Short Communication

Foaming and Emulsifying Properties of Rice Bran Extracts Obtained by Subcritical Water Treatment

Pramote Khuwijitjaru[1*], Panit Nualchan[1] and Shuji Adachi[2]

[1]*Department of Food Technology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhon Pathom, Thailand*
[2]*Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan*
[*]*Corresponding author. E-mail address: kpramote@su.ac.th*

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Abstract

Rice bran, a by-product from rice milling process, contains a number of valuable components that impart either functionality or nutritive value. This study explored the properties of rice bran extract that obtained by treating the defatted rice bran with subcritical water at different temperatures (100, 150 and 200°C) and times (5, 10 and 15 min) in a batch-type vessel. The liquid extracts were analyzed for a protein content, foaming activity and emulsifying and emulsion stabilizing activities. We found that increasing the treatment temperature increased all of those values. Although treatment time affected trivially on those properties, long treatment time (15 min) at 200°C reduced the foaming activity and emulsion stabilizing activity of the extract significantly.

***Key Words*:** Defatted rice bran; Subcritical water treatment; Superheated water; Protein content; Foaming property; Emulsifying properties

Introduction

Treating low-cost bio-materials with subcritical water (i.e. water at temperature 100 to 347°C under pressure high enough to maintain its liquid state) was found to be a promising way for production of more valuable products. Previous studies have shown that amino acids and other organic acids could be produced from fish meat by-products (Yoshida et al., 1999), glucose and other oligomers could be produced from cellulose (Sasaki et al., 1998) and fatty acids could be obtained from vegetable oil (Holliday et al., 1997; Pinto and Lancas, 2006). In addition, subcritical water treatment of by-product may result in a new functional ingredient, for example, an okara that treated with subcritical water could reduce the blood pressure in hypertensive rats (Wakita et al., 2004).

Rice bran, a by-product which obtained from rice milling process, contains a number of valuable components, for example, proteins, carbohydrates and other phytochemicals that exhibit health benefits (Cheruvanky, 2003). Parrado et al. (2006) reported that the enzymatic extract of rice bran had a potential to be used as a functional food for the treatment and prevention of chronic disease such as cancer. To now, however, the bran is used commercially only for edible oil extraction and animal feed production. Treating rice bran with subcritical water has been studied by a few researchers. Sereewatthanawut et al. (in press) demonstrated that rice bran extract, that

obtained by subcritical water treatment, contained protein and reducing sugar, and could be used as a medium for yeast fermentation. Wiboonsirikul et al. (in press) reported that defatted Japanese black rice bran extract obtained by this method could function as antioxidant, emulsifier and emulsion stabilizer.

In this study, Thai rice bran was used as a raw material for investigating the effects of temperature and time of subcritical water treatment on some functional properties, particularly foaming activity and emulsifying and emulsion stabilizing activities, of the obtained extracts. This information should be useful for further optimization study of the process.

Materials and Methods

Materials

Rice bran was purchased from a local market in Nakhon Pathom, Thailand and defatted by mixing with hexane for 20 hours at ambient temperature (ratio of bran to hexane equals 1 to 20). Defatted samples were stored at -20°C until use. Protein content of the defatted bran was 142 mg/g-rice bran (Kjeldahl method, conversion factor of 5.95). To remove dissolved air, the distilled water was purged with nitrogen gas for 3 hours before use in extraction. Folin-Ciocalteu reagent, sodium dodecyl sulphate (SDS) and bovine serum albumin (BSA) were purchased from Fluka (Switzerland).

Subcritical water treatment

Subcritical water treatment of the rice bran was conducted using a batch-type vessel made of stainless steel (SUS 314) with a net volume of 94 mL (Taiatsu Techno Corporation, Japan). A 3.0 g of rice bran sample and 80.0 g of distilled water were added to the vessel. The vessel was closed and heated with a mantle heater. Treatment time was counted after heating up the vessel to the desired temperature (100, 150 and 200°C). Times need to heat up the vessel were 10 min for 100°C, 19 min for 150°C and 30 min for 200°C. After a specific treatment time (5, 10, and 15 min), the vessel was removed from the heater and cooled immediately with running tap water. Obtained liquid was filtered through a filter paper (Whatman No.2) and the volume was adjusted to 100 mL with distilled water. To evaluate the effects of temperature and time, the extracts were used for analyses without further concentration. The heat treatment was performed in triplicate for each condition with completely randomized design.

Analysis of Rice Bran Extract

Protein Content

Protein content was determined using Lowry method (Wrolstad, 2005). Bovine serum albumin (BSA) was used for preparation of a standard curve and the value was expressed as mg protein/g-rice bran.

Foaming Activity

Foaming activity was determined using the method described by Lawal and Adebowale (2006) with some modifications. Twenty milliliters of liquid extract was added into a 50 mL-cylinder. The aeration was performed using a rotor/stator homogenizer (Ultra Turrax disperser, IKA, Germany) at speed number 2 (ca. 13,000 rpm) for 2 min. The foaming activity (% volume) was calculated from the following equation:

$$\%\ \text{volume} = \frac{(\text{Volume of prepared foam} - \text{Volume of liquid extract})}{\text{Volume of liquid extract}} \times 100\ \% \quad (1)$$

Emulsifying and Emulsion Stabilizing Activities

Emulsifying activity and emulsion stabilizing activity were determined using the methods described by Uruakpa and Arntfield (2005) with some modifications. Oil-in-water type emulsion was prepared using the rice bran extract (4 mL) and palm oil (6 mL). The oil and liquid were emulsified using a rotor/stator homogenizer (Ultra Turrax disperser, IKA, Germany) at speed number 2 (ca. 13,000 rpm) for 1 min. For emulsifying activity evaluation, a 30 µL of emulsion was diluted with 20 mL of 0.1 % SDS solution and the emulsion activity index was determined from the absorbance of the diluted

emulsion at 500 nm. For an emulsion stabilizing activity evaluation, the remained portion of prepared emulsion was poured into a 10 mL test tube and allowed to stand at room temperature for 24 hr before the height of emulsion layer and the height of all mixture were measured. Emulsion stabilizing activity (ES) was calculated from the following equation:

$$ES = \frac{\text{Height of emulsion layer}}{\text{Heigh of all mixture}} \times 100\,\% \qquad (2)$$

Statistical Analysis

Factorial analysis of variance (two-way ANOVA) was used to indicate significance of two main effects and their interaction effect on each response. If the interaction was not significant and thus main effects can be used to interpret the effects of temperature and time separately, Tukey's HSD multiple comparison (P = 0.05) was used for comparing means for each main effect. If the interaction was significant, the main effects could not be used to summarized the effects of temperature and time separately and therefore the data were treated as one-way ANOVA with nine different temperature and time combinations and Tukey's HSD multiple comparison (P = 0.05) was used for treatment means comparison (Neter et al., 1990). All statistical analyses were performed with a statistical software R version 2.4.1 (R Development Core Team, 2006).

Results and Discussion

The color of rice bran extracts obtained by subcritical water treatment changed from white to yellow and to brown as the extraction temperature increased from 100 to 150 and to 200°C. The extracts at 200°C also had burnt smell.

Two-way ANOVA indicated that temperature-time interaction effect was not quite significant (P = 0.07) for the protein content, while both temperature and time affected the protein content significantly (P < 0.0001 and P = 0.0002, respectively). Protein content in the extracts largely depended on the extraction temperature. Tukey's HSD multiple comparisons showed that increasing temperatures from 100 to 200°C increased the protein content in the extracts from about 30 to about 130 mg/g-rice bran (Figure 1). The increasing of protein content agreed with the result of Wiboonsirikul et al. (in press) and that of Sereewatthanawut et al. (in press). Subcritical water treatment is known to promote hydrolysis reaction, thus more soluble peptides could be produced from low soluble protein in rice bran. The effect of time, however, was trivial and the treatment time longer than 10 min did not increase the protein content significantly. Longer time might allow further degradation of peptides into low-weight molecules (Yoshida et al., 1999). The maximum protein content was 136 ± 6 mg/g-rice bran, which obtained at 200°C, 15 min.

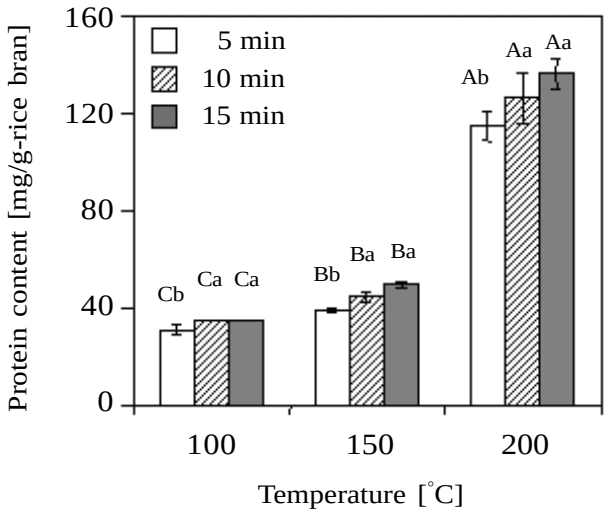

Figure 1 Protein content of rice bran extracts obtained by subcritical water treatment at different conditions. Bars within the same treatment time with different capital letters were significantly different and bars within the same treatment temperature with different small letters were significantly different (Tukey's HSD, P < 0.05).

Foaming activity is a measurement of ability of liquid to form foam after aeration. Proteins can help forming the foam because of their surface active property. Rice bran protein concentrate showed good

foaming and emulsifying properties comparable to those of casein (Chandi and Sogi, 2007). Two-way ANOVA indicated that temperature-time interaction effect was significant ($P = 0.04$) for foaming activity. Treatment time significantly affected the foaming activity only at 200˚C. The data were further analyzed using one-way ANOVA as described above. Treatments with higher temperature resulted in higher foaming activity of the extracts (Figure 2). All treatments at 150˚C gave the same foaming activity and we found that the foam obtained at this temperature was not stable and rapidly collapsed. Treatment at 200˚C, 10 min appeared to be the best treatment for giving foaming activity. Decreasing of foaming activity of the treatment at 200˚C, 15 min may be explained from degradation of peptides into small molecules.

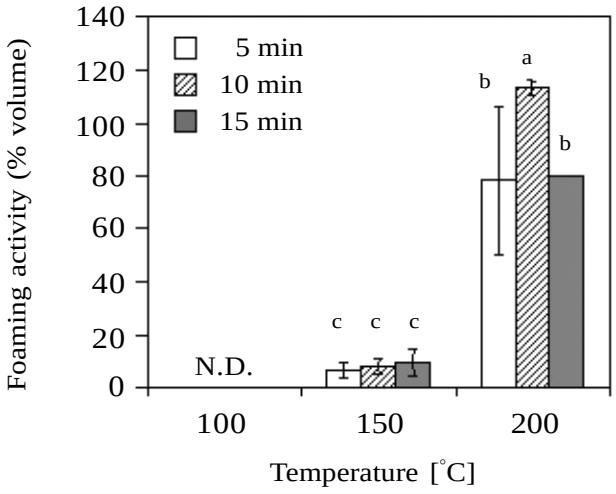

Figure 2 Foaming activity (% volume) of rice bran extracts obtained by subcritical water treatment at different conditions. Bars with different letters were significantly different (Tukey's HSD, $P < 0.05$). N.D. means that no foam layer was observed.

Two-way ANOVA indicated that temperature-time interaction effect was also significant for emulsion activity and emulsion stabilizing activity ($P < 0.0001$). Therefore the data were further analyzed using one-way ANOVA as described above. Treatments with higher temperatures resulted in higher emulsifying activity and emulsion stabilizing activity of the extracts (Figure 3 and Figure 4). All treatments at 200˚C and treatment at 150˚C, 15 min seemed to give equal highest emulsifying activity. Treatments at 200˚C, 5 and 10 min gave the highest value for emulsion stabilizing activity. At 200˚C with the longest treatment time of 15 min significantly reduced emulsion stabilizing activity. Wiboonsirikul et al. (in press) also reported that treatment of rice bran at higher than 200˚C reduced emulsifying and emulsion stabilizing activities and the authors demonstrated that loses of both activities related to disappearing of the high molecular-mass substances. As discussed above, subcritical water at high temperature can promote hydrolysis reaction of protein and produce smaller peptides. It was reported that larger peptides provide emulsion stability while smaller peptides reduced emulsion stability (Panyam and Kilara, 1996). Tuncturk and Zorba (2006) demonstrated that proteolysis of casein at lower level slightly increased emulsion stability, but higher proteolysis level decreased the value significantly.

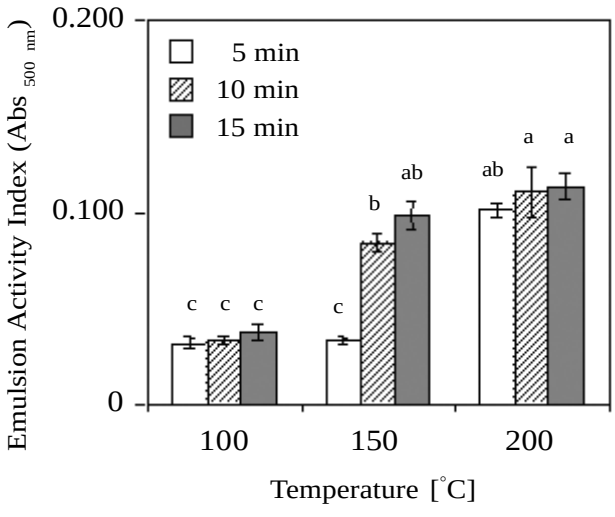

Figure 3 Emulsifying activity (Abs 500 nm) of rice bran extracts obtained by subcritical water treatment at different conditions. Bars with different letters were significantly different (Tukey's HSD, $P < 0.05$).

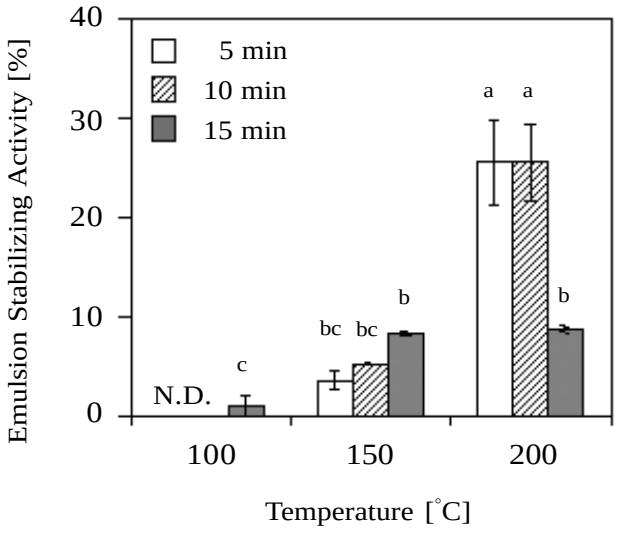

Figure 4 Emulsion stabilizing activity (%) of rice bran extracts obtained by subcritical water treatmen at different conditions. Bars with different letters were significantly different (Tukey's HSD, $P < 0.05$). N.D. means that no emulsion layer was observed.

Conclusion

Rice bran extract obtained by subcritical water treatment imparts some functional properties that are advantageous to food industry. Increasing treatment temperatures increased the protein content, foaming activity and emulsifying and emulsion stabilizing activities of the extracts. Long treatment time seemed to adversely affect those properties.

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