Quality and stability of rice bran protein powder at different storage condition

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Abstract Rice bran contain higher amount of protein, vitamins, essential fatty acids, minerals, dietary fiber and sterol attribute to large food application. The possible application of rice bran protein in food industry are hampered due to the limited information on its stability which may be caused by the rancidity due to the exposure of its oil to lipase. Therefore, stability study for protein extracted form rice bran is essential to evaluate the protein quality at different possible storage condition. Three different storage condition was evaluated; 1. Sample 1 (Store in freezer), 2. Sample 2 (Store in close container in room temperature) and 3. Sample 3 (Store in open container in room temperature). Protein was extracted using hot water process at temperature of 120°C for 20 minutes and dried using spray drying process. All sample are monitored for 5 days for its protein composition, water activity, moisture content and pH. The functional group of the sample where analysed using Fourier transform infrared radiation (FTIR). As a conclusion, the storage condition gives an effect on the water activity and moisture content of the rice bran protein product. However, it not given the significance effect on protein concentration and pH.

1. Introduction
The annual world rice production is approximately about 460 million metric tons per years. The rice milling process will yield major product that are 70% of rice endosperm and minor product consisting of 20% of rice husk, 8% rice bran and 2% rice germ [1]. Rice bran is one of the valuable by product in milling process which normally utilized as feed stock of animal [2].

Rice bran has potential as a protein source as it contains a significant concentration of high-value components in nutraceutical and pharmaceutical applications. The quality of rice bran protein powder can be affected from the environmental condition due to the existence of lipids. The activation of lipase in rice bran can cause faster development of rancidity during the milling process since it is break down lipids’ substances into glycerol and fatty acids. This hydrolysis reaction is occurred right after rice milling process affected by the existence of lipase enzyme as a catalyst.

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The free fatty acids produced in this reaction are harmful compounds and make it unsuitable for human consumption and unfit for edible use. Due to this matter, the rice bran product has a limited application in food. The high fiber content in the rice bran also caused a possible hull contamination to the rice bran which usually not suitable to consume as a routine food [3]. However, by removing this oil and fiber element, a good quality of rice bran protein maybe produced. The produce rice bran protein can be utilized as a source of hypoallergenic proteins and may provide as a good ingredient for formulations of infant food. The previous study done by Nurul Izzah et al (2016) on the extraction method had conclude several promising results where about 14 % of soluble protein can be extracted using various water extraction method such as hot water extraction and microwave extraction [4]. Therefore, the stability study of the rice bran protein is essential to evaluate the effect of various storage conditions on the quality of this products.

2. Material and Methods

2.1 Sample source and preparation

Raw rice bran fresh was collected from a local rice mill in Perlis, Malaysia. The collected rice bran was packed in air tight double-layered polythene bag and stored at -4°C prior to experimental procedure to prevent the hydrolysis. For extraction process, 100g of rice bran was weighed by using analytical balance before mix it with two litres of distilled water. The mixture were subjected to autoclaving process at temperature of 120°C for about 20 minutes [5]. After that, the extracted rice bran would be cooled down at room temperature. The vacuum filter was used to separate the rice bran protein extract from the slurry. Rice bran protein was proceeded for the spray drying method to produce a rice bran protein powder at the temperature of 150°C and feed flow rate of 30%. After drying process, the rice bran protein powder was stored in air tight double-layered polythene bag prior to experimental procedure.

2.2 Stability study at different storage condition

To evaluate the stability of rice bran protein powder, the simulated storage condition was created as tabulated in Table 1. The protein powder in each condition was observed for 5 days.

| Type of sample | Storage condition |
|---------------|------------------|
| Sample 1      | The sample was being sealed with polyethylene bag and stored in freezer at 4°C and 1 atm |
| Sample 2      | The sample was being sealed with polyethylene bag and placed in room temperature at 25°C and 1 atm |
| Sample 3      | The sample would be exposed freely to the surrounding at 25°C and 1 atm |

2.3 Protein concentration

The protein concentration in the extracted rice bran solution was measured using UV-Vis Spectrophotometer (Brand: Shimadzu, Model: UV-1800, Japan) based on Bradford reagent as indicator. A standard calibration curve of bovine serum albumin (BSA) was developed for this purpose.

2.4 Water activity

The water activity (aw) of the powders was determined at 25°C using Aqualab Series 3TE water activity meter (Decagon Devices, Pullman, Washington, US) equipped with thermolectric system that allows the instrument to maintain a set chamber temperature throughout the measurement [6].
2.5 Moisture content and pH
The moisture content of rice bran powder was determined by using moisture analyser. It consists of weighing units and heating units (infrared technology). A highly sensitive precision balance was built into the unit that allows for detection of weight changes of 1.0 mg and 0.01% moisture loss. About 1.0-1.5 grams of rice bran powder for each moisture reading. The pH values of each sample was measured in homogenate prepare of 1 gram of rice bran protein powders with 80 ml distilled water using pH meter (Model 340, Mettler-Toledo GmbH, Schwerznbach, Switzerland). All the sample were repeated for three times to get average reading of diluted powders [6].

2.6 FTIR analysis
The secondary structures of rice protein powder after 5 day for sample 1, 2 and 3 was characterized by using Fourier Transform Infrared (FTIR). The dried protein samples was mixed with KBr and grounded, and then pressed into a pellet. FTIR spectra was obtained in the wave number range from 600 cm⁻¹ to 4,000 cm⁻¹ during 32 scans with 4 cm⁻¹ resolution using a Nicolet 5700 FTIR spectrometer (Thermo Nicolet Corporation, USA).

3. Results and Discussion
3.1 Water activity on rice bran powders in different storage condition
The water activity (aw) of the rice bran powders at different storage condition was showed in Figure 4.1. The rice bran powders were analysed in three different storage conditions which has a values between 0.4 to 0.62. Sample 1 and 2 which keep in freezer and container, respectively had a range values between 0.40 to 0.47, and were thus relatively stable from a microbiological point of view, which is no microbial growth occurs in food products with aw<0.6. Meanwhile, sample 3 which exposed to surrounding showed the values between 0.60 to 0.62. The presented values in exposed surrounding showed that there were microbial growth in the powders [7], for example Osmophilic yeasts (Saccharomyces rouxii), few molds (Aspergillus echinulatus, Monascus bisporus). From a physicochemical point of view, aw can be defined as the ratio of vapour pressure of water in a food and the vapour pressure of pure water at the same temperature [8]. The aw describes the extent to which water present in a food is bound, and therefore its availability to participate in certain reactions, which were having a major influence on microbial, chemical and physical stability of food.

![Figure 1. Water activity of rice bran protein powder in three different storage conditions](image-url)
3.1 Protein concentration in rice bran protein powder in different storage condition

Protein content in rice bran powder is essential nutrient in which bran have a nutritionally and commercially values. Thus, the processing method need to ensure the non-deterioration of protein content [9]. Figure 2 showed the protein concentration for three sample at different storage condition for five days observation. The values of protein concentration were varied from 8.16 to 12.65%. The protein content of rice bran (control) was observed in Day 0 which were 11.45%, whereas the highest protein content showed in freezer was 12.65%. Compared to these three different conditions, it was predicted that freezer and container storage would be the best condition to store the samples.

![Figure 2: Protein concentration at different storage condition.](image)

3.3 Characterization of Rice Bran Protein Powder using FTIR

The FTIR spectrum of rice bran protein powders stored at different conditions from 4000-600 cm\(^{-1}\) were showed in Figure 3 and functional group observed in powders as shown in Table 2. Based on Figure 3, the general spectra profile of the rice bran powders shown FTIR spectra of the rice bran powders with different conditions were similar, but a few of characteristics band is different in wavenumbers or absorbance. There was a wide band that is indicated at 3600 shifted to 3000 cm\(^{-1}\) which was allocated a O-H stretching bond of hydrogen bond hydroxyl group mainly from cellulose.
The three different conditions were studied in Figure 3 shown that rice bran powder that kept in freezer represented higher peak of hydrogen bond compare to others conditions. This may due to hydration or rehydration of water on the surface of powder. The band around at 2922 cm\(^{-1}\) represented the C-H vibration of cellulose. Furthermore, the band at 1645 cm\(^{-1}\) in the spectrum of rice bran powders with different conditions were assigned as the adsorbed water bending vibration in cellulose. The band around at 1370 cm\(^{-1}\) were attributed as characteristic bending or stretching of different groups of cellulose and lignin [10]. The cellulose are a lignin structure in rice bran was probably due to the possible cellulosic material that contain in rice bran. It was known that rice bran consist of cellulose, fat, high quality of oil and protein, mineral, dietary fibre and others components. The food waste especially agricultural residue like rice bran contains lignocellulose as a major component [11].

![Figure 3 Comparison of FTIR spectra for different sample. a) Sample 1 b) Sample 2 c) Sample 3](image)

**Table 2. Functional observed in rice bran powder**

| Wavenumber, cm\(^{-1}\) | Functional Group                                      |
|-------------------------|-------------------------------------------------------|
| 3232                    | O-H stretching hydrogen bond                           |
| 2922                    | CH and CH2 stretching                                  |
| 1645                    | COO- stretching                                       |
| 1370                    | C=O stretching or bending of the cellulose and lignin  |
| 893                     | C-O-C stretching at β-glycosidic linkage              |

### 3.4 Rancidity and moisture content

Table 3 show the pH and moisture content of rice bran protein at different storage condition. The pH values of rice bran protein approximately about 6.85 maintain for 5 days all storage condition. This possibly due to the thermal effect on the drying process which act as the sterilization process. The water base extraction process also reduced the possible for rice bran oil residue in the protein powder. Residue oil in the product are main caused the of the powder rancidity due enzymatic hydrolysis of this oil. The rice bran oil has shown nearly neutral pH level which is 6.9 after minimum days of storage. Several studies have shown that the stabilization of rice bran oil, by retaining different
criteria, but a new stabilization method has reported that by controlling the pH level of the rice bran oil can reduce the activity of lipase in rice bran oil for longer period of storage, where lipase activity of rice bran oil is increasing during storage, while the pH value is decreasing.

**Table 3.** The pH and moisture content of the rice bran protein for different sample.

| Day | Sample 1 | Sample 2 | Sample 3 |
|-----|----------|----------|----------|
|     | pH       | Moisture content (%) | pH       | Moisture content (%) | pH       | Moisture content (%) |
| 1   | 6.88     | 7.41     | 6.82     | 7.41     | 6.81     | 7.41     |
| 2   | 6.84     | 7.30     | 6.85     | 7.02     | 6.81     | 12.83    |
| 3   | 6.85     | 7.59     | 6.86     | 7.52     | 6.86     | 12.09    |
| 4   | 6.80     | 6.67     | 6.86     | 7.03     | 6.85     | 13.00    |
| 5   | 6.83     | 7.76     | 6.80     | 8.91     | 6.79     | 14.29    |

The moisture content of the powders varied between 6.67 to 8.91 for sample 1 and sample 2. The low moisture content will produce a good quality of rice bran protein powders by improving the shelf life of the products and maintain its microscopic structure. Meanwhile, sample 3 show the rapid increase of moisture content from 7.41 to 14.29 % in five-day storage condition. The existence of pore in the powder particle allow water from the environment to absorbed increase its moisture content. The protein and sugar degradation may also give water as a reaction product, in effect increasing the moisture and water content [12].

4. Conclusion

The objective of the research study was to evaluate the effect storage condition on the rice bran powders quality with three different storage condition that are kept in freezer (sample 1), kept in container (sample 2) and kept exposed it to surrounding (sample 3). Water activity for sample 3 are increased as the time increased up to 65 % due to the microbial growth in the powders. Therefore, it can be concluded that the rice bran protein powder is not suitable to exposed into the room temperature. Besides that, the storage condition also observed are not given the significant effect on the protein concentration. The FTIR spectrum showing the existence of functional group that is useful information for rice bran powders such as OH groups, C=O stretching and vibration group and others group.

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