Comparison in Beta-Glucan Extraction from Mixed Defatted Rice Bran and Khao Dawk Mali Defatted Rice Bran Cultivars Using Taguchi Method of Experimental Design

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ABSTRACT

This research aims to compare the extraction of beta-glucan from mixed defatted rice bran and Khao Dow Mali defatted rice bran cultivars by studying factors affecting beta-glucan extraction. Five factors (four levels) were particle size of defatted rice bran (mm) (<75, 75-149, 150-250, non-separated), ratio of water (ml) to defatted rice bran (g) (5:1, 10:1, 15:1, 20:1), pH (4, 7, 8, 10), temperature (°C) (35, 45, 55, 60), and duration (h) (1, 3, 5, 7). According to the Taguchi method, the experiment was designed as an L16 (45) orthogonal array. The results found that the similarly optimal factors for extracting total beta-glucan from both defatted rice brans were 75-149 mm, 20:1, 55 °C, and 3 h. However, it has been shown that the optimal pH for both is different. The mixed defatted rice bran prefers to be extracted at pH 4, obtaining a total beta-glucan of 5.26 ± 0.05 mg/100 g of defatted rice bran. This is less than Khao Dow Mali defatted rice bran cultivars that prefer to extract at pH 10, which obtained a total beta-glucan of 12.35 ± 0.38 mg/100 g defatted rice bran. Based on the mean S/N ratio, the most influential factors were the ratio of water to defatted rice bran, followed by particle size, pH, temperature, and extraction time.

Keywords: *Extraction, Beta-glucan, Defatted rice bran, Taguchi method*

INTRODUCTION

Beta-glucan is a soluble dietary fiber that is found in cereal grains, mushrooms, and yeast, each of which has a different type of structural bond. As a result, there are different physical, chemical, and biological properties (Zhu et al., 2016). Beta-glucan from cereals is found mainly in the grain and bran parts of both oats and barley. It is also found in wheat, rice, and rye, among other cereals (Biliaderis, & Izydorczyk, 2007; Ain et al., 2019; Mejía et al., 2020). Currently, beta-glucan is widely used in pharmacology, cosmetics, and healthy foods. The advantages of beta-glucan in pharmacological fields were that it helped to control blood glucose levels, reduce the amount of cholesterol and LDL, increase HDL in the blood, and stimulate the body's immune system (Mejía et al., 2020; Atanasov et al., 2020; Shoukat & Sorrentino, 2021). In cosmetics, they are extensively used as an ingredient in skincare and sun protection products as they help the skin by moisturizing, firming, increasing

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elasticity, and reducing the inflammation of radiation-damaged skin (Shoukat & Sorrentino, 2021; Zhu et al., 2016; Glass, 2020, Du et al., 2014). In addition, beta-glucan is used as a functional ingredient in many healthy diets to replace the fat content and increase the benefits of the fiber content. For example, the beta-glucan extract from rice bran and barley flour is used to replace coconut milk in Thai desserts, as well as fat substitution with beta-glucan extract in non-fat yogurt and beef burger products (Inglett et al., 2014; Nikoofar et al., 2013; Szpicer et al., 2020).

The structure of cereal beta-glucan, usually called mixed-linkage (1,3)(,4)-β-D-glucan (hereafter referred to as "beta-glucan"), is a linear homopolysaccharide of D-glucopyranosyl residues, which are connected by a combination of β-(1,3) and β-(1,4) glycosidic linkage. Blocks of oligomeric cellulose that are consecutively β-(1,4)-linked to D-glucose and joined by a single β-(1,3) linkage make up the structure. While trimers (G3) and tetramers (G4) constitute the majority of the cellulose segments, longer cellulosic oligosaccharides (G5-G9) are also present in the polymeric chains (Lazaridou & Biliaderis, 2007). The amount of beta-glucan extracted from cereals depends on particle size, solvent, pH, temperature, and time (Benito-Román et al., 2011; Asif et al., 2010, Phuwadolpaisarn, 2017). It also depends on the type and strain of grains, which contain different amounts of beta-glucan in each part of the cereal grains, such as the endosperm, bran, and hull (Michaela & Kraic, 2006; Phuwadolpaisarn, 2021).

Defatted rice bran is a waste from rice bran oil production plants. According to King Rice Oil Group (2022), "Standard defatted rice bran.", it contains dietary fiber and protein (approximately 12% and 15%, respectively), which are higher than rice bran, but with a small amount of fat. Therefore, it can be used as a raw material for extracting beta-glucan. Thailand is one of the ASEAN countries that produces a large amount of rice bran oil, resulting in a lot of defatted rice bran waste. These wastes are mainly used as feedstock for high-protein animal feed and also used to make mushroom fertilizer. However, there are still many defatted rice bran wastes that have not been reused. In Thailand, most rice bran oil companies employ mixed cultivars of rice bran as raw materials to produce rice bran oil, for example, Thai Edible Oil Company. On the other hand, Surin Rice Bran Oil Company produces rice bran oil only from Khao Dawk Mali rice since Surin is one of the provinces in the Northeast that mainly grows this cultivar. The Taguchi method used in this study is a popular experimental design for studying the appropriate factors of substance extraction. By allowing optimization with a minimal number of trials, the Taguchi method is a special statistical design of experiments that offers a reliable design solution, enhances quality, and lowers costs. It is possible to simultaneously optimize many variables and get more quantitative data from fewer experimental trials (Idris et al., 2020; Chen et al., 2017). Therefore, this research aims to study the differences in beta-glucan extraction from mixed defatted rice bran cultivars and the Khao Dow Mali defatted rice bran cultivar by using the Taguchi method of experimental design. The factor affecting beta-glucan extraction from both defatted rice bran was studied. The five factors studied were the ratio of water to rice bran residue, rice bran particle size, pH, temperature, and extraction time. Each factor was studied at four levels. Using rice

bran as a raw material for beta-glucan extraction is another alternative for the application of defatted rice bran waste, which is a significant amount of industrial factory waste.

METHODOLOGY

Preparation of defatted rice bran

The defatted rice bran was washed by boiling it with 80% ethanol (100 g of defatted rice bran per 1 L of ethanol) at 80°C for 1 h. After that, the ethanol was removed by filtration with a vacuum system. The defatted rice bran was dried at 50°C. The defatted rice bran was processed through a sieve to separate the sizes of the bran before being used for beta-glucan extraction.

Taguchi Method of Experimental Design

The initial stage was identifying the variables that affected the beta-glucan extraction procedure. Five criteria, each with four values, were taken into consideration to find the best conditions for beta-glucan extraction from both defatted rice bran cultivars. The selected factors and their levels are shown in Table 1. The next stage was to build the matrix of experiments and choose the data analysis method after choosing the parameters and their values. The Minitab 18 program was updated to include the factors and their levels. The L_{16} (4^5) orthogonal array was created using the software's Design of Experiment (DOE) feature using a combination of the factors that were taken into consideration and their levels (Chen et al., 2017). The L_{16} orthogonal array information is shown in Table 2

Table 1 Process factors and their levels for beta-glucan extraction

Factor	Level			
	1	2	3	4
Particle size of defatted rice bran (μm)	<75	75-149	150-250	NS*
Ratio of water (ml) to defatted rice bran (g)	5:1	10:1	15:1	20:1
pH	4	7	8	10
Temperature (°C)	35	45	55	60
Time (h)	1	3	5	7

*NS is non-separated

Using the larger-the-better quality characteristics, the experimental data were analyzed to identify the optimum beta-glucan extraction factor and estimate the total beta-glucan content at the optimum conditions (Neag et al., 2022). Equation 1 can be used to determine the S/N ratio for larger-the-better quality characteristics by using

Minitab software. Verifying the results of the tests is the last stage of the Taguchi method. As a result, three tests were conducted utilizing the identified optimum conditions to validate the results. The confirmation experiment data were averaged, and this average value was compared to the expected average value.

$$S/N = -10\ log\left[\left(\Sigma \frac{1}{Y^2}\right) / n\right] \quad \ldots\ldots\ldots\ldots 1$$

where Y is the value of the response and n is the number of repetitions

Beta-glucan extraction

Following the L_{16} orthogonal array presented in Table 2, the experiments were conducted by extracting 1 g of defatted rice bran of various sizes and soaking them in different volumes of water. The water was adjusted for acidity with 2 M HCl or alkalinity with 2 M NaOH. The samples, placed in conical flasks, were subjected to an incubator shaker at 150 rpm with various temperatures and durations. After extraction, the sample was adjusted to pH 4.5 with 2 M HCl for the precipitation of proteins. Then, defatted rice bran and proteins were separated from the supernatant by centrifugation at 4000 g for 10 min, followed by filtration. The supernatant was neutralized by 2 M NaOH and then centrifuged at 4000 g for 10 min to remove the remaining residue. The supernatant containing beta-glucan was precipitated with 95% ethanol, adding a volume equal to the extract volume and then mixing well. The supernatant was allowed to stand at 4°C overnight for complete precipitation of beta-glucan. The precipitate was collected by centrifugation at 4000 g for 15 min and dried at 50°C for 2 days.

Analysis of beta-glucan content

The determination of the beta-glucan content was done using a mixed-linkage beta-glucan assay for cereal grains kit (K-BGLU, Megazyme, Wicklow, Ireland) according to the standard method of McCleary and Glennie-Holmes (McCleary & Glennie-Holmes, 1985). For the hydrolysis of cereal mixed-linkage beta-glucan with this approach, other polysaccharides are unaffected. 0.1 g of the extract was combined with 0.2 ml of 50% (v/v) aqueous ethanol and 4 ml of 20 mM sodium phosphate buffer (pH 6.5) to start the reaction. After mixing the sample on a vortex mixer, it was then incubated at 100°C for 1 min. The sample was vigorously shaken and incubated again for 6 min. The sample was then treated with 0.2 ml of lichenase (lichenase from *Bacillus subtilis*; 50 U/ml) and incubated for one hour at 50°C. During incubation, the sample was vigorously shaken every 15 min. 5 ml of 200 mM sodium acetate buffer (pH 4.0) was added to the sample and allowed to stand at room temperature for 5 ml, then centrifuged at 1000 g for 10 min. Three of the four supernatant tubes (0.1 ml) were added to 0.1 ml of beta-glucosidase (beta-glucosidase from *Aspergillus niger*; 40 U/ml) and 50 mM sodium acetate buffer (pH 4.0), In the fourth supernatant tube, add 0.1 ml of 50 mM sodium acetate buffer (beta-glucosidase was not added) to obtain the initial glucose content. These samples were then incubated for 15 min at 40°C. After 20 min at 40°C, 3.0 ml of the glucose oxidase/peroxidase (GOPOD) reagent was added. The amount of beta-glucan was determined at 510 nm (within 1 h). The beta-

glucan content was analyzed using standard D-glucose, and the positive control was oat and barley flour instead of the beta-glucan extract. The beta-Glucan content was calculated using the determined glucose quantity in Equation 2 and expressed as total beta-glucan (mg) in 100 g of defatted rice bran using Equation 3.

$$\text{Beta-glucan (\% w/w)} = \Delta A \times \frac{F}{mg} \times 8.46 \quad \ldots\ldots\ldots\ldots 2$$

where ΔA is the absorbance difference between the sample before and after beta-glucosidase treatment, mg is the sample's weight (in mg), and F is a factor for converting absorbance values to g of D-glucose.

$$\text{Total beta-glucan (mg/100 g defatted rice bran)} = G \times Y \times 10 \quad \ldots\ldots\ldots\ldots 3$$

where G is the beta-glucan content (% w/w), and Y is the yield of extracted beta-glucan (g/100g defatted rice bran)

Statistical Analysis

The experimental design was performed using the Taguchi method with Minitab software. (version 18, LEAD Technologies, Inc.). Statistical analyses were conducted using SPSS (v. 22; IBM, Amonk., New York, NY, USA). The results were calculated as the average of three replicate samples, and the results are expressed as the mean ± standard deviation. A significant difference between the total beta-glucan of mixed and Khao Dow Mali cultivars was determined using Duncan's new multiple range test at the $p < 0.05$ level.

RESULTS AND DISCUSSION

The beta-glucan content in mixed defatted rice bran and Khao Dawk Mali defatted rice bran extracts

The total amount of beta-glucan in the extract is more or less, depending on the extraction conditions. The factors that are important for the extraction of beta-glucan from cereal grains are the particle size of defatted rice bran, the ratio of water to defatted rice bran, pH, temperature, and duration (Benito-Román et al., 2011; Phuwadolpaisarn, 2017). As shown in Table 2, the 16 experiments according to the L_{16} orthogonal array revealed that the total beta-glucan content of Khao Dawk Mali defatted rice bran in experiment 8 is the highest at 8.00 ± 0.34 mg/100 g defatted rice bran, followed by experiments 12, 4, and 11 with contents of 5.17 ± 0.27, 4.39 ± 0.49 and 4.08 ± 0.38 mg/100 g defatted rice bran, respectively. In mixed defatted rice bran, the total beta-glucan is greatly lower than in the Khao Dawk Mali cultivar. Experiment 4 showed the highest total beta-glucan content of 3.93 ± 0.11 mg/100 g defatted rice bran, followed by experiment 8 with a content of 2.81 ± 0.13 mg/100 g defatted rice bran. Mixed defatted rice bran is a waste material obtained from rice bran oil

production from the mixture of rice bran and rice germ (King Rice Oil Group, 2022). This is the reason that the extracts from mixed cultivars had a lower total beta-glucan content than the Khao Dawk Mali cultivar. The Khao Dawk Mali defatted rice bran cultivar is exactly the wasted rice bran from the Khao Dawk Mali cultivar (Surin Bran Oil, 2022). In addition, the heterogeneity of the mixed defatted rice bran of various cultivars causes the total amount of beta-glucan to be unstable. Because each variety of rice bran contains a varied amount of beta-glucan, corresponding to the study of Phuwadolpaisarn (2021), it is important to determine the proportion of each variety when using mixed defatted rice bran for beta-glucan extraction.

The optimal condition for beta-glucan extraction of mixed defatted rice bran and Khao Dawk Mali defatted rice bran cultivars

The average values of the signal-to-noise (S/N) ratio for larger-the-better quality characteristics for each factor are presented in Figure 1 for beta-glucan extraction from both defatted rice bran cultivars. As shown in Figure 1, the level of factors that contribute to the highest total beta-glucan content is the factor level with the highest mean S/N ratio. Therefore, the optimal conditions for beta-glucan extraction from mixed defatted rice bran are 75-149 mm of particle size, a ratio of 20 ml of water to 1 g of defatted rice bran, and pH 4 at 55°C for 3 h. While the Khao Dawk Mali cultivar had the best extraction at the same level as the mixed cultivars but a different pH value of 10. The predicted total beta-glucan at optimal conditions for mixed defatted rice bran and Khao Dawk Mali defatted rice bran cultivars are 5.38 and 12.62 mg/100 g defatted rice bran, respectively. When the beta-glucan was extracted from defatted rice bran again according to the appropriate conditions. It was found that the mixed defatted rice bran extract had a beta-glucan content of 0.20 ± 0.04 % w/w, whereas the total beta-glucan content was 5.26 ± 0.05 mg/100 g defatted rice bran, which was a deviation from the predicted amount, representing 2.31% of error. In Khao Dawk Mali, the defatted rice bran extract had a beta-glucan content of 0.24 ± 0.04 % w/w, whereas the total beta-glucan content was 12.35 ± 0.38 mg/100 g defatted rice bran, which was a deviation from the predicted amount, representing 2.14% of error. A comparison of the optimal conditions between this study and other research is shown in Table 3. The optimal factors for beta-glucan extraction from defatted rice bran and barley were different in particle size, the ratio of water to defatted rice bran, and pH. The optimal temperature and time are at the same level. Although the beta-glucan content of defatted rice bran was lower than that of barley flour because barley is the major source of beta-glucan, the defatted rice bran could be used as a beta-glucan source for reducing industrial waste.

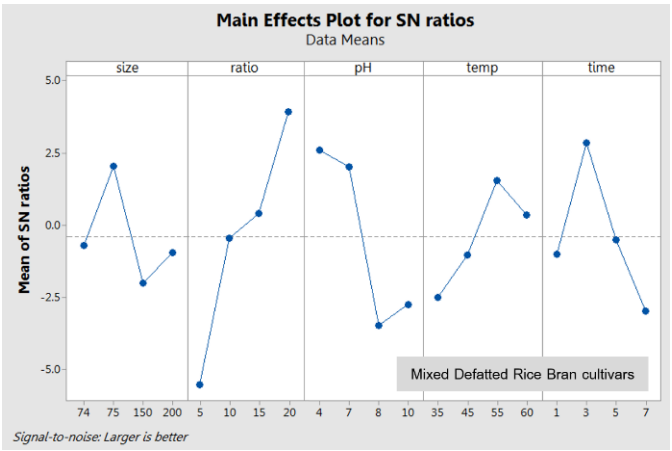
The factors with the highest average S/N ratio of both types of defatted rice bran were the same: particle size, the ratio of water to defatted rice bran, temperature, and duration. However, the optimum pH for extraction was different, i.e., mixed defatted rice bran was 4, while Khao Dawk Mali defatted rice bran was 10. This might be caused by a specific production procedure employed in some steps of the production of rice bran oil. As a result, the Khao Dawk Mali defatted rice bran and the mixed defatted rice bran from two producers of rice bran oil were different (King Rice Oil Group, 2022; Surin Bran Oil, 2022). According to the literature, beta-glucan

extraction from different strains of barley showed that the amount of beta-glucan extracted in alkaline conditions was mostly higher than that obtained in acidic conditions. In an acidic environment, there are fewer contaminants such as proteins and other carbohydrates (Mishra et al., 2020). The optimum temperature to extract beta-glucan from both types of defatted rice bran, corresponds to the optimum temperature for beta-glucan extraction from oats and barley, which is in the range of 45-63°C (Limberger-Bayer, 2014).

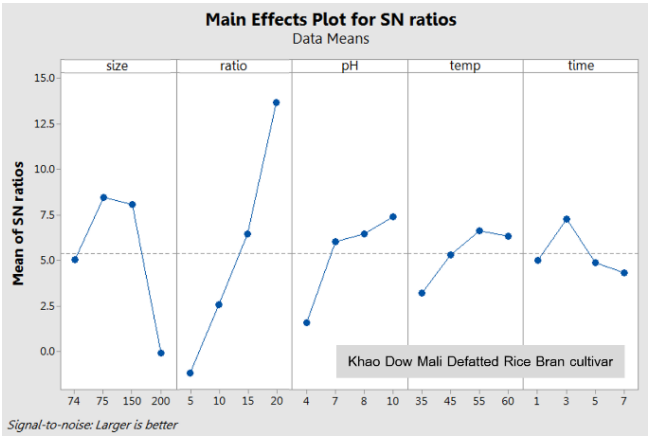
Table 2 The experimental L_{16} orthogonal array information and total beta-glucan content from mixed defatted rice bran and Khao Dawk Mali defatted rice bran cultivars for each of the sixteen trials.

Exp.	Factor					Total beta-glucan* (mg/100 g defatted rice)	
	Size	Ratio	pH	Temp	Time	Mixed cultivars	Khao Dow Mali cultivar
1	<75	5	10	60	7	0.32 ± 0.03^{i}	1.13 ± 0.32^{gh}
2	<75	10	8	45	1	0.56 ± 0.01^{h}	1.43 ± 0.27^{fg}
3	<75	15	7	35	5	1.04 ± 0.01^{f}	1.65 ± 0.26^{fg}
4	<75	20	4	55	3	3.93 ± 0.11^{a}	4.39 ± 0.49^{c}
5	75-150	5	8	55	5	0.61 ± 0.03^{h}	1.60 ± 0.32^{fg}
6	75-150	10	10	35	3	1.10 ± 0.03^{de}	2.41 ± 0.35^{e}
7	75-150	15	4	45	7	1.36 ± 0.06^{c}	1.76 ± 0.27^{f}
8	75-150	20	7	60	1	2.81 ± 0.13^{b}	8.00 ± 0.34^{a}
9	150-250	5	7	45	3	0.79 ± 0.03^{g}	1.60 ± 0.13^{fg}
10	150-250	10	4	60	5	1.21 ± 0.02^{d}	1.28 ± 0.19^{fgh}
11	150-250	15	10	55	1	0.81 ± 0.13^{g}	4.08 ± 0.38^{c}
12	150-250	20	8	35	7	0.54 ± 0.01^{h}	5.17 ± 0.27^{b}
13	mixed	5	4	35	1	0.52 ± 0.01^{h}	0.23 ± 0.02^{j}
14	mixed	10	7	55	7	1.11 ± 0.06^{de}	0.88 ± 0.26^{i}
15	mixed	15	8	60	3	1.10 ± 0.01^{de}	1.80 ± 0.23^{f}
16	mixed	20	10	45	5	1.04 ± 0.03^{f}	3.07 ± 0.20^{d}

*Mean with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.



(a)



(b)

Figure 1 Mean S/N ratio for beta-glucan extraction from defatted rice bran (a) mixed cultivars (b) Khao Dawk Mali cultivar

Table 3 The optimal conditions and beta-glucan content in this study and other research on the aqueous beta-glucan from cereal.

Source	Optimal factor					Beta-glucan content (% w/w)	Ref
	Size (μm)	Ratio (water : bran)	pH	Temp (°C)	Time (hr)		
Defatted rice bran (mixed cultivars)	75-149	20:1	4	55	3	0.20	This study
Defatted rice bran (Khao Dawk Mali)	75-149	20:1	10	55	3	0.24	
Barley flour (D24 cultivars)	100	5:1	8	55	3	4.42	Lazaridou & Biliaderis, 2007
Barley flour (H13 cultivars)	100	5:1	8	55	3	2.89	

Comparison of the influence of factors affecting beta-glucan extraction

The mean S/N ratio was calculated to analyze the influence of factors on beta-glucan extraction. It is calculated based on the difference between the mean S/N ratios of the highest and lowest factors. As shown in Table 4 and Table 5, it was found that the factors at levels 1, 2, 3, and 4 (according to Table 1) influencing beta-glucan extraction were different. The factor that most influenced the extraction of total beta-glucan was the ratio of water to defatted rice bran. This has more influence in Khao Dawk Mali defatted rice bran, which is 42.17%, than in mixed defatted rice bran, which is 32.07%. On the other hand, the particle size factor has more influence on beta-glucan extraction from mixed cultivars (24.00%) than from Khao Dawk Mali (13.72%). The mean S/N ratio of mixed cultivars in level 4 (non-separated) is -0.07, which is a really low value compared to others. This may be because mixed defatted rice bran contains many varieties of rice bran cultivars. Each variety has a different size of rice bran. This is consistent with the research of Abrahamsson (2020) and Salah et al. (2020), who found that particle size affects beta-glucan extraction from oat bran. Non-separated oat bran contains less total beta-glucan content than the individually separated particle sizes of oat bran that were extracted with high-temperature water. It reveals that the particle size separation process of defatted rice bran must be performed before beta-glucan extraction. It is more important for the extraction of beta-glucan from mixed cultivars than that of the Khao Dawk Mali cultivar. However, particle size separation is another important step in the aqueous extraction of beta-glucan. That will help filter the contaminated waste from the cereal (Salah et al., 2020). The pH value (20.69%), had a greater influence on the extraction of beta-glucan from the Khao Dawk Mali cultivar than mixed cultivars. While the duration had the least influence on beta-glucan extraction from both defatted rice brans, which is consistent with Abrahamsson (2020).

Table 4 Analysis of the influence of factors on the extraction of beta-glucan from mixed cultivars defatted rice bran.

Level	Mean S/N ratio of the beta-glucan extraction				
	Size	Ratio	pH	Temp	Time
1	5.04	-1.19	1.61	3.22	5.03
2	8.49	2.57	6.04	5.32	7.29
3	8.09	6.49	6.46	6.66	4.89
4	-0.07	13.69	7.44	6.36	4.34
(%)**	24.00	41.72	16.34	9.65	8.28

****** Influence (%) = 100 - (($\Sigma_{\text{main effect}}$ - main effect)/ $\Sigma_{\text{main effect}}$) x 100; $\Sigma_{\text{main effect}}$ = sum of main effect of factor; main effect = S/N ratio_{max}- S/N ratio_{min}

Table 5 Analysis of the influence of factors on the extraction of beta-glucan from Khao Dawk Mali defatted rice bran.

Level	Mean S/N ratio of the beta-glucan extraction				
	Size	Ratio	pH	Temp	Time
1	-0.71	-5.52	2.61	-2.50	-1.00
2	2.04	-0.46	2.01	-1.03	2.85
3	-2.01	0.41	-3.49	1.55	-0.50
4	-0.96	3.94	-2.76	0.35	-2.98
(%)**	13.72	32.07	20.69	13.75	19.77

****** Influence (%) = 100 - (($\Sigma_{\text{main effect}}$ - main effect)/ $\Sigma_{\text{main effect}}$) x 100; $\Sigma_{\text{main effect}}$ = sum of main effect of factor; main effect = S/N ratio_{max}- S/N ratio_{min}

CONCLUSIONS

The factor affecting the total amount of beta-glucan extracted from the two defatted rice bran cultivars that differed was the acid-base condition. Beta-glucan extraction from defatted mixed rice bran was well done at a pH of 4, but that from the Khao Dawk Mali cultivar was well done at a pH of 10. Thus, the total beta-glucan contents of both cultivars were different, with the amount of Khao Dawk Mali being higher than the mixed cultivars. From the mean S/N ratio, it was found that the most influential factor for beta-glucan extraction was the ratio of water to defatted rice bran. Other factors influenced the extraction of beta-glucan from the two types of defatted rice bran differently. The size of the defatted rice bran particles had a greater influence on the mixed cultivars. Therefore, the significant factors that would be of concern when using defatted rice bran as a raw material for beta-glucan extraction are the ratio of solvent (water) to defatted rice bran, the particle size of rice bran, and the pH value.

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