THE INFLUENCE OF CONDITIONS ON BETA-GLUCAN EXTRACTION FROM THAI RICE BRAN CULTIVARS AND THEIR BIOLOGICAL PROPERTIES

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Abstract - Rice bran is one of the important source of cell wall polysaccharides. This research purposes to study of the influence of several operational factors on the aqueous extraction of beta-glucan from three Thai rice bran cultivars, Khao Dawk mali 105 (KD-RB), Khao Pathum Thani 1 (KP-RB), and Khao Chinat 1 (KC-RB). Solvent:bran ratio, extraction time, pH, and temperature were identified as the important parameters affecting the beta-glucan extraction. Among four parameters, temperature seemed to be most influence. The maximum extractable beta-glucan from rice bran was obtained at s:r ratio 12.5; extraction time 3, pH 10, and temperature 60°C, that are defined as an optimal condition. The highest extractable beta-glucans are in the following order, KD-RB, KP-RB, and KC-RB with 1.905±0.06, 1.758±0.05, and 1.415±0.07 mg/100 g of rice bran, respectively. In vitro antioxidant and prebiotic activities assays indicated that three beta-glucan extracts had significant scavenging effect on DPPH radical and inhibition capacity of lipid peroxidation whereas they showed increased growth of probiotic bacteria such as Lactobacillus casei, Enterococcus faecium, and Streptococcus thermophilus. Their biological activity demonstrated that three rice bran beta-glcan extracts should potentially be a natural antioxidant and might serve as a probable ingredient for cosmetic and food products.

Keywords - Beta-Glucan, Extraction, Biological Properties, Thai Rice Bran

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple food and is grown in almost every part of Thailand. It is also the main export product of Thailand that produces about 19 million tons per year of rice. About 1.5 million tons of rice bran [1], which is the main by-product produced during milling of whole rice grain are produced. Rice bran is a source of several non-starch polysaccharides, which located in plant cell wall, especially (1-3),(1-4)-β-D-glucans or beta-glucans. Beta-glucans are a soluble dietary fiber and has been reported to have beneficial physiologic effects on human health and potential importance as an ingredient for the functional food industry. They occur in starchy endosperm, aleurone and bran cell walls of cereal grains such as barley, oats and rice. In rice, although bran contains 6% w/w beta-glucan that is less than starchy endosperm (20% wt beta-glucan), the beta-glucan of bran is free-formed structure whereas such of starchy endosperm bind with xylan in form of xyloglucan/mannan [2].

Cereal beta-glucans are unbranched homopolysaccharides comprised of β-D-glucopyranose linked by a mixture of β-(1-3),(1-4) glycosidic bonds. Their structures are specific that is the β-(1-4)-linked glucose chain is interrupted with the β-(1-3)-linkages. Most of consecutive β-(1-4)-linked segments are trimers and tetramers, but there is evidence for a minor amount of longer cellulose-like segments which have more than tetramer up to 14-mers. Two major oligomer units of cereal beta-glucan are 3-O-β-cellobiosyl-D-glucose (DP3), and 3-O-β-cellotriosyl-D-glucose (DP4), containing about 58-72% and 20-34%, respectively [3]. The cereal beta-glucan have become more interesting because they exhibits a diverse range of health benefits such as blood cholesterol lowering effect, anticancer effect and skin health promotion effects which include antioxidant activity [4]- [7].

Researches on rice bran were mostly focused on isolation of oil-soluble fraction, acting as antioxidant such as γ-oryzanol [8], tocopherols and tocotrienols [9]. Additional, a small portion of rice bran is processed into edible oil [10]. Although extraction of beta-glucan from rice bran was very few reports, many considerable works have been done with oat, barley and wheat brans [11]-[14]. As beta-glucan is a minor composition [2] of cereal bran, it is necessary to study on extraction condition for enhancing the extraction yield. Previous reports found that solvent:bran ratio, extraction time, pH and temperature have been identified as the important parameters in the beta-glucan extraction condition [15]. The genotype and growing-year of the cereal also affect the beta-glucan content [16]. Therefore, the main objective of this work was to study of the effect of several parameters on the performance of aqueous beta-glucan extraction from rice bran. Solvent:bran ratio, extraction time, pH and temperature, was studied in this work for three different rice bran cultivars, namely *Oryza Sativa* L. CV. Khao Dawk mali 105; KD-RB, *O. Sativa* L. CV. Khao Pathum Thani 1; KP-RB and *O. Sativa* L. CV. Khao Chinat 1; KC-RB. Furthermore, the biological effect of rice bran beta-glucan extracts were evaluated in vitro by scavenging activity to DPPH radical, inhibition of lipid peroxidation and prebiotic activity assay.

II. DETAILS EXPERIMENTAL

2.1. Chemicals

2,2'-diphenyl-1-picrylhydrazyl (DPPH), synthetic antioxidant butylatedhydroxyanisole (BHA), polyoxyethylene sorbitan monolaurate (Tween 20), and ferrous chloride were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA). Linoleic acid (analytical grade) was obtained Fluka (Buchs, Switzerland). The analytical grade ethanol and ammonium thiocyanate were purchased from Merck (Darmstadt, Germany). Lactobacillus MRS agar and broth (De Man, Rogosa, Sharpe) were purchased from Hi-Media Pvt. Ltd. (Mumbai, India). An analytical mixed-linkage beta-glucan kit was from Megazyme Int. (Wicklow, Ireland).

2.2. Preparation of rice bran

Rice bran powders of three Thai rice cultivars, namely KD-RB, KP-RB and KC-RB were obtained by milling rice grain in a local grinding mill, followed by sieving to separated residual grain and husk from rice bran. Rice bran was stabilized by heating at 100°C for 15 min. Each rice bran sample was packed in a polyethylene bag.

2.3. Beta-glucan Extraction Procedure

The extraction procedure was modified from methods of Wood et al. [17] and Ahmad et al. [18]. 20 g of rice bran powder were suspended in the desired volume of water, adjusting pH by 2 M (basic pH) at various extraction time and temperature. Mixture was continuously shaked at 200 rpm in a incubator shaker. The resultant mixture was added 2 M HCl to bring the pH to 5.0 to precipitate protein and centrifuged for 10 min at 6000 rpm. Solid material was separated and supernatant was adjusted to pH 7.0 by addition of 2 M NaOH. The residue in supernatant was removed by centrifuging. Beta-glucan was precipitated from the supernatant by the addition of the equal volume of ethanol (99.9% v/v) and left overnight at 4°C. The precipitate was collected after centrifugation for 15 min at 6000 rpm and dried in the vacuum oven at 50°C for 48 h. The dried rice bran beta-glucan extracts were stored in a sealed plastic tube and kept in desiccators until analysis.

Four conditions were used for study on the factor affecting the beta-glucan extraction of three varieties of rice bran:1) extraction at various volume of water (100, 150, 200, 250, and 300 ml) to reach the suitable solvent (water):rice bran ratio, obtaining a highest extractable beta-glucan, at constant pH, extraction time and temperature. 2) extraction at various extraction time (1, 3, 5, and 7 h) at suitable s:r ratio and constant pH and temperature 3) extraction at various pH (4, 7, 10, and 11) at suitable s:r ratio and extraction time at constant temperature. 4) extraction at various temperature (35, 45, 55, and 60°C) at suitable s:r ratio, extraction time and pH.

2.4. Analysis of beta-glucan content

Beta-glucan content was analyzed using the beta-glucan enzymatic assay kit "*Mixed-linkage beta-glucan*", (Megazyme Int.) by hydrolysing with lichenase (1000 U/ml) and beta-glucosidase (40 U/ml). The glucose produced was measured toward standard glucose using a glucose oxidase (>12,000 U)/peroxidase (>650 U/ml) reagent and then analyzed at absorbance 510 nm. All values of beta-glucan content were expressed as extractable beta-glucan was estimated following Eq. (1):

Extractable beta-glucan (mg/100 g of rice bran) = [beta-glucan extract (mg) x beta-glucan content (% db)]/100 **(1)**

2.5. DPPH radical scavenging activity

Free radical scavenging activity of beta-glucan extracts was determined by using a stable DPPH radical according to the Yen et al. [19], with slight modification. Samples were dissolved in absolute ethanol at 0.2-1.0 mg/ml. 1 ml test samples were mixed with 1 ml of freshly prepared ethanolic DPPH solution (0.1 mM). After shaking vigorously, the mixture was incubated at room temperature for 30 min in the dark and prior to measurement of the absorbance at 517 nm. BHA and ethanol were used as a standard antioxidant material and a control, respectively.

The scavenging activity of the DPPH radicals was calculated following Eq. (2):

Scavenging ability (%)

$$= [1/(A_{517\ nm,\ sample} - A_{517\ nm,\ control})] \times 100 \quad (2)$$

2.6. Inhibition of lipid peroxidation

The inhibition of lipid peroxidation was slightly modified from Mitsuda et al. [20]. Linoleate emulsion was prepared by mixing of linoleic acid (0.02 M), and Tween 20 (0.02 M) in 50 ml of sodium phosphate buffer (0.2 M, pH 7.0). The Tween 20 was add to obtain a stable emulsion. 0.5 ml of each beta-glucan extract (1 mg/ml) in distilled water was mixed with 2.5 ml of the freshly linoleate emulsion. This mixture was continuously shaked in incubator shaker and kept in darkness at 37°C. The accelerated oxidation of linoleic acid was measured after 4 and 7 days of thermal treatment. The determination of oxidation degree (as peroxides formation) was performed according to the ferric-thiocyanate method: 0.1 ml of the reaction mixture was added to 4.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 35% hydrochloric acid. After shaking vigorously, the mixture was kept at room temperature for 3 min, and then the absorbance was measured at 500 nm.

The percentage of lipid peroxidation inhibition was derived following Eq. (3)

Lipid peroxidation inhibition (%)

$$= [1/(A_{500\ nm,\ sample} - A_{500\ nm,\ control})] \times 100 \quad (3)$$

2.7. Prebiotic activity assay

The growth simulation of probiotic bacteria, namely *L. casei*, *E. faecium* and *S. thermophilus* in presence of rice bran beta-glucan extracts were determined according to modified method of Das, Baruah and Goyal [21]. The overnight grown probiotic bacterial culture (~ 1.5 x 10^8 CFU/ml) was inoculated separately to the 10 ml MRS broth, containing 2% (w/v) of glucose (as positive control) or 0.02% (w/v) of each beta-glucan extract and incubated at 37°C under anaerobic conditions for 24 h. The growth of the bacterial culture was classified as CFU/ml, by counting colonies which appeared on spreading 100 µl of culture after 24 h of incubation on MRS agar plate (55 g/l agar) and the plates were incubated at 37°C for 18 h. The prebiotic activity assay was expressed as total counts of probiotic bacteria (log_{10} CFU/ml).

2.8. Statistical analyses

All measurements were done in triplicate and data were express as mean ± standard deviation. Linear regression analysis for calculation of EC_{50} values were performed by using Frees statistical regression calculation online (https://www.easycalculation.com/statistics/regression.php). Statistical analyses were done using R program ((freeware, https://www.r-project.org/).

III. RESULTS AND DISCUSSION

3.1. The aqueous beta-glucan extraction from three rice bran cultivars

Effect of solvent (water):rice bran ratio

The s:r ratio was varied in the range 5-7.5 for investigation the effect of solvent content on beta-glucan extraction from the three varieties of tested rice brans, KD-RB, KP-RB, and KC-RB. As shown in Fig.1, the extractable beta-glucan of three rice brans showed a relatively constant increase when enhancing the water content until s:r ratio of 12.5. Similar result has been reported by Bhatty [11] that extracted the beta-glucans from oat and barley brans. Among four levels of s:r ratio, rice bran extracts at s:r ratio of 12.5 revealed the highest extractable beta-glucan value, that are 0.974±0.17, 0.969±0.03 and 0.793±0.09 mg/100 g of rice bran, for KD-RB, KC-RB and KP-RB, respectively.

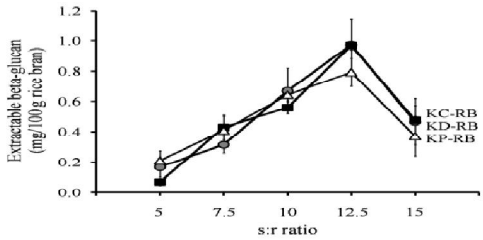


Fig.1. Effect of solvent (water):rice bran ratio on extractable beta-glucan for three different varieties of rice brans (extraction time 5 h; pH 10 and temperature 55°C). Each value is expressed as mean ± standard deviation (n=3, independent extractions).

Effect of extraction time

The influence of the extraction time was studied, as is shown in Fig.2. The maximum extractable beta-glucans of all extracts were performed at 3 h, that are 1.500±0.07, 0.966±0.04, and 0.965±0.02 mg/100 g of rice bran, for KD-RB, KC-RB, and KP-RB, respectively. Longer extraction time did not increase the amount of extractable beta-glucans, revealing that, after 3 h, equilibrium had been reached. However, at 5 and 7 h, displayed dramatically decreased beta-glucan content because of the activity of beta-glucanase (or Lichenase), locating in rice bran [22]. The enzyme specifics to β-(1-3),(1-4)-linkage of the polymer chain of beta-glucan, therefore it might be reactive at longer incubation time, affecting to reduce the amount of beta-glucan.

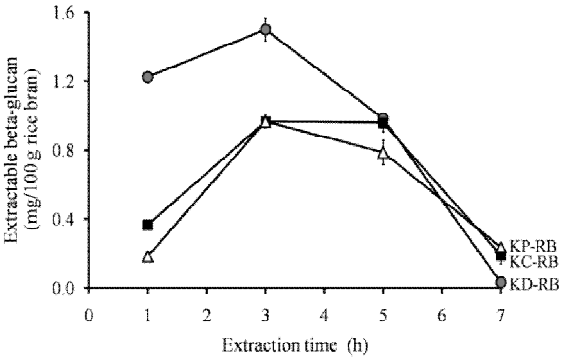


Fig.2. Effect of extraction time on extractable beta-glucan for three different varieties of rice brans (s:r ratio 12.5; pH 10 and temperature 55°C). Each value is expressed as mean ± standard deviation (n=3, independent extractions). Some error bars are too small to be visible.

Effect of pH

The pH value is also a important factor for beta-glucan extraction. In these experiment, pH was adjusted as acidic by addition of 2 M HCl whereas basic was adjusted by addition of 10% w/v Na_2CO_3. The effect of pH value on the beta-glucan extraction presents a similar trend for the three tested rice bran cultivars, as depicted in Fig.3.

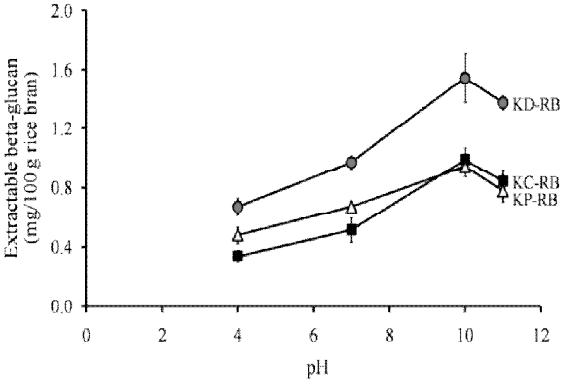


Fig.3. Effect of pH on extractable beta-glucan for three different varieties of rice brans (s:r ratio 12.5; extraction time 3 and temperature 55°C). Each value is expressed as mean ± standard deviation ((n=3, independent extractions). Some error bars are too small to be visible.

A slight increase in the extractable beta-glucan content was observed when water was adjusted from pH 7 to 10 that is in agreement with some results found in Bhatty [11]. In that work, extractable beta-glucan recovery from Azhul bran at pH 10 was higher than pH 7. However, a further pH was raised to 11, that caused a moderate reduction in the amount of beta-glucan extracted. The extractable rice bran beta-glucans at pH 4 presented lower value than pH 10, corresponding to result of Ahmad et al. [18]. All beta-glucan extracts showed the highest content at pH 10, that are 1.544±0.16, 0.989±0.07, and 0.945±0.08 mg/100 g of rice bran, for KD-RB, KC-RB, and KP-RB, respectively.

Effect of temperature

Three varieties of tested rice brans were carried to extract beta-glucan in the range 35-60°C for study the effect of temperature, As shown in Fig. 4, experimental result presents a relatively constant increase in the extractable beta-glucan content when increasing temperature similar to result of Dawkins and Nnanna [23] that has been used oat bran as raw material. At temperature 60°C, including s:r ratio 12.5; extraction time 3, pH 10 gave the highest contents of extractable beta-glucans that are 1.905±0.06, 1.758±0.05, and 1.415±0.07 mg/100 g of rice bran for KD-RB, KP-RB, and KC-RB, respectively. Therefore, these factors suggested as an optimal extraction condition. Even though, higher temperatures increasing the amount of beta-glucan extracted, lead to rise in the concentration of other contaminated polysaccharides in the extracts.

3.2. Scavenging ability to DPPH radical

The DPPH free radical, a stable free radical, is the most widely used and accepted assay for evaluation of the total antioxidant ability of natural compounds [24]. DPPH plays a role as an electron or hydrogen radical acceptor to became a diamagnetic molecule [25]. As shown in Fig.5, all beta-glucan extracts showed a dose-dependent scavenging abilities. Beta-glucan extracts of KD-RB, KC-RB, and KP-RB scavenged DPPH radicals by 66.5±3.9, 58.12±3.5 and 33.3±2.5% at 1 mg/ml, respectively whereas the scavenging ability toward DPPH radicals of the BHA was over 80% at 0.4-1 mg/ml. In a previous study, Glucagel, the high purity beta-glucan extracts showed lower free radical scavenging power than low purity beta-glucan extracts, barley balance and barley fibre rich fraction, because it had a lower polyphenol content compared with both low purity beta-glucan extracts [26]. Similarly, the scavenging ability toward DPPH radicals of among KD-RB, KC-RB, and KP-RB beta-glucan extracts at 0.2 mg/ml also displayed higher powder than Glucagel extracted by 70% ethanol [26]. This data could explain that not only beta-glucan appeared in the extracts but also other polyphenols, which contaminated in extracts, play an important role on antioxidant activity toward DPPH radical.

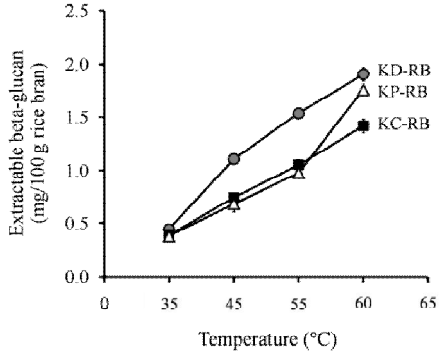


Fig.4. Effect of temperature on extractable beta-glucan for three different varieties of rice brans (s:r ratio 12.5; extraction time 3 and pH 10). Each value is expressed as mean ± standard deviation ((n=3, independent extractions). Error bars are too small to be visible.

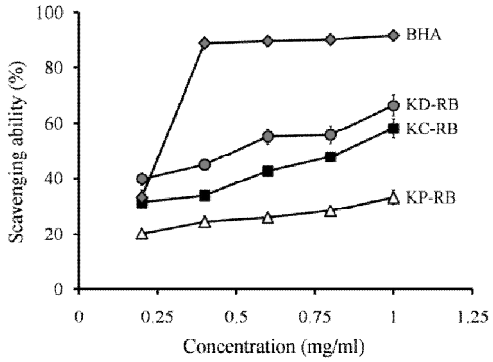


Fig.5. Free radical scavenging ability on DPPH of rice bran beta-glucan extracts and BHA. Each value is expressed as mean ± standard deviation (n=3). Error bars are too small to be visible.

According to linear regression analyses, the effective concentration of each beta-glucan extract was expressed as EC_{50} (mg/ml) value that is required to show 50% antioxidant ability. EC_{50} values of the scavenging ability on DPPH radicals for KD-RB and KC-RB were 0.55±0.02 and 0.73±0.08 mg/ml, respectively, revealing higher scavenging ability than EC_{50} value of KP-RB that was predicted as 2.62±0.54 mg/ml. The results indicated that rice bran beta-glucan extracts had scavenging ability toward DPPH radicals although they appeared lower ability than BHA.

3.3. Antioxidant activity in a linoleic acid emulsion system

The antioxidant effects of the beta-glucan extracts from three rice brans and BHA on the peroxidation of linoleic acid were studied at 1 mg/ml of each sample, as depicted in Fig.6. The peroxidation inhibition of linoleic acid of all beta-glucan extracts incubated between 4 and 7 days were no different. However, that of samples at 7 days revealed slightly lower than 4 days because the lipid peroxide occurred form linoleic acid emulsion system continuously decreased

at longer incubation time, attributing to small difference of absorbance between control and sample [27]. After incubation time for 4 days, beta-glucan extracts of KD-RB, KC-RB, and KP-RB inhibited lipid peroxide by 62.0±3.3, 65.3±2.9 and 63.1±0.1%, respectively whereas inhibition of BHA against lipid peroxide by 61.6±2.8%. The results indicated that rice bran beta-glucan extracts had ability to inhibit peroxidation of linoleic acid. Moreover, they were as effective as BHA in inhibiting the peroxidation of linoleic acid.

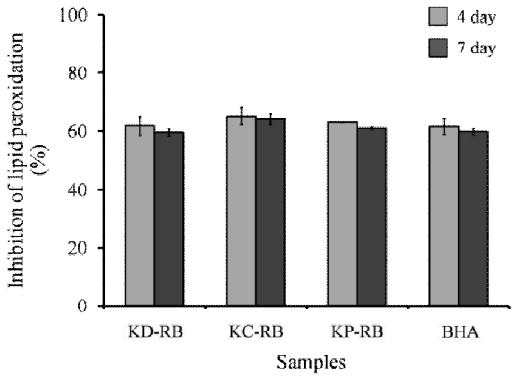


Fig.6. Inhibition of lipid peroxidation evaluated in the linoleic acid emulsion system of rice bran beta-glucan extracts and BHA (1 mg/ml) after incubation for 4 and 7 days. Each value is expressed as mean ± standard deviation (n=3). Some error bars are too small to be visible.

3.4. In vitro prebiotic activity of beta-glucan extracts

The enhancement in population of three probiotic bacteria, namely *L. casei, E. faecium,* and *S. thermophilus* in the presence of 2% (w/v) glucose and 0.02% (w/v) beta-glucan extracts are shown in Table 1. The growths of these probiotic bacteria in presence of beta-glucan and glucose were comparable at 37°C after 24 h incubation. The results showed that the beta-glucan extract from KD-RB stimulated the growth of *L. casei, E. faecium,* and *S. thermophilus*, by increasing its number from 10.14±0.024 to 10.45±0.009, 9.99±0.021 to 10.16±0.021, and 10.08±0.048 to 10.25±0.017 $\log_{10}$CFU/ml, respectively. The growth of *L. case* and *E. faecium*, were also simulated by the beta-glucan extract from KP-RB, increasing its number from 10.14±0.024 to 10.36±0.019, and 9.99±0.021 to 10.17±0.049 $\log_{10}$CFU/ml, respectively but that of *S. thermophilus* was not simulated. Similarly, the growth of *S. thermophilus* were very slightly simulated by the beta-glucan extract from KC-RB whereas that of *L. case* and *E. faecium*, were simulated by increasing its number from 10.14±0.024 to 10.35±0.03 and 9.99±0.021 to 10.42±0.031 $\log_{10}$CFU/ml, respectively. The data suggested that three rice bran beta-glucan extracts had ability to be prebiotics for these probiotic bacteria, especially showing affect on the growth of *L. case* and *E. faecium.*

Table 1: Growth of probitic bacteria in presence of rice bran beta-glucan extracts

Samples	Total counts of probiotic bacteria (log10 CFU/ml)		
	L. casei	*E. faecium*	*S. thermophilus*
Glucose	10.14±0.024 [b]	9.99±0.021 [a]	10.08±0.048 [a]
KD-RB	10.45±0.009 [d]	10.16±0.021 [b]	10.25±0.017 [c]
KP-RB	10.36±0.019 [c]	10.17±0.049 [b]	10.08±0.040 [a]
KC-RB	10.35±0.030 [c]	10.42±0.031 [d]	10.13±0.025 [b]

Each value is expressed as mean ± standard deviation (n=3). The means in the same column with different subscripts letters (a-) are significantly difference at $p < 0.05$ (paired t-test using R program).

CONCLUSIONS

The present study has revealed the influence of parameters on the operation of aqueous beta-glucan extraction from different Thai rice brans. Solvent:bran ratio, extraction time, pH and temperature was studied in this work. Of the four parameters compared for beta-glucan extraction from three rice bran cultivars, temperature seemed to be most influence. Moreover, experiments showed that the extractable beta-glucan of all rice brans, operating at varied levels of each parameter, showed a similar trend. The optimal condition in this study that maximizes the extractable beta-glucan form rice bran beta-glucan extracts was s:r ratio 12.5; extraction time 3, pH 10, and temperature 60°C. According the optimal condition, extractable beta-glucans from KD-RB, KC-RB, and KP-RB are 1.905±0.06, 1.415±0.07, and 1.758±0.05 mg/100 g of rice bran, respectively. The results of antioxidant activities found that the rice bran beta-glucan extracts had moderate ability toward stable DPPH free radical and lipid peroxide. Moreover, they could stimulate the growth of probiotic bacteria, namely namely *L. casei, E. faecium,* and *S. thermophilus.* It is first time on study of extraction and biological properties of beta-glucan from Thai rice bran cultivars. Therefore, rice bran should be explored as a potential source for beta-glucan production.

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