Environment and Ecology 39 (3) : 533—536, July—September 2021
ISSN 0970-0420

Ultrasound Assisted Aqueous Enzymatic Extraction of Rice Bran Oil

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Received 10 April 2021, Accepted 12 June 2021, Published on 7 July 2021

ABSTRACT

In the present study, the effects of ultrasonic pre-treatment along with aqueous enzymatic extraction on oil yield were investigated. Four different ultrasonic treatment durations 15, 30, 45, 60 min with 60s on and 5-20s off pulse intervals were evaluated for their effectiveness in releasing oil from rice bran. The oil recovery from ultrasound treated sample was compared with conventional extraction of rice bran oil. The highest oil recovery of 88.15% was obtained with ultrasound treatment period of 30 min with 60s on and 5s off timings.

Keywords Rice bran oil, Ultrasound assisted aqueous extraction, Enzyme assisted aqueous extraction, Rice bran.

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INTRODUCTION

Rice (*Oryza sativa*) is the most important cereal crop cultivated around the world, which feeds more than half of the world's population. It is the agricultural commodity with worldwide production of 741.5 million tonnes (FAOSTAT 2017). Rice grain or rice kernel composes of hull (20%), bran and germ (10%) and starchy endosperm (70%) (Orthoefer 2005). Rice bran is the by-product of rice processing, accounted for 5-8% in grain weight.Typically, rice bran contains 12-22% oil, 11-17% protein, 8-17% ash, 10-15% moisture and 6-14% fiber. Rice Bran Oil (RBO) is unique edible oil produced from the rice bran. 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. It is popular as a cooking oil in several Asian countries, including, Bangladesh, Japan, India and China because of its high smoking point and suitable for high temperature cooking foods (Orthoefer 2005).

Rice bran oil extraction methods include mainly mechanical press method, solvent (hexane) extraction, enzymatic extraction and supercritical CO_2 extraction method (Wang *et al.* 2013, Zhang *et al.* 2015). 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 oflow quality in terms of unwanted free fatty acids, unsaponifiable matter. These considerations prompted attempts to develop aqueous extraction methods. In aqueous extraction, there is no chemical potential for oil dissolution and extraction is based on the insolubility of oil in water rather than on the dissolution of oil.

However, aqueous extraction processes are usually characterized by low oil yields. The low extraction yields can be overcome by using enzymes, such as cellulase, amylase, protease that hydrolyze the structural polysaccharides forming the cell wall of oilseeds or that hydrolyze the proteins, which form the cell and lipid body membranes and facilitate oil release from the oil bodies. Thus, the soluble components diffuse into water and the released oil forms a separate liquid phase.

In recent years, ultrasound-assisted extraction (UAE) has become an effective method for edible oils and fats (Lin *et al.* 2008, Wu *et al.* 2011, Zhang *et al.* 2009, Chen and Diosady 2010, Huang *et al.* 2013). UAE is an inexpensive, simple and efficient alternative to conventional extraction techniques. Mechanism of UAE is attributed to mechanical and cavitation efficacies which can result in disruption of cell wall and enhanced mass transfer across cell membrane and less extraction time (Hossain *et al.* 2012, Wang *et al.* 2013).Thus, the main aim of the present workis to study the enhancement of aqueous enzymatic extractionof rice bran oil by ultrasound treatment.

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≥1000 U/g, α-Amylase from *Aspergillus oryzae* ≥800 U/g and Protease from *Aspergillusoryzae*≥1000 U/g were purchased fromSigma–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).

Yield determination

The yield of rice bran oil was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of flask with concentrated oil- Empty weight of flask}}{\text{sample weight}}$$

This method gave 8 ± 0.59 g of oil per 50 g of rice bran, which was set as100 % oil recovery for comparison.

Aqueous enzymatic extraction

The rice bran was stabilized 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 aratio of 1:6 (w/v) and pH was adjusted to 7.0 with 0.1 N NaOH. Sample was centrifuged (C-23BL, REMI Instruments Ltd) at 8000 rpm for 20 min to separate supernatant. The supernatant was concentrated to get rice bran oil and the obtained rice bran oil was dried in hot air oven to eliminate the traces of water.

Ultrasound-assisted enzymatic extraction

Ultrasound treatment was carried out on an ultrasound processor. The prepared sample was placed in a 500 mL beaker. The beaker was placed on the stand of ultrasound processor (probe sonicator, DP 120). The ultrasound probe of 12 mm diameter was attached to the ultrasound transducer. A constant frequency of 20 kHz and ultrasound power of 120 W was used for treatment of samples. The samples were subjected to treatments with treatment time of 15,30,45 and 60 min with pulsation of 60s 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.

Statistical analysis

All experiments were performed in triplicate. The values reported are the mean ± SD. Analysis of variance was conducted to determine statistical significance ($p<0.05$) based on the CRD. SPSS STATISTICS version 23 was used for the statistical analysis of the data.

RESULTS AND DISCUSSION

The effect of ultrasonic time on oil yield was shown in Fig. 1. The oil yield was found to increase (from 80% to 88.15%) with the increase of time from 5 to 30 min and then decreases when the time continuously increased. The highest oil recovery (88.15% ± 2.12) was obtained from the combination of 30 min with 60s on and 5s off. This was due to the ultrasonic processing; there action process was strengthened rapidly in a certain period of time. As the ultrasound treatment time was increased, cavitations effect weakened slowly and thus, reduction in oil recovery was observed.

Analysis of variance table indicated that the significance value is lower than the chosen significance level ($p<0.05$). So that, the null hypothesis was discarded. Thus, there was a significant difference between operation time, on and off timings and extraction efficiency. It was concluded that operation time and on and off timings (operation time × on and off timings) significantly influenced the extraction efficiency of rice bran oil.

CONCLUSION

The results indicated that ultrasound-assisted aqueous enzymatic extraction of rice bran oil was an efficient extraction method withthe highest oil recovery of 88.15% ± 2.12. More importantly, ultrasound assisted aqueous enzymatic extraction was an environmental-friendly alternative to conventional solvent extraction methods.

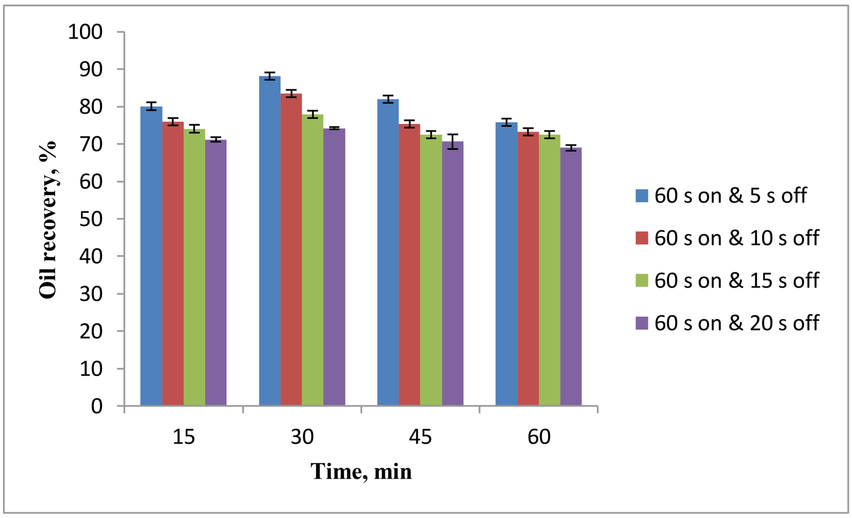

Fig. 1. Oil recovery from aqueous enzymatic extraction of rice bran oil with different ultrasound treatment time.

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