A parametric study for the enzymatic extraction of rice bran oil and its optimization

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Abstract— The technology for the enzyme-assisted aqueous extraction of rice bran oil has been regarded as an eco friendly process which produces good quality oil. Rice bran oil has been identified as "heart oil" for the last few years due to presence of natural antioxidants and beneficial micronutrients like γ-oryzanol, tocopherols, tocotrienols, phytosterols, polyphenols and squalene. The nutritional qualities and health benefits of rice bran oil have been established for the presence of these components. So the extraction process parameters are quite significant for having these beneficial constituents in extracted oil. Conventional solvent extraction procedure uses hexane as solvent which is identified as an air pollutant. Therefore, enzyme-assisted aqueous extraction procedure is a good alternative one. In the present research investigation, effects of different extraction parameters like pH, temperature, concentration of enzymes, mixing time, mixing speed and ratio of bran to water has been studied using combination of enzyme Neutrase and Celluclast for the extraction of rice bran oil from bran. The parameters were optimized in terms of oil recovered and oil extractability. Extracted oil was characterized with regard to free fatty acid, unsaponifiables, phosphorous content, peroxide value, anisidine value and γ-oryzanol content. Our study identified the optimized extraction parameters for the rice bran oil from bran which may also be applicable for the enzymatic extraction of other oils too.

Keywords- *Rice bran oil; Enzymatic extraction; γ-oryzanol; Neutrase; Celluclast.*

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I. INTRODUCTION

Rice bran is one of the valuable by products of rice milling industry [1] and a potential source of edible oil. Rice bran oil (RBO) from bran has gained considerable importance in the last few years due to presence of natural antioxidants and micronutrients like γ-oryzanol, tocopherols, tocotrienols, phytosterols, polyphenols and squalene which potentially provide benefits to health [2,3,4,5]. γ-oryzanol and phytosterols can reduce blood cholesterol and decrease cholesterol absorption [3,6,7]. Tocopherols, tocotrienols and squalene are powerful antioxidants and these are associated with the prevention of cardiovascular diseases [4,5,8]. Due to presence of these beneficial components, RBO has a high nutritional value and has been considered as specialty oil.

Extraction process of RBO from rice bran is quite important for retaining these nutritional components in the extracted oil. Conventional extraction method includes hexane as solvent but the use of hexane is unfavourable due to its toxicity and flammability [9]. Moreover, hexane extraction requires high temperature which results undesirable components in the extracted oil due to oxidative deterioration and development of rancid and off flavours [10]. For this reason, it is of considerable interest to investigate suitable alternative process which must be eco-friendly and has the capacity to retain the healthy components in the extracted oil.

Aqueous oil extraction method is becoming an important alternative to hexane oil extraction. The use of water as medium for separation of oil and protein from different oil bearing seeds has been investigated by several researchers [11,12,13]. But due to low oil yield, aqueous oil extraction method was unsuccessful in the past in spite of its cleaner environmental friendly technology. But recently, another current development involves the use of enzymes to assist oil extraction from seeds. Application of aqueous enzymatic extraction process is undoubtedly a promising technology in the fats and oils industry due to its many advantages compared to conventional methods. So the challenge of producing high quality oil can be met through the innovative enzymatic extraction process [14]. Among the advantages, for instance, it lowers investment costs [15], reduces energy requirements [16] and can minimize further refining operations [17] along with its environmental-friendly nature.

Enzyme-assisted aqueous extraction of RBO from full fat bran has been studied by several researchers. Sharma *et al.* [18] reported 77% recovery of RBO from rice bran using mixture of enzymes protease, α-amylase and cellulose. Single stage enzyme assisted aqueous extraction of soybean was studied by De Moura *et al.* [19] to yield free oil, skim, cream and insoluble portions. Enzymatic extraction of onion oil [20], sunflower oil [14], borage oil [21], rice bran oil [22,23,24], corn oil [25], rapeseed oil [26], coconut oil [27], soybean oil [28] have also been investigated recently. In the present research investigation, combination of enzymes, Neutrase and Celluclast for the aqueous extraction of rice bran oil from bran has been studied and the effects of different extraction parameters like pH, temperature, concentration of enzymes, mixing time, mixing speed and ratio of bran to water has been analyzed. Optimization of extraction parameters have been done with regard to oil recovered and oil extractability. Moreover, quality of extracted oil have been evaluated with respect to free fatty acid, unsaponifiables, phosphorous content, peroxide value, anisidine value and γ-oryzanol content in our analysis.

II. MATERIALS

Full fat rice bran used in this study was provided by M/s. Sethia Oils Ltd. (Burdwan, West Bengal, India).The enzymes used in the present study were Neutrase (Protease from

Bacillus amyloliquefaciens) and Celluclast (Liquid cellulase from *Trichoderma reesei*). These enzymes were kind gift from Novozyme South Asia Private Ltd, Bangalore, India. Except otherwise specified all other chemicals used were A.R. Grade.

III. EXPERIMENTAL METHODS

A. Purification of rice bran by sieving

Full fat rice bran was screened to pass through a 40 mesh (422 µm openings) sieve. The fraction (-40 mesh), passing through the sieve was used in our study.

B. Enzyme-assisted aqueous extraction of RBO frombran

25gm microwave stabilized full fat rice bran (–40 mesh) was taken in a 0.5 L conical flask and was stirred in aqueous medium maintaining a definite ratio of bran and water (w/w) for different time intervals. A definite temperature and stirrer speed were maintained in the presence of combined enzymes Neutrase and Celluclast at different concentrations at a particular pH. After the completion of extraction process of RBO from bran, the mixture was allowed to reach at room temperature. Then 0.03 L food grade hexane was added and the solvent-laden mixture was stirred for 15 minutes to dissolve the extracted oils in hexane. After that, the overall mixture was centrifuged at 5000 rpm for 10 minutes and liquid layer was isolated. The hexane layer was separated through a separating funnel, desolventised under vacuum and the extracted oil was preserved.

Oil yield (%) = (Amount of extracted oil/amount of bran taken) X 100.

Oil extractability (%) = (Amount of extracted oil /amount of oil in bran) X 100.

C. Solvent extraction of RBO using hexane from bran

The full fat rice bran (50 g) was fed to Soxhlet extractor fitted with a 0.5 L round-bottom flask and a condenser. The extraction was carried out for 4 h with 0.3 L of n-hexane on a water bath. After extraction, hexane was distilled off under vacuum at 4mm Hg pressure at 45°C and the oil was stored under refrigeration (4°C) for further analysis.

Acid value, unsaponifiables, phosphorous content, peroxide value, anisidine value were determined by Official and Tentative Method of American Oil Chemists' Society. Oryzanol content was determined according to the method of Gopala Krishna *et al*. [29]. For the determination of oryzanol, samples of accurately weighed RBO (about 10 mg each) in triplicate were dissolved in hexane and volume made up to 10 mL and mixed well in a vortex. The O.D. was measured in 1 cm cell at 314 nm in a UV-VIS spectrophotometer (UV-1601, SHIMADZU, Japan). Solution having O.D. greater than 1.2 were further diluted before reading O.D. The oryzanol content in the oil was calculated using the following formula. A blank was performed with 0.02 L hexane only. The specific extinction coefficient of oryzanol is 358.9.

$$\text{Oryzanol, g\%} = \frac{\text{OD of hexane solution}}{\text{weight (g) of oil} \times 10} \times \frac{100}{358.9}$$

All experimental values are reported as mean ± s.d., where n=3 (n=no of observations).

IV. RESULTS AND DISCUSSIONS

Table 1 shows the initial characteristics of sieved (-40 mesh) full fat rice bran. Oil content through Soxhlet extractor was obtained 22.38±0.41%. Bran contains 16.17±0.17% protein which indicates that it is a good source of protein along with carbohydrates and fibers.

A. Effect of pH for enzyme-assisted aqueous extraction of RBO from bran

Enzyme shows its optimum activity at a definite pH and therefore, pH of the extraction medium plays an important role for optimum separation of oil from bran using enzymes which was investigated in our study. Fig. 1 shows the effect of pH for enzyme-assisted aqueous extraction of RBO from sieved bran in the presence of combined enzymes Neutrase and Celluclast (1% each) at 55^{0}C for 4hrs with a mixing speed of 60 rpm maintaining a ratio of bran: water 1:10 (w/w). From Fig.1, it revealed that increasing pH in the extraction medium enhances the oil recovery and maximum oil recovery (66.08%) was obtained at pH 7. After that, increasing pH did not contribute any enhanced oil yield. This may be due to the fact that at pH 7, enzymes are most active for cell rupturing process which helps to release oil from ruptured cell of bran. So, this was the optimum pH for the extraction of RBO from full fat sieved bran in our experiment.

B. Effect of temperature for enzyme-assisted aqueous extraction of RBO from bran

Temperature is one of the significant extraction parameters for separation of oil from bran. Fig. 2 shows the effect of temperature for enzyme-assisted aqueous extraction of RBO from sieved bran in the presence of combined enzymes Neutrase and Celluclast (1% each) for 4hrs with a mixing speed 60 rpm maintaining a ratio of bran: water 1:10 (w/w) at pH 7. It has been observed from Fig. 2 that at a temperature of 55^{0}C, combined enzymes contributed maximum extraction of oil from bran. Further enhancing temperature did not increase the percent oil yield rather decreased. This may be due to the fact that enzymes are active at a particular temperature for cell rupturing process beyond that enzymes are deactivated and rupturing process is incomplete.

TABLE 1. CHARACTERISTICS OF SIEVED RICE BRAN

Characteristics (wt %)	Permeated (-40 mesh sieved bran)
Oil content	22.38±0.41
Moisture	5.96±0.11
Protein	16.17±0.17
Ash	10.73±0.34
Other fractions*	44.14±0.25

Other fractions include fiber, carbohydrates, starch etc.

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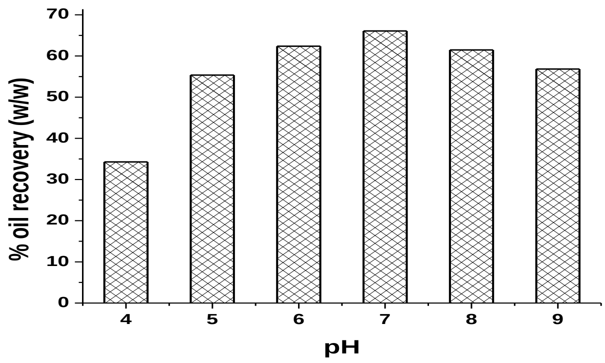

Fig.1 Effect of pH on oil recovery by enzyme-assisted aqueous extraction of RBO from sieved bran.

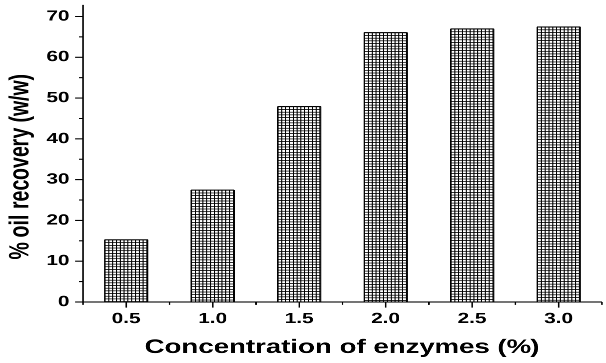

Fig. 3 Effect of concentration of enzymes on oil recovery by enzyme-assisted aqueous extraction of RBO from sieved bran.

C. Effect of enzyme concentration for enzyme-assisted aqueous extraction of RBO from bran

Fig. 3 shows the effect of concentration of combined enzymes Neutrase and Celluclast for enzyme-assisted aqueous extraction of RBO from sieved bran for 4hrs at 55^0C with a mixing speed of 60 rpm maintaining a ratio of bran: water 1:10 (w/w) at pH 7. As combined enzymes contribute better extraction efficiency than using single enzyme, so in our study, both the enzymes were used in equal concentration with respect to bran. It has been observed from Fig. 3 that increasing concentration of enzymes increases the rate of extraction of RBO from bran up to certain limit. At 2% concentration of enzymes (1% each), maximum extraction of oil was obtained as evidenced from Fig. 3. But after that, enhancing concentration of enzymes did not increase the percent oil yield and increasing concentration has no effect on the oil extractability. This may be due to the fact that at an optimum concentration of enzymes Neutrase and Celluclast, rupturing cell walls of rice bran and releasing free oil to be washed out of cells with water occurred. Therefore, as optimum cell rupturing occurs at a specific concentration of enzymes (2%), so further increasing concentration of enzymes does not have any significant effect in the extraction process.

D. Effect of time of stirring for enzyme-assisted aqueous extraction of RBO from bran

The effect of time of stirring for enzyme-assisted aqueous extraction of RBO from sieved bran has been investigated at 55^0C with a mixing speed 60 rpm maintaining a ratio of bran: water 1:10 (w/w) at pH 7 in the presence of combined enzymes Neutrase and Celluclast (1% each). It has been observed from Fig. 4 that 4 hrs is the optimum time of stirring for the enzyme-assisted aqueous extraction of RBO. Further enhancement of time of stirring at specified conditions did not contribute any significant results. This may be due to the fact 4hrs is the optimum time for all cell rupturing of bran by the enzymes and release of oil from the internal ruptured cell of rice bran. So, after stipulated time, no further improvement with regard to oil yield occurs if extraction continues.

E. Effect of mixing intensity for enzyme-assisted aqueous extraction of RBO from bran

Effect of mixing intensity or stirring speed is other important parameters for optimum extraction of RBO from bran using aqueous enzymatic approach. Here, the extraction of RBO from bran has been evaluated at 55^0C maintaining a ratio of bran: water 1:10 (w/w) at pH 7 in the presence of combined enzymes Neutrase and Celluclast (1% each) for 4hrs. Fig. 5 shows that maximum oil recovery was obtained at an optimum stirrer speed of 60 rpm in the given reaction conditions. Increasing mixing intensity beyond 60 rpm did not contribute any considerable increase of oil recovery as evidence from Fig. 5. This may be due to the fact that at an optimum stirrer speed, contact between enzyme and bran along with cell rupturing process occurs at an adequate level and the process of cell rupturing results release of oil from ruptured cell of bran at a significant amount.

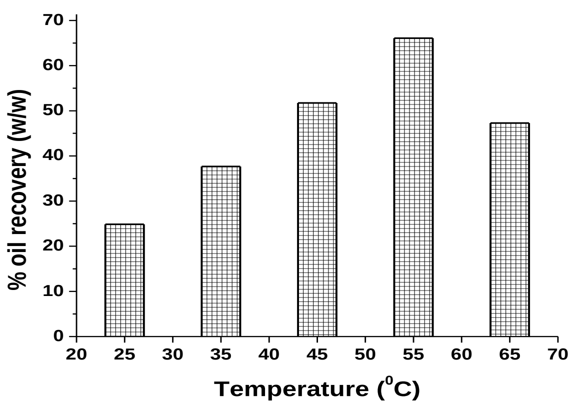

Fig. 2 Effect of temperature on oil recovery by enzyme-assisted aqueous extraction of RBO from sieved bran.

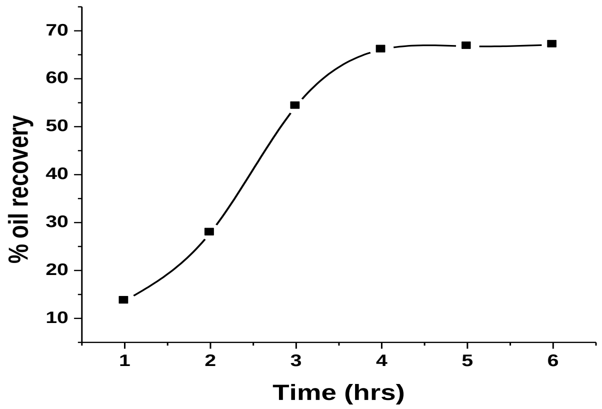

Fig. 4 Effect of time of stirring on oil recovery by enzyme-assisted aqueous extraction of RBO from sieved bran.

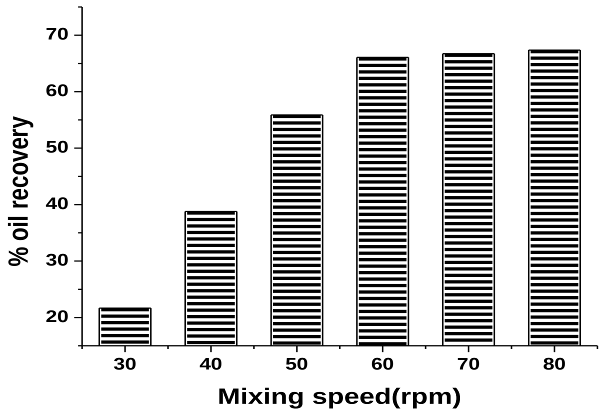

Fig. 5 Effect mixing intensity on oil recovery by enzyme-assisted aqueous extraction of RBO from sieved bran.

F. Effect of ratio of bran to water for enzyme-assisted aqueous extraction of RBO from bran

Effect of ratio of rice bran to water for the extraction of RBO from bran has been evaluated here at 55^0C maintaining at pH 7 in the presence of combined enzymes Neutrase and Celluclast (1% each) for 4hrs at 60 rpm. Fig. 6 shows that 1:10 ratio of rice bran to water contributed maximum oil recovery in the given reaction conditions. Further increasing amount of water in the extraction process did not contribute any significant effect on oil recovery.

Table 2 shows the characteristics of extracted RBO from bran using combined enzymes Neutrase and Celluclast. It has been observed from the Table that though oil yield is higher (22.38±0.41%) in solvent extraction method compared to enzymatic extraction process (19.07±0.26%) but the quality of oil is better in the latter technology. Enzymatic aqueous extraction of RBO contains lesser amount of FFA (0.79±0.03%) than hexane extracted RBO (3.78± 0.33%). Peroxide value and anisidine value are also less in enzyme-assisted aqueous extracted oil which conforms to the good quality of RBO along with containing sufficient quantity of oryzanol which is equal to solvent extracted oil. Apart from the results obtained from Table 2, enzyme-assisted aqueous extraction method is less energy intensive and more environment friendly.

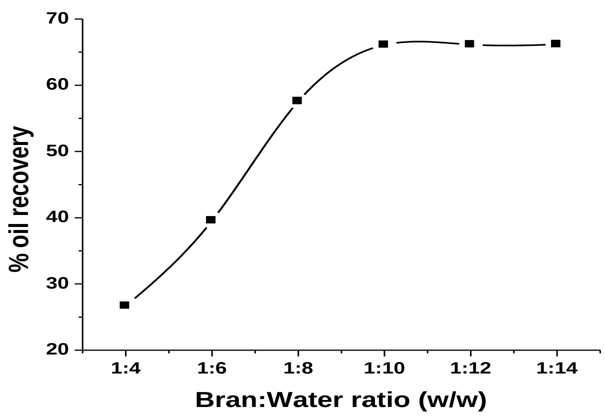

Fig. 6 Effect bran to water ratio on oil recovery by enzyme-assisted aqueous extraction of RBO from sieved bran.

TABLE 2. CHARACTERISTICS OF RBO FROM ENZYME-ASSISTED AQUEOUS EXTRACTION OF BRAN

Characteristics (%, w/w)	RBO from Enzyme-assisted aqueous extraction	RBO from n-hexane extraction
Oil yield	19.07±0.26	22.38±0.41
FFA	0.79±0.03	3.78± 0.33
Oryzanol	1.98 ± 0.04	1.99± 0.21
Unsaponifiables	1.66 ± 0.33	2.11± 0.27
P content	0.11 ± 0.01	0.11± 0.06
Peroxide value	2.36 ± 0.17	5.87± 0.47
Anisidine value	1.03±0.05	2.67± 0.41

V. CONCLUSION

The present study deals with the evaluation and quantification of the effects of different extraction parameters in aqueous enzymatic applications on the quality of oil during rice bran oil extraction. Study includes the process of enzyme assisted aqueous extraction of rice bran oil from bran and also the extraction parameters like pH, temperature, concentration of enzymes, mixing time, mixing speed and ratio of bran to water have been optimized. Here, combined enzymes Neutrase and Celluclast have been applied and these enzymes contribute significantly towards oil extractability. Study also shows that extraction parameters have a noteworthy role for the maximum production of rice bran oil from bran. Quality of the enzyme-assisted aqueous extracted rice bran oil is also analyzed and it has been concluded from the analysis that the quality of the oil is better than conventional one indicating that the hazardous solvent can be avoided in the extraction process. Again, compare to enzymatic aqueous extraction method, solvent extraction method gives better result with respect to oil yield, but the former process consumes less energy along with its eco friendly nature. The outcome of our research analysis will assist the future researchers to optimize the extraction parameters for different edible and non edible vegetable oils with the help of aqueous enzymatic method.

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