KOH

N= Normality of KOH solution

#### Peroxide Value

Peroxide value (PV) is the measure of the lipid oxidation in foods, it assess the primary oxidative changes in the system. Five grams of sample was weighed into a 500 mL of conical flask. Glacial acetic acid-chloroform mixture of 30 mL was added to it and the oil was dissolved completely. An amount

of 0.5 mL saturated potassium iodide solution was added to the solution obtained earlier, mixed well and was allowed to stand for 1 min in dark with occasional shaking. Then, 430 mL of water and 3-4 drops of starch indicator were added to it. Following this, titration was carried out against standard 0.01 N sodium thiosulphate with vigorous shaking to liberate all iodine from chloroform layer until the blue colour disappeared. Blank was treated similarly in the absence of oil (AOAC, 2006) [2].

Peroxide value expressed as milli equivalent of peroxide oxygen per kg of sample (meq/kg)

$$\text{Peroxide value (meq/kg of oil)} = \frac{\text{Titre value} \times N \times 100}{\text{weight of oil}}$$

Where

Titre value = mL of sodium thiosulphate

N= Normality of sodium thiosulphate solution

#### Colour

The colour of the oil was measured by using a colorimeter (Lovibond PFX-995 Tintometer). The colour was expressed as red (R), yellow (Y) and blue (B) units as per the standard procedure (AOAC, 2000) [1]. The small volume of sample was filled in the 10 mm optical glass chamber and placed into the chamber channel. The colour reading was noted down given by instrument.

#### Viscosity

The viscosity of the oil was measured by using viscometer (Brookefield DV1 Viscometer). The viscosity was expressed in centipoises (cP), Pascal second (Pa.s), milli pascal second (mPa.s) and poise (P). The sample was filled in the beaker and the spindle was attached to the lower shaft. Spindle number was entered into the viscometer; the spindle was inserted and centered in the test material. End condition of the test was selected using the RUN UNTILL function. MOTOR ON key was pressed for viscosity measurement.

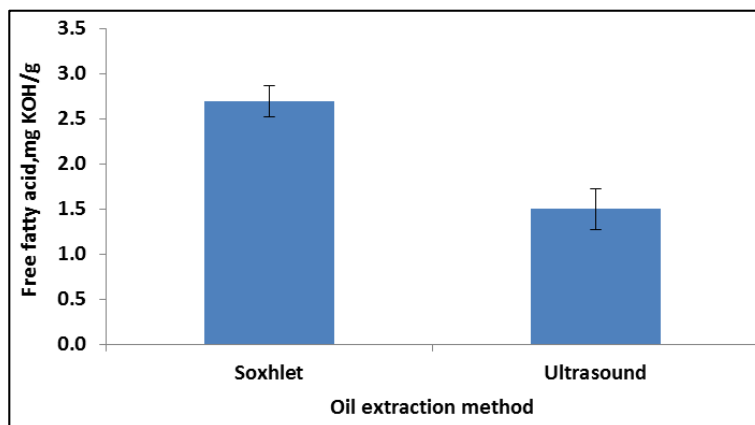
## Results and Discussion

From ultrasound assisted aqueous enzymatic extraction, the highest oil recovery ( $88.15\% \pm 2.12$ ) was obtained from the combination of 30 min with 60 s on and 5 s off. The quality parameters of ultrasound assisted aqueous enzymatic extraction and conventional extraction of rice bran oil was compared as follows.

#### Free Fatty Acid

The free fatty acid values of oil from conventional and ultrasound assisted aqueous enzymatic extractions of rice bran oil were compared. From Figure 1, it was evident that ultrasound assisted aqueous enzymatic extraction of oil has a substantially lower content of free fatty acid (1.49 mg KOH/g) than that of conventionally (hexane) extracted oil (2.69 mg KOH/g). Similar results were reported by Ming *et al.* (2014) [10].

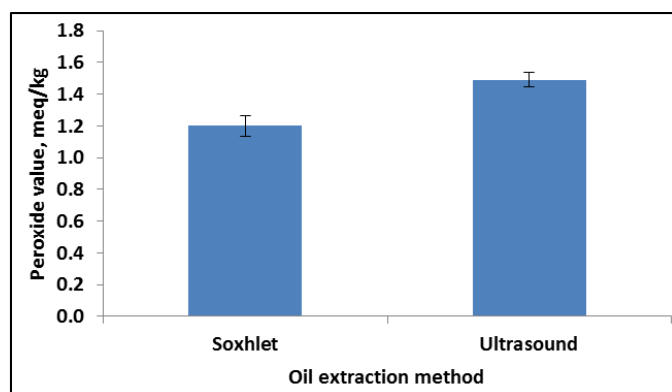
This was due to the fact that FFA content in the bran is saponified with added base during the extraction process. This implies that a lower amount of neutralizing agent is needed in the refining stage. The F-test table showed that  $P < 0.05$ , so that there was a statistical significant difference between the free fatty acid value of both ultrasound and solvent extraction methods.



**Fig 1:** Free fatty acid value of conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil

### Peroxide value

The peroxide values of conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil was shown in Figure 2. The peroxide values of oil from conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil was compared. The peroxide value of the oil obtained from ultrasound assisted aqueous enzymatic extraction (UAEE) of rice bran oil was little higher (1.5678 meq/kg of oil) than the peroxide value of conventionally (hexane) extracted oil (1.220 meq/kg of oil). Hanmoungjai *et al.* (2000) [5] reported the similar observation on the value of peroxide value. It was noteworthy that even though the peroxide value of UAEE oil was higher than hexane extracted oil, the remaining qualities were better than the hexane extracted oil. It only exceeded the industrially specified limit (1.0-1.2 meq/kg of oil) by a small margin. The F-test table showed that  $P < 0.05$ , so that there was a statistical significant difference between the peroxide value of both ultrasound and solvent extraction methods.



**Fig 2:** Peroxide value of conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil

### Colour

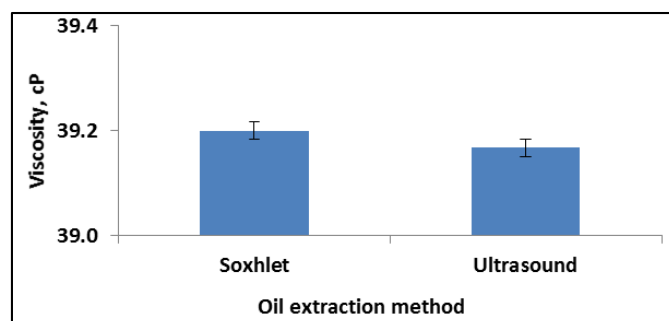
The colour of oil obtained from conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil was compared. It was observed that ultrasound-assisted aqueous-extracted oil had a lower content of colouring substances when compared with hexane extracted oil (Table 1). This indicated that less bleaching earth was needed to refine the oil. Juliano (1985) [8] reported that due to the presence of carotene, chlorophyll and high temperature during recovery of hexane made darker colour in conventional soxhlet extracted oils. The F-test table showed that  $P < 0.05$ , so that there was a statistical significant difference between the colour value of both ultrasound and solvent extraction methods.

**Table 1:** Colour of conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil

Rice bran oil	Colour
Conventional	20Y+2.8R
UAEE	15Y+2R

### Viscosity

The viscosity of oil from conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil was compared. It was observed that the viscosity of ultrasound assisted aqueous enzymatic extraction of rice bran oil was almost similar to that of conventional (hexane) extraction of oil (Fig. 3). No change in viscosity of oil was noted. Ming *et al.* (2014) [10] reported similar observation on viscosity of oil. The F-test table showed at  $P < 0.05$ , there was no statistical significant difference between the viscosity value of both ultrasound and solvent extraction methods.



**Fig 3:** Viscosity of conventional and ultrasound assisted aqueous enzymatic extraction of rice bran oil

### Conclusion

With regard to quality, the rice bran oil extracted by ultrasound assisted aqueous enzymatic extraction had a lower content of free fatty acid, imparting components than the hexane-extracted oil; higher content of peroxide value and viscosity than conventional (hexane) extracted oil. Thus, More importantly, ultrasound assisted aqueous enzymatic extraction was an environmental friendly alternative to conventional solvent extraction methods.

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