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Optimization of Acid Hydrolysis Conditions for Feruloylated Oligosaccharides from Rice Bran through Response Surface Methodolgy

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Response surface methodology (RSM) was employed to optimize the hydrolysis conditions with trifluoroacetic acid (TFA) to obtain the maximum amount of feruloylated oligosaccharides from rice bran. The TFA concentration and hydrolysis time effects on feruloylated oligosaccharides recovery are studied. The optimum hydrolysis conditions for maximizing feruloylated oligosaccharides recovery were 193 mM TFA concentration and 1.36 h of hydrolysis time. Under these conditions the corresponding acyl ferulic group quantity was 78.63 μg in 1 mL of hydrolysate. The model was experimentally verified with a satisfactory coefficient of R^2 (= 0.96). The quantity of acyl ferulic group in the feruloylated oligosaccharides, purified using Amberlite XAD-4, was 916.12 μg/g of rice bran under the optimum hydrolysis conditions. The proposed method accounted for 54.08% of the total acyl ferulic group in rice bran. The results suggest that the proposed conditions were useful in maximizing recovery of feruloylated oligosaccharides from rice bran.

KEYWORDS: Rice bran; feruloylated oligosaccharides; response surface methodology

INTRODUCTION

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one of the most abundant, ubiquitous hydroxycinnamic acids derived from phytochemical phenolic compounds. This acid exists widely in plants and is esterified with the arabinose of arabinoxylans in the cell wall (*1*).

Some studies have recently isolated feruloylated oligosaccharides from the hydrolysate of grain bran prepared with enzymatic or mild acid hydrolysis (*2*−*4*). The feruloylated oligosaccharides, 5-*O*-feruloyl-L-arabinofuranose (FA) and *O*-(5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX), were obtained from the enzymatic hydrolysate of refined corn bran insoluble dietary fiber. Feruloylated oligosaccharides have shown higher antioxidant characteristics than free ferulic acid in the microsomal lipid peroxidation system (*2*), reduced the oxidation of low-density lipoproteins (*5*), and protected rat erythrocytes against oxidative damage in vitro (*4*). Katapodis et al. (*6*) reported that the feruloylated arabinoxylotrisaccharide possessed strong antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl reduction assay and inhibited the copper-mediated oxidation of human low-density lipoprotein. These functions suggest that feruloylated oligosaccharides might have potential use in atherosclerosis prevention or other applications involving its antioxidant capacity to gain importance in the health food, pharmaceutical, and cosmetic industries.

Rice bran is a part of the brown rice kernel outer layer, produced from the brown rice milling process. Rice bran occupies about 10% of brown rice weight. A total of 40−45 million tons of rice bran is produced annually, mainly in the Far East and Southeast Asia (*7*). Rice bran contains a variety of nutrients and bioactive compounds. However, only a small amount has been used for oil production or stock feed or fertilizers. The arabinoxylan content in the rice bran cell wall amounts to about 50−55% (*8, 9*). The amount of ferulic acid is about 0.9% w/w rice bran dry matter (*10*). At present not much research has investigated the isolation of feruloyl arabinoxylans or developed techniques for producing feruloylated oligosaccharides from rice bran (*11*). Yuan et al. (*12*) found the reaction conditions for optimum feruloylated oligosaccharide production

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from wheat bran insoluble dietary fiber using response surface methodology (RSM).

This study used two-level factorial factor experimental designs, the steepest ascent method, and the central composite to determine the optimal condition for rice bran acid hydrolysis to produce feruloylated oligosaccharides. Trifluoroacetic acid (TFA) was used in rice bran hydrolysis, and feruloylated oligosaccharides were collected after the removal of TFA from the hydrolysate. We hope that the results of this research will provide the technique to recover feruloylated oligosaccharides from rice bran more efficiently and make some contribution to the creation of an economical value of rice bran, which has long been considered as conventional agricultural waste.

MATERIALS AND METHODS

Materials. TaiKung 9 (TK9) rice bran was provided by Hung Chang Rice Mill Factory. The bran was milled and passed through a 0.5 mm sieve. Rice bran powder was defatted immediately using hexane with the Soxhlet apparatus. The dry defatted rice bran was then kept in a sealed container until further treatment/analysis.

Mild Acid Rice Bran Hydrolysis. Defatted rice bran (0.25 g) was treated with 5 mL of TFA at 100 °C. The hyrdrolysate was centrifuged, and the supernatant was used for further studies.

Determination of Acyl Ferulic Group. The hydrolysate (100 μL) and defatted rice bran were saponified with NaOH (100 μL, 1 M) in the dark at room temperature for 2 and 18 h, respectively. The reaction was stopped by the addition of H_3PO_4 (150 μL, 1 M) ((*12*)). The acyl ferulic group of this solution was then analyzed using HPLC (Hitachi, Japan) with a chromatography column [Mightysil RP-18 GP 250−4.6 (5 μm), Kanto Chemical, Tokyo, Japan]. The column was maintained at 30 °C. The 20 μL sample was injected into the HPLC column and eluted with methanol/water/acetic acid (50:50:0.5) at a flow rate of 0.8 mL/min for 15 min. The absorbance of the eluate was continuously monitored at 320 nm. The ferulic acid cleaved from feruloylated oligosaccharides was identified by comparison of its relative retention time with that of the standard compound (ferulic acid).

Feruloylated Oligosaccharide Preparation. The supernatant prepared with the hydrolysate of 1 g of rice bran was applied onto an open column packed with Amberlite XAD-2 [previously washed with 95% (v/v) ethanol and then water]. Elution was carried out successively with 2 column volumes of distilled water, 3 column volumes of 50% (v/v) methanol, and 2 column volumes of methanol. The fraction eluted using methanol/water was concentrated and lyophilized to prepare the dry water-soluble feruloylated oligosaccharides for the acyl ferulic group determination (*12*).

Experimental Designs. *Factorial Design and Steepest Ascent.* A factorial design was used to approach the optimal region in the first optimization step. Two independent variables (x_1, TFA concentration; x_2, hydrolysis time) were employed at two equidistant levels (−1 and +1). The acyl ferulic group (μg/mL) quantity was determined as the response variable (Y). The rice bran concentration was 5% (w/v) in each run. In the factorial design, low and high factor settings were coded as −1 and +1, and the midpoint was coded as 0. Each experiment was performed three times at different times. The test data of the experimental design provide the estimation of the main effects of the factors (*14*), and a first-order regression model fitted to the data was obtained. The regression equation was adopted to approach the vicinity of the optimum through steepest ascent (*15*).

Central Composite Designs (CCD). A central composite design with five coded levels ($-\alpha$, −1, 0, +1, $+\alpha$) was performed to determine the contour of the response surface in the optimum region (*15*). For the two factors, this design was made up of a full 2^2 factorial with four cube points, augmented with three replications of the center points (all factors at level 0) and the four star points, that is, points having for one factor an axial distance to the center of $\pm\alpha$, whereas the other factor is at level 0. The axial distance α was chosen to be 1.414 to make this design orthogonal. To predict the optimal point, a second-order polynomial function was fitted to the experimental results. For two factors this equation is

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 \quad (1)$$

where Y, predicted response, is the quantity of acyl ferulic group in rice bran hydrolysate.

Data Analysis. Statgraphics Centurion XV (StatPoint, Inc.) was used for obtained experimental data regression analysis. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and its statistical significance was checked using an F test. The significance of the regression coefficient was tested using a t test.

RESULTS AND DISCUSSION

Factorial Design 2^2 and the Path of Steepest Ascent. The factorial design was used to determine the influence of TFA concentration and hydrolysis time on the acyl ferulic group yield (μg/mL) (**Table 1**). A linear regression equation was obtained:

$$\text{acyl ferulic group} = 49.22 + 15.30 \times \text{TFA concn} + 14.60 \times \text{hydroylsis time} \quad (2)$$

The R^2 statistic indicated that the model corresponded to 0.99 of the variability of data (**Table 2**).

Parameters of this equation have shown that two factors had a positive effect on feruloylated oligosaccharide production (**Table 2**). A steepest ascent experimental procedure was designed to approach the optimal condition of these two factors on the basis of this regression equation. The experimental design and data are shown in **Table 3**. The acyl ferulic group quantity increased step by step until run 3. The data for run 4 decreased, which implied that the values of the variables were close to the optimal region.

Table 1. Experimental Design and Results of the 2^2 Design

run	TFA concn (mM)	hydrolysis time (h)	acyl ferulic group[a] (μg/mL)
1	−1 (50)	−1 (0.5)	17.61 ± 2.06
2	1 (200)	−1 (0.5)	52.63 ± 1.46
3	−1 (50)	1 (1.5)	50.24 ± 1.31
4	1 (200)	1 (1.5)	77.41 ± 0.67

[a] Each datum represents the mean ± SD of triplicate tests.

Table 2. Results of the Regression Analysis of the 2^2 Experimental Design[a]

parameter	estimate	T statistic	P value
constant	49.22	72.87	0.0000
TFA concn (mM)	15.30	22.65	0.0000
hydrolysis time (h)	14.60	21.62	0.0000

[a] $R^2 = 0.99$.

Table 3. Experimental Design and Results of the Steepest Ascent

run	TFA concn (mM)	hydrolysis time (h)	acyl ferulic group (μg/mL)
(1) base point (zero level in the 2^3 design)	125	1	
(2) unit (range of unity level)	75	0.5	
(3) slope (estimated coefficient ratio from eq 2)	1	1	
(4) correspondent range ((2) × (3))	75	0.5	
(5) proportion of (4) ((4) × 0.5)	37.5	0.25	
(6) expt no.			
1	125	1	46.33
2	163	1.25	64.67
3	201	1.5	78.17
4	239	1.75	55.43
5	277	2	52.11

Table 4. Experimental Design and Results of the 2^2 Full Factorial Central Composite Design

			acyl ferulic group (μg/mL)	
test set	TFA concn (mM)	hydrolysis time (h)	observed	predicted
1	0 (200)	0 (1.5)	76.97	77.10
2	0 (200)	0 (1.5)	76.52	77.10
3	0 (200)	0 (1.5)	77.80	77.10
4	−1 (150)	−1 (1)	64.73	61.21
5	1 (250)	−1 (1)	64.45	52.43
6	−1 (150)	1 (2)	62.94	44.11
7	1 (250)	1 (2)	36.47	35.33
8	−1.41 (129)	0 (1.5)	59.80	65.38
9	1.41 (270)	0 (1.5)	53.88	52.96
10	0 (200)	−1.41 (0.8)	61.82	62.58
11	0 (200)	1.41(2.2)	34.48	38.40

Table 5. Regression Analysis Results for the 2^2 Experimental Design[a]

	regression analysis		
parameter	estimate	*T* statistic	*P* value
constant	77.10	30.44	0.0000
TFA concn	−4.39	−2.83	0.0367
hydrolysis time	−8.55	−5.51	0.0027
(TFA concn)^2	−8.97	−4.86	0.0046
TFA concn × hydrolysis time	−6.55	−2.98	0.0306
(hydrolysis time)^2	−13.31	−7.21	0.0008

[a] $R^2 = 0.96$, $F = 21.50 > F_{5,5,0.0} = 10.97$, P value = 0.0022.

Therefore, the conditions for run 3 (201 mM TFA and 1.5 h) were chosen as the center point to proceed with the CCD.

Central Composite Design. TFA concentration and hydrolysis time were further optimized using a CCD. The variables were at three levels in the experimental design. The data gained from the test was fitted to a second-order response surface model. The center point of this design was repeated three times to allow for better estimation of the experimental error and provide extra information about the response in the interior of the experimental region (*13*). The experimental design and results are presented in **Table 4**.

A full second-order polynomial model was obtained from the experimental data regression analysis as follows:

$$\text{acyl ferulic group} = 77.10 - 4.39 \times \text{TFA concn} - 8.55 \times \text{hydrolysis time} - 8.97 \times (\text{TFA concn})^2 - 6.55 \times \text{TFA concn} \times \text{hydrolysis time} - 13.31 \times (\text{hydrolysis time})^2 \quad (3)$$

The regression model analysis is shown in **Table 5**. The adequacy of the model was examined using the Fisher F test and the coefficient of determination R^2. The $F = 21.50$ was greater than the critical value for F at the 0.01 level, $F_{5,5,0.01} = 10.97$, and the P value was 0.0022. These two statistical values demonstrated that this regression was statistically significant at the 99% confidence level. The R^2 was 0.96, which implies that the sample variation of 96% for the production of acyl ferulic group is attributable to the independent variables.

All of the variable parameters in the quadratic model were negative, which refers to a maximum point existing. The maximum point (193 mM TFA; 1.36 h) was observed in the RSM contour plot **Figure 1**, and the predictive production of acyl ferulic group was 78.63 μg/mL. The maximum point was verified using a triplicate test. The data were 76.61, 77.47, and 76.42 μg/mL of acyl ferulic group, which are close to the predicted values.

According to the average maximum production (76.83 μg/mL) of 4 mL of hydrolysate prepared from 0.25 g of rice bran,

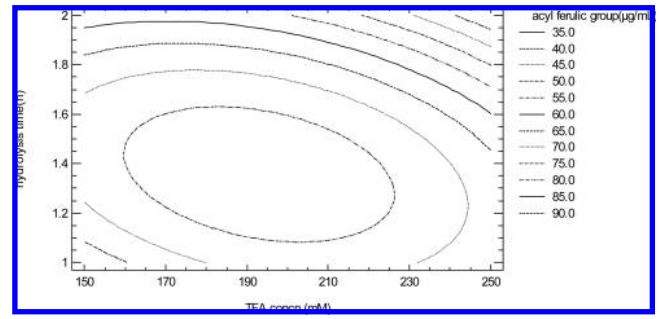

Figure 1. Contour plot of the central composite design.

the acyl ferulic group content in 1 g of rice bran was determined as 1258.08 μg. In reality, the acyl ferulic group content in the untreated rice bran sample was determined as 1694.20 μg/g of rice bran by chemical assay. The acyl ferulic group recovery from hydrolysate was about 73%. The amount of feruloylated oligosaccharides collected from the Amberlite XAD-4 column was quantified as 0.022 g/g of rice bran, and the acyl ferulic group of the partially purified feruloylated oligosaccharides was quantified as 916.14 μg/g of rice bran. The acyl ferulic group recovery from feruloylated oligosaccharides was about 54.08% per gram of rice bran. Yuan et al. (*12*) reported that the feruloylated oligosaccharide yield from wheat bran insoluble dietary fiber by xylanases for 35 h was 1.55 mM. The wheat bran contained around 36% insoluble dietary fiber (*14*). The estimated acyl ferulic group quantity in feruloylated oligosaccharide isolated from 1 g of wheat bran was about 900 μg, a value close to our data. Fang et al. (*15*) also showed a similar result that was about 10% of the total feruloylated oligosaccharides obtained from coba husk hemicellulose using enzymatic hydrolysis.

Many papers have revealed that feruloylated oligosaccharides exhibit a variety of biological activities. Rice bran is one of the most abundant and available agricultural wastes and a potential economic resource for feruloylated oligosaccharide production. However, rare studies have investigated techniques for exploiting rice bran to produce feruloylated oligosaccharides. This research investigated the optimum conditions for preparing feruloylated oligosaccharides from rice bran using TFA hydrolysis. To use feruloylated oligosaccharides to develop healthy foods, we need to know more about the structure, stability, and biological activities of this oligosaccharide.

ABBREVIATIONS USED

RSM, response surface methodology; TFA, trifluoroacetic acid; CCRD, central composite rotatable design; TK9, TaiKung 9; CCD, central composite design.

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