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Aqueous enzymatic extraction of rice bran oil

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Abstract
An aqueous enzymatic extraction method was developed to extract rice bran oil. Different concentrations of individual enzymes, combinations of enzymes and incubation time were studied. The effect of hexane and aqueous enzymatic extraction on the oil yield was compared. The combination of cellulase (270 U), amylase (216 U) and protease (135 U) for four hours of incubation yielded significantly more oil than the other combinations. Under the optimal reaction conditions, a free oil recovery of 76.48% was obtained. The findings suggest that the critical selection of AEE parameters is key to high oil recovery.

Keywords: Rice bran oil, aqueous enzymatic extraction, extraction of oils, enzyme concentrations, enzyme assisted aqueous extraction, rice bran

1. 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). 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) [1]. 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.

Commercial rice bran oil is extracted using organic solvents such as hexane, petroleum ether, ethanol etc. Hexane has been used as the solvent for rice bran oil extraction due to high oil extractability and easy availability. This process, however, has some constraints with respect to the oil quality (Amarasinghe *et al*., 2009) [2].

Aqueous enzymatic extraction eliminates the problems associated with the use of organic solvents and possibly improves the oil quality. It also enables the simultaneous recovery of oil and valuable materials, such as proteins and polysaccharides from oilseeds. Some conventional refining process, e.g., degumming, can be eliminated. Aqueous enzymatic-extracted oils have higher content of nutrients such as tocopherols, phenolics, and oxidative stability than that of hexane-extracted oils (Li *et al*., 2013) [5]. The present study was undertaken to optimize the aqueous enzymatic extraction method for rice bran oil and the oil recovery of aqueous enzymatic-extracted with hexane-extracted rice bran oil were compared.

2. Materials and Methods
2.1 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 *Aspergillus Oryzae*, ≥1000 U/g were purchased fromSigma–Aldrich Co.

2.2 Methods
2.2.1 Oil 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) [3]. The oil yield from this method was set as 100% oil recovery for comparison.

The basic procedure for aqueous enzymatic extraction of rice bran oil is as follows: (1) Stabilise 50 g of rice bran at 110 °C for 20 min in hot air oven to inactivate lipase enzyme; (2) add enzyme (v/w); (3) Incubate the mixture at 37 °C for an hour; (4) add 300 ml of distilled water (bran: water as1; (5) adjust pH to 7.0 with 0.1 N NaOH or 0.1 N HCL; (6) centrifuge at 8000 rpm for 20 min in a Refrigerated centrifuge; (7) Carefully separate the supernatant; (8) evaporate supernatant to obtain oil; (9) oil obtained was dried in a hot air oven at 100 °C for 30 min to eliminate the traces of water. The free oil yield is calculated by the following equation,

$$\text{Oil yield, \%} = \frac{\text{weight of oil}}{\text{weight of rice bran}} \times 100$$

For individual enzyme screening, three enzymes (cellulase, amylase, protease) of different concentrations i.e., cellulase of 90 U, 144 U, 270 U, 360 U; amylase of 72 U, 180 U, 216 U, 135 U and protease of 45 U, 90 U, 135 U, 180 U were selected and treated. For enzyme combinations, combination of three enzymes of different enzyme concentrations i.e., cellulase, amylase, protease as 90 U, 72 U, 45 U; 144 U, 180 U, 90 U; 270 U, 216 U, 135 U and 360 U, 288 U, 180 U were used. The optimized combination was used for further experiment and carried out process for incubation periods of 1, 2, 3 and 4 h.

2.3 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

3. Results and Discussion

The solvent extraction gave 8.0 g of oil per 50 g of seeds, which was set as 100% oil recovery for comparisons.

Enzymes are utilized to facilitate the degradation of the cell wall and the extraction of oil from the oil bodies. The effects of five individual enzymes and a control with no enzyme on free oil yield were investigated

The effect of different concentrations of individual enzymes on the amounts of oil extracted by aqueous extraction was shown in Fig.1. It was showed that among the four concentrations with three enzymes, 270 U of cellulase gave highest oil recovery (35.22% ± 2.48). The high oil yielded with cellulase suggests that cellulose surrounding the oil-rich liposomes is the main barrier that hinders the liberation and coalescence of oil from the oil bodies. Treatments with amylase caused intermediate oil yields and protease resulted in the lowest yield.

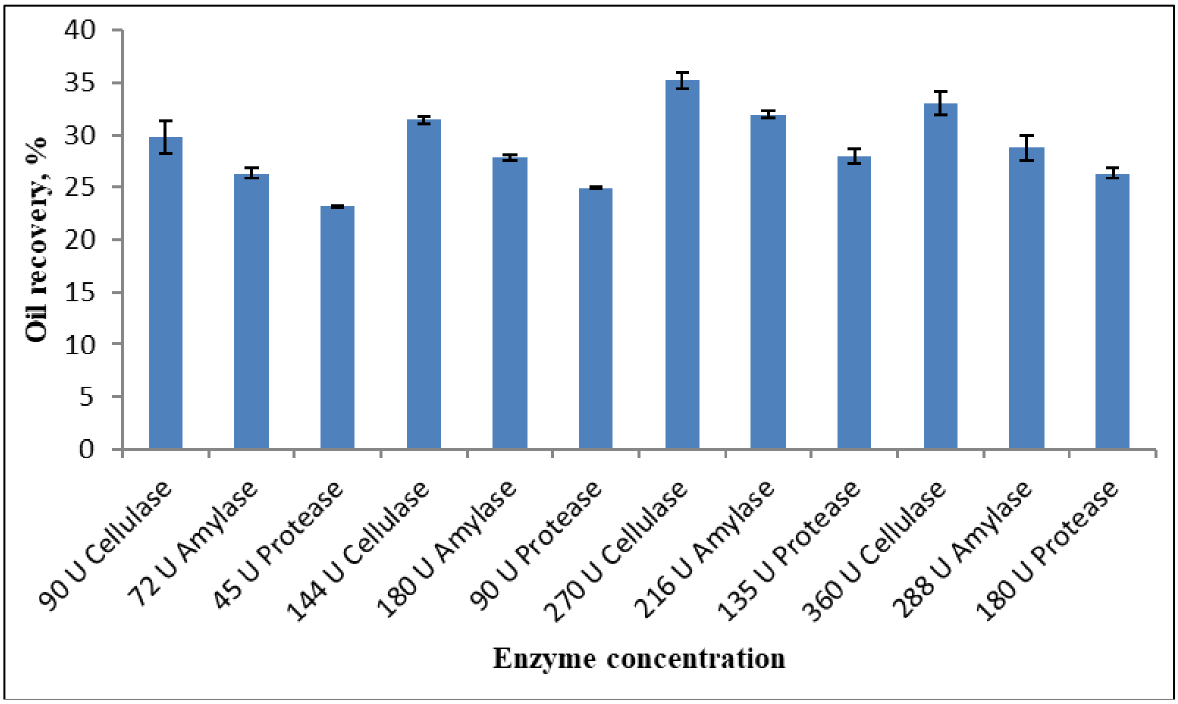
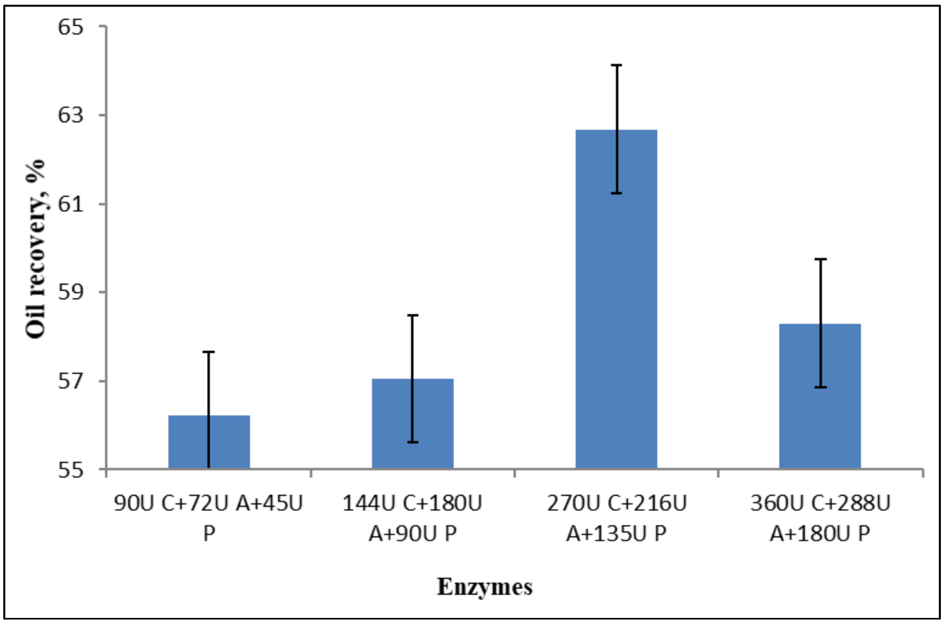


Fig 1: Oil recovery from aqueous enzymatic extraction of rice bran oil with individual enzymes

The effect of combination of enzymes on the amount of oil extracted by aqueous extraction was shown in Fig.2. The highest oil yield (62.68% ± 2.18) was obtained from the combination of cellulase, amylase and protease concentrations of 270 U, 216 U and 135 U, respectively. This was due to concentration of combination of enzymes. Hence, this combination was used for further experiments.



*C- cellulase; A- amylase; P- protease

Fig 2: Oil recovery from aqueous enzymatic extraction of rice bran oil with combination of enzymes

The effect of combination of enzymes with different incubation period on the amounts of oil extracted by aqueous extraction was shown in Fig.3. The highest oil recovery (76.48% ± 2.09) was obtained for four hours incubation, because the reaction of enzymes with the rice bran for more time gives greater yield.

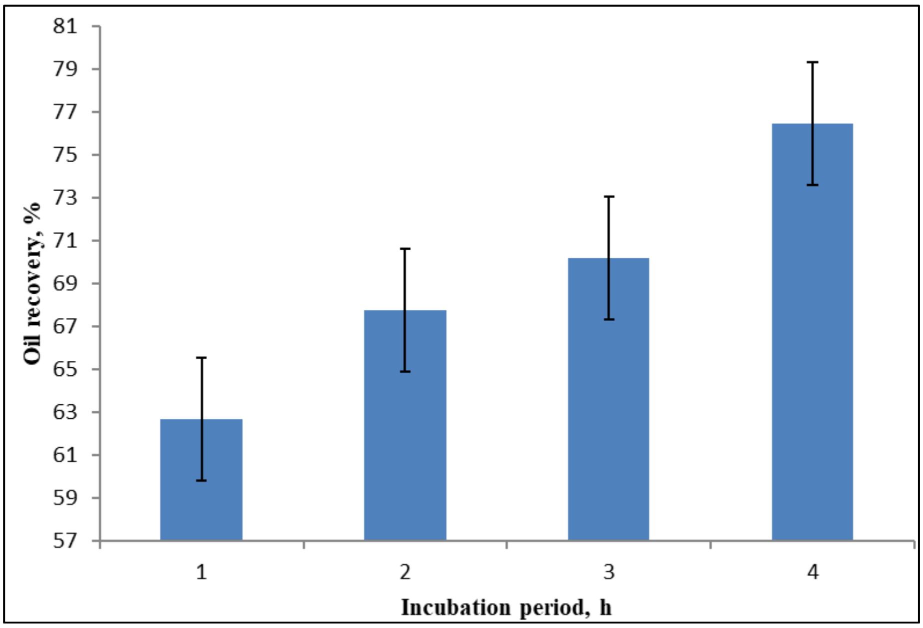


Fig 3: Oil recovery from aqueous enzymatic extraction of rice bran oil with combination of enzymes for different incubation period

4. Conclusion

The aqueous enzymatic extraction process has been shown to be effective for extracting rice bran oil. The combination of enzyme concentrations of 270 U+216 U+135 U for four hours of incubation gave highest oil recovery of 76.48% ± 2.09. Thus, the combination of aqueous and enzymes for extraction of oil is an economical alternative to solvent extraction.

5. References

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