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### **Optimization of Rice Bran Fermentation Conditions Enhanced by Baker's Yeast for Extraction of Protein Concentrate**

\*Chinma, C.E.<sup>1,2</sup>, Ilowefah, M.<sup>2</sup> and Muhammad, K.<sup>2</sup>

#### ABSTRACT

The rice bran fermentation conditions for extraction of protein concentrate was enhanced by the use of baker's yeast at optimized conditions using response surface methodology (RSM). A central composite design with three independent variables: fermentation temperature (25 to 35°C), yeast concentration (1 to 5%) and fermentation time (10 to 24 h) was used to study the response variable (protein yield). Results indicated that the generated regression model represented the relationship between the independent variables and the responses. Also, all linear terms, two quadratic terms (fermentation temperature and time) and all interactive terms had significant (p < 0.05) effect on the protein yield. The optimum conditions for yeast pretreatment of rice bran protein extraction were achieved at 30°C for 17 h using 3% yeast concentration to obtain a protein yield of 23.37%, which showed no significant difference (p  $\geq$  0.05) from the response surface methodology predicted protein yield (23.02%). The use of baker's yeast in the fermentation of rice bran for extraction of protein concentrate can be more effectively used to improve the extraction yield compared to natural fermented (15.43%) and untreated rice bran (10.16%).

Keywords: Rice bran, fermentation, baker's yeast, protein extraction, response surface methodology.

#### Introduction

Rice bran is the by-product obtained during milling of rice grain which contains 12 to 15% protein (Zhang *et al.*, 2012). Rice bran protein is a good source of well-balanced amino acid and contains hypoallergenic protein which is desirable in infant food formulation (Xia *et al.*, 2012). Rice bran protein has enormous health benefits and good potentials in food industry applications (Fabian and Ju, 2011). Rice bran protein concentrate and isolate are not commercially produced due to lack of commercially feasible extraction method (Fabian and Ju, 2011). Fabian and Ju, (2011) reported that several treatment methods (physical, heat, enzymic and chemical) have been used in the extraction of protein from rice bran. The authors further reported that the use of enzyme and subcritical water treatment showed promising protein yield among all the available pretreatment methods, but the relative expensive cost of enzyme and the quality of protein extracted at relatively high temperatures for subcritical water treatment constitutes limitations to the use of the methods due to reduced protein functionality (Fabian and Ju, 2011). Shih (2003) found that treatment of rice bran by chemical, enzyme and heat methods prior to protein extraction could affect, often adversely, the protein functionality.

Fermentation of wheat bran with baker's yeast enhanced protein extraction and functionality

<sup>&</sup>lt;sup>1</sup> Department of Food Science and Technology, Federal University of Technology, Minna, Nigeria

<sup>&</sup>lt;sup>2</sup> UPM-BERNAS Research laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

<sup>\*</sup> corresponding author: chinmachiemela@yahoo.com

(Katina et al., 2006; Katina et al., 2012). There is paucity of information in literature on optimization of the fermentation conditions enhanced by the use of baker's yeast for extraction of protein concentrate. In this study, the use of response surface is important since the efficiency of protein extraction of fermented rice bran may be affected by fermentation temperature, time and yeast concentration, and their effects may be independent or interactive. According to Rustom et al. (1991), RSM is employed when many factors and interactions affect desired response. Also, RSM provides relevant information in the shortest time with the least number of experiments (Firatligil-Durmus and Evranuz, 2010) and generates a mathematical model which describes the chemical or biochemical process (Bas and Boyaci, 2007). The study investigated the production of rice bran protein by fermentation of rice bran using baker's yeast under optimized conditions.

# Materials and Methods *Materials*

Bran from rice variety MR 219 was obtained from KBB Sekinchan, Malaysia. Commercial baker's yeast (Eagle QS6540 2801 0001, China) was purchased from a supermarket in South City Serdang, Selangor Malaysia.

### Optimization of rice bran fermentation conditions for extraction of protein concentrate

The effect of three independent variables  $x_1$  (fermentation temperature),  $x_2$  (yeast concentration) and  $x_3$  (fermentation time) on protein yield (Y<sub>1</sub>) was evaluated using response surface methodology (RSM). A central composite design (CCD) was used to study the main and combined effects of fermentation process variables on the protein yield in order to create model between the variables; and use variables to optimize rice bran fermentation conditions for extraction of protein concentrate. Twenty runs based on CCD and six center points with three independent variables were used (Table 1). The independent variables range studied were fermentation temperature (25 to 35°C), yeast concentration (1 to 5%) and fermentation time (10 to 24 h). Based on the fermentation conditions as specified in the RSM design (Table 1), 100 g of rice bran, 350 ml of water and a specific amount of baker's yeast (based on the concentration specified in the RSM design) was mixed in a 1000 ml beaker and fermented according to the experimental design in a fermenting chamber (Binder KBF-240 Germany) to obtain yeast fermented rice bran batter. Fermented rice bran batter was dried in an air oven at 45 °C for 6 h and then ground to pass 100  $\mu$ m sieve size to obtain yeast fermented rice bran flour (YFRBF).

# Preparation of naturally fermented rice bran flour

In order to compare the yield of protein concentrate from optimized yeast fermented rice bran flour with protein concentrates from natural and unfermented rice bran flour, 100 g of rice bran and 350 ml of water without baker's yeast was used for the preparation of natural fermented rice bran flour (NFRBF)(at room temperature of 25°C for 20 h) based on the method described by Katina *et al.* (2012) while untreated rice bran flour (UFRBF).

### Preparation of rice bran protein concentrates

The method of Chandi and Sogi (2007) was adopted in the preparation of rice bran protein concentrate with slight modification in terms of centrifugation speed (at  $3000 \times g$ ) and time (20 min), and drying method (freeze drying). Yeast fermented rice bran flours obtained (based on RSM design), NFRBF and UFRBF were separately defatted using petroleum ether (in flour to solvent ratio of 1:3) at setting 7 in a lab stirrer for 30 min, and centrifuged at 3000× g for 20 min at room temperature. The defatted rice bran flours were dried overnight in a fume hood to remove residual solvent, sieved through 100 µm sieve and stored in a plastic bag at 5°C. The defatted flours were separately suspended in distilled water (1:10). The slurry was set at pH 9 using 4 M NaOH solution, stirred for 30 min and the resultant slurry was adjusted to pH 4.5 using

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