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Treatment of corn bran dietary fiber with xylanase increases its ability to bind bile salts, in vitro

Ye-Bi Hu, Zhang Wang *, Shi-Ying Xu

School of Food Science and Technology, Southern Yangtze University, Box 98, No. 170 Hui He Road, Wuxi 214036, PR China

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Abstract

A corn bran fiber (CDF) was further treated by xylanase and the product – XMF was obtained. Response surface methodology (RSM) was used to optimize the hydrolysis conditions (pH, time and enzyme dosage), binding of cholate (B_{SC}), chenodeoxycholate (B_{SCDC}), deoxycholate (B_{SDC}) and taurocholate (B_{STC}) by XMF were determined. The influence trends of 3 factors were dissimilar, pH affected the binding capacity most significantly, then hydrolysis time, lastly the dosage. The optimized conditions were pH 5.3, 1.75 h and enzyme dosage 0.70 g/100 g CDF, the values for B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} were increased to 1.88, 2.34, 1.67 and 2.08 fold of CDF, respectively, which were not significantly different from those predicted ($p < 0.05$). There was not correlation between the bindings of any two bile salts by XMF, which indicates that the binding mechanisms of different bile salts by XMF studied here are different. The TDF, IDF and SDF content of XMF were increased by 12%, 12% and 285%, respectively. The WHC, SW and OBC of XMF were 1.11, 1.34 and 1.87 fold of CDF, respectively.
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Keywords: Corn bran dietary fiber; Xylanase hydrolysis; Increase; Bile salt-binding; In vitro; Response surface methodology; Correlation

1. Introduction

Dietary fiber has demonstrated benefits for health maintenance and disease prevention, and is component of medical nutrition therapy. It is now well established that certain sources (such as psillium, pectin and oats) of dietary fiber, independent of the fat or carbohydrate content of the diet, can lower serum cholesterol concentrations. Fiber specifically affects the concentration of cholesterol in blood, which is carried by low-density lipoproteins (LDL). However, blood concentrations of triglycerides and high-density lipoproteins are unaffected by these fibers. Dietary fiber decreases bile acid and cholesterol absorption in the intestinal tract through increasing bile acid and cholesterol excretion thus enhancing bile acid synthesis from cholesterol, and as a result acts as a hypocholesterolemic source (Marlett, 2001). Humble (1997) and Anderson (1995) summarized the evidence supporting an inverse relationship between cardiovascular disease and dietary fiber.

Lots of dietary fibers processed from wheat bran, fruits, pea hulls and bagasse among others have been incorporated into food products such as bread and fish products (Sanchez-Alonso, Haji-Maleki, & Borderias, 2007; Sudha, Vetrimani, & Leelavathi, 2007). Corn bran, which originates from the aleurone layer, testa, pericarp and residual endosperm tissue, is a by-product of the starch industry. In China the production of corn bran is nearly 2×10^7 tons per year, most of them are cheaply used as animal feed (Yu, 2005). In corn bran, about 40% (w/w) is heteroxylan, followed by cellulose and some phenolic acids, but it is almost devoid of lignin. The heteroxylans are generally not extractable using water and are thought to be linked in the cell wall to cellulose through hydrogen bonding and physical entanglements (Chanliaud, Saulnier, & Thibault, 1995). There are more abundant total fiber, cellulose, hemicellulose, and much less lignin content in corn bran than in

* Corresponding author. Tel./fax: +86 510 85884496.
E-mail address: ZW@sytu.edu.cn (Z. Wang).

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wheat bran and rice bran (Wang & Liu, 2000). Therefore, corn bran is a good source for dietary fiber. Dong et al. (2000) fed Wistar male rats with fibers extracted by amylases from coat of corn (CDF), wheat bran (WDF) and red beans (RDF), and found out that the arteriosclerosis index (AI) of CDF was significantly lower than that of WDF and RDF while the high-density lipoprotein cholesterol (HDL-C) was higher than that of WDF and RDF. Zhang and Wang (2005) prepared corn dietary fiber from corn residue using α-amylase, and alkaline proteinase hydrolysis, and fed it to hyperlipaemic mice. The feeding results showed that with the addition of corn dietary fiber up to 8% of total feed, the levels of serum total cholesterol and total triglycerides were lower, and the HDL-C was higher, compared with the control. The in vitro binding capacities of bile acids by lots of fruits, vegetables and cereal brans have been studied (Kahlon & Smith, 2007; Story & Kritchevskv, 1976). Nevertheless, except the study by Hu and Wang (2006), in which xylanase hydrolysis was found efficient to improve the binding of bile salts in vitro by dietary fiber extracted from corn bran, there is no other systemic research on the binding of bile acids by corn bran dietary fiber.

The main advantages of response surface methodology (RSM) are reduced number of needed experimental trials, and the reliability and reproducibility of the model parameters. It enables simultaneous and efficient evaluation of the effects of many factors and their interactions on response variables with reduced and manageable experimental runs (Myers & Montgomery, 2002; Yuan, Wang, & Yao, 2006). Therefore, it has been widely applied in food process design and optimization. However, RSM has not been used for evaluating and optimizing the influence of xylanase hydrolysis on the binding of bile acids by corn bran fiber in vitro.

The purpose of this study was to evaluate the effects of xylanase hydrolysis on the binding of bile salts by corn bran dietary fiber, prepared by enzymatic extraction method, and to optimize the hypocholesterolemic function of the studied dietary fiber through the treatment of xylanase, a five-level, three-variable central composite rotatable design (CCRD) of RSM.

2. Materials and methods

2.1. Enzyme assay

One gram of xylanase (Xylanase NCB X50, 5000 IU/g, from *Bacillus subtilis*, main enzyme activity EC 3.2.1.8) supplied by Hunan New Century Biochemical Co., Ltd., PR China was dissolved in 100 mL of 50 mmol/L phosphate buffer (pH 6.5) with continuous stirring for 30 min at 25 °C. The precipitate was removed by centrifugation at 10,000*g* for 20 min (Model TG16-WS, Changsha Xiang Yi Centrifuge Co., Ltd, PR China), whereas the resulting supernatant was used as the enzyme solution. Xylanase activity was routinely assayed in a reaction mixture (3.5 mL) containing boiled 1 g/dL oat spelt xylan (Sigma Company, St. Louis, MO), 50 mmol/L phosphate buffer (pH 6.5) and appropriately diluted enzyme solution. After 15 min incubation at 50 °C, the reducing sugar produced in the reaction mixture was assayed by the dinitrosalicylic (DNS) acid method with D-xylose as the standard (Miller, 1959). All activity measurements were performed at least in triplicates and the mean calculated.

2.2. Preparation of corn bran dietary fiber (CDF)

Corn bran was provided by Dancheng Caixin Group Co., Ltd. (Henan, PR China) and was milled through a 250 μm screen (Ebihara & Nakamoto, 2001), then processed mainly according to Wang and Liu (2000) with slight modification. Briefly, a sample of 50 g corn bran was autoclaved for 45 min at 121 °C in order to destroy endogenous enzymatic activities (Zilliox & Debeire, 1998) and subsequently swollen at 50 °C for 3 h in water (500 mL) with continuous stirring. Then, 0.2 mL of α-amylase Termamyl 120 L (EC 3.2.1.1, from *B. licheniformis*, 120 KNU/g, Novozymes (China) Investment Co., Ltd., Beijing, PR China) was added to the suspension. Beakers containing corn bran suspension were heated in a 100 °C boiling water bath for 30 min and shaken gently every 5 min. Then 0.6 mL amyloglucosidase AMG 300 L (EC 3.2.1.3, from *Aspergillus niger*, 300 AGU/g) from Novozymes (China) Investment Co., Ltd., Beijing, PR China was added, and the mixture incubated at 60 °C on a super water bath thermostatic vibrator (Model 501, Shanghai Experimental Instrument Co., PR China) for 60 min with 145 rpm agitation. Next pH was adjusted to 7.5 with 300 mmol/L NaOH, and the samples were incubated with 1.6 g of proteinase Neutrase 3.0 BG (EC 3.4.24.28, from *B. amyloliquefaciens*, Novozymes (China) Investment Co., Ltd., Beijing, PR China) at 50 °C for 60 min with 145 rpm agitation. After the enzyme hydrolysis, 95% ethanol (4 times of the volume of the hydrolysate) was added to precipitate polysaccharides, and left for 12 h at ambience. The precipitate was collected by centrifuge (1000*g*, 15 min), and vacuum-dried to obtain the dietary fiber (CDF) used in this study.

2.3. Xylanase hydrolysis

Hydrolysis of CDF was performed in a 250 mL stoppered Erlenmeyer flask with a working volume of 100 mL of 50 mmol/L phosphate buffer at the required pH values (4.3–8.7), containing the required amount (0–2.06 g/100 g CDF) of xylanase (NCB X50, 5000 IU/g, from *B. subtilis*, main enzyme activity EC 3.2.1.8), supplied by Hunan New Century Biochemical Co., Ltd., PR China. Ten grams CDF were added to the freshly prepared xylanase enzyme solution. The reaction mixture was incubated on a super water bath thermostatic vibrator at 50 °C with 145 rpm agitation for required time (0–7.7 h), then operated as Section 2.2. to obtain the xylanase modified fiber (XMF).

2.4. Binding of bile salts in vitro

Sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and sodium taurocholate were purchased from the Sigma Company (St. Louis, MO, USA). The in vitro binding procedure of XMF to bile salts was a modification of that by Yoshie-Stark and Wasche (2004). Each bile salt (as substrate) was dissolved in physiological saline (pH 6.5) to make a 2 μmol/mL solution. Forty milligrams of the XMF sample were added to each 5 mL bile salt solution, and the individual substrate solution without samples was used as blank. Then tubes were incubated for one hour in a 37 °C shaking water bath. Mixtures were centrifuged at 60,000*g* for 20 min at 10 °C in an ultracentrifuge (Model J-26XPI, Beckman, USA). The supernatant was removed into a second set of tubes and frozen at −20 °C for bile salts analysis. Bile salts were analyzed using HPLC (Model 1525, Waters, USA) on a Sunfire C_{18} column (4.6 × 150 mm i.d., 5 μm particle size, Waters, USA), maintained at 35 °C. The injected sample volume was 10 μL for each bile salt. Sodium cholate, sodium chenodeoxycholate and sodium deoxycholate were eluted with methanol: 0.04 g/dL formate acid (88:12) at a flow rate of 0.8 mL/min for 10 min. Sodium taurocholate was eluted with methanol: 0.04 g/dL KH_2PO_4 (80:20) at a flow rate of 1.0 mL/min for 10 min. The absorbance of the eluate was monitored continuously at 220 nm for sodium cholate, sodium chenodeoxycholate and sodium deoxycholate, and 205 nm for sodium taurocholate, respectively (Model 2996 PDA detector, Waters, USA).

2.5. Main compositions and some physical properties

Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) in CDF and XMF were determined using AACC 32-07 method (32-07, 2000). Water holding capacity (WHC) and oil binding capacity (OBC) were determined using the method of Sangnark and Noomhorm (2003).

2.6. Experimental design and statistical analysis

A five-level, three-variable RSM-CCRD according to Myers and Montgomery (2002) using Design-Expert® Version 6.0.11 (State-Ease, Inc., Minneapolis, MN) was applied to determine the best enzymatic hydrolysis conditions as explained by Cheison, Wang, and Xu (2006). The factorial design consisted of 8 factorial points, 6 axial points and 3 central points.

Based on our preparatory investigations on xylanase characterization and the effect of enzyme dosage on the binding of sodium taurocholate and sodium deoxycholate (data not shown), the variables considered in the CCRD were hydrolysis pH 5.2–7.8, time 1–6 h and xylanase dosage 0.05–1.55 g/100 g CDF, while 50 °C was chosen as the temperature. Direct binding amount of XMF against sodium cholate (B_{SC}), sodium chenodeoxycholate (B_{SCDC}), sodium deoxycholate (B_{SDC}) and sodium taurocholate (B_{STC}) were determined as response variables. Variable factors with both the coded and actual values are presented in Table 1. The quadratic response surface analysis was based on the multiple linear regressions taking into account the main, the quadratic and the interaction effects, according to Eq. (1). As three parameters were varied, 10 β-coefficients were to be estimated, i.e. coefficients for the 3 main effects, 3 quadratic effects, 3 interactions and 1 constant.

Table 1
Variables and their levels employed in a central composite rotatable design for optimization of xylanase hydrolysis conditions

Variable	Coded levels				
	−1.682	−1	0	+1	+1.682
Hydrolysis pH	4.30 (4.31)[a]	5.2	6.5	7.8	8.70 (8.69)
Hydrolysis time (h)	0 (−0.7)	1	3.5	6	7.7
Enzyme dosage[b]	0 (−0.46)	0.05	0.8	1.55	2.06

[a] Values in bracket represent actual factor values that were not practically useable.
[b] g/100 g CDF.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + e \qquad (1)$$

where Y is the response variable, $\beta_0, \beta_i, \beta_{ii}$, and β_{ij} are constant coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i/X_j is the independent variables, e is the error.

For the models, the linear regression analysis of variance (ANOVA) was performed. The total model, R^2 value, adjusted R^2 value, the residual error, the pure error and the lack of fit were calculated (Myers & Montgomery, 2002).

Comparison of the means was performed by one-way ANOVA using the honestly significant difference (HSD) of Tukey's ad-hoc test. The linear correlation of every two of binding capacity was also analysed using linear regression analysis on the CCRD experimental data. These statistical analyses were done using SPSS 13.0 for Windows software (SPSS Institute Inc., Cary NC).

2.7. Verification of the model

Optimization of xylanase hydrolysis in terms of hydrolysis pH, time and enzyme dosage was calculated using the predictive equation obtained from RSM. The hydrolysis of CDF was carried out at the optimized conditions. Binding level of every bile salt by XMF was analyzed and compared with the predicted value and CDF.

3. Results and discussion

3.1. Statistical analysis

Experimental data obtained in the study are summarized in Table 2. Multiple regression analysis was performed on

Table 2
Summarized general statistics for experimental data obtained in the study

Response		All Runs	Center Runs
B_{SC}[a]	Range	29.40–85.78	69.70–72.03
	Average	56.34	71.10
	RSD[e]	26.61%	1.24%
B_{SCDC}[b]	Range	12.90–75.50	26.15–33.65
	Average	35.42	29.31
	RSD[e]	44.97%	3.89%
B_{SDC}[c]	Range	7.78–90.98	78.48–90.98
	Average	48.02	85.33
	RSD[e]	55.83%	6.34%
B_{STC}[d]	Range	19.68–81.70	71.55–74.80
	Average	51.97	73.65
	RSD[e]	34.50%	1.82%

[a,b,c,d] The binding amount of sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and sodium taurocholate, respectively, in μmol/g xylanase modified fiber (XMF).
[e] Relative standard deviation of mean.

the experimental data. The coefficients of the models' variables and the ANOVA for the CCRD are shown in Table 3. The *p*-values of the four models for B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} are significant ($p < 0.05$). The behaviour of B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} can be explained by 89.13%, 74.67%, 82.81% and 85.76% by each model, respectively. Moreover, the adjusted R^2 correlating to B_{SC} and B_{STC} are 0.7515 and 0.6745, which are high enough to assure the accuracy of these models, although the "lack of fit" for them is not ideal. Thus these models adequately represent the relationships among the parameters chosen.

3.2. Binding of sodium cholate (B_{SC})

The values for B_{SC} ranged between 29.4 and 85.78 μmol/g XMF (Table 2). Neglecting the non-significant terms summarized in Table 3, Eq. (2) was the best description for B_{SC}, where hydrolysis pH and time were the most important factors with *p*-value of 0.0171 and 0.0176, while enzyme dosage influenced at the least extent with a *p*-value greater than 0.1.

$$B_{SC} = 70.73 + 6.29A + 6.25B - 6.76B^2 - 9.26C^2 + 8.99AB \quad (2)$$

where A, B and C are the hydrolysis pH, time (h) and xylanase dosage (g/100 g CDF), respectively, while B_{SC} is the binding amount of sodium cholate (μmol/g XMF).

Fig. 1a shows that the general influence of pH between the ranges studied was linear, B_{SC} increased with the increase of pH, and this trend became more apparent when the hydrolysis time was over than 3 h. The influence of pH may be due to that pH affects the activity and stability of xylanase as shown in Fig. 2, furthermore, different enzyme components may have different hydrolysis efficiency when pH is out of its most suitable range for a long time (Biely, 2003).

The effects of the hydrolysis time and enzyme dosage are shown in Fig. 1b, B_{SC} increased with the increase in enzyme dosage and hydrolysis time up to the optimum, while fell with further increase in dosage and time beyond the optimum. Yuan et al. (2006) also observed such an influence trend of enzyme amount and hydrolysis time in his extraction of feruloyl oligosaccharides from wheat bran using xylanase from *B. subtilis*. B_{SC} reached a maximum value at about 4.6 h and dosage 0.8 g/100 g CDF. The reasons for such a phenomenon might be of two-fold. One, with longer times, more and more hemicellulose–heteroxylan of the corn bran (Chanliaud et al., 1995) is hydrolyzed into smaller molecules or segments by xylanase. More and smal-

Table 3
Regression coefficients, their *p*-values of the second-order polynomial equations and the analysis of variance

Coefficient[a]	B_{SC}[b]		B_{SCDC}[c]		B_{SDC}[d]		B_{STC}[e]	
	β-coefficient	*p*-value	*β*-coefficient	*p*-value	*β*-coefficient	*p*-value	*β*-coefficient	*p*-value
β_0	70.73	<0.0001	35.42	<0.0001	83.97	<0.0001	73.46	<0.0001
β_1	6.29	0.0171	−6.27	0.0456	−12.97	0.0246	7.03	0.0387
β_2	6.25	0.0176	5.94**	0.0559	5.39*	0.2745	−5.26**	0.0992
β_3	2.35*	0.2825	0.99*	0.7475	−10.06**	0.0626	4.53*	0.1460
β_{11}	−1.89*	0.4244	–	–	−18.85	0.0070	−3.72*	0.2613
β_{22}	−6.76	0.0189	–	–	−11.21**	0.0520	−12.77	0.0041
β_{33}	−9.26	0.0042	–	–	−14.19	0.0252	−10.26	0.0120
β_{12}	8.99	0.0114	13.35	0.0040	5.28*	0.4042	3.75*	0.3339
β_{13}	−3.62*	0.2125	3.15*	0.4006	2.07*	0.7372	−4.06*	0.2988
β_{23}	−3.47*	0.2306	7.91**	0.0526	−2.30*	0.7099	7.46**	0.0781
Others statistics	Sum of squares	–	Sum of squares	–	Sum of squares	–	Sum of squares	–
Total model	3204.35	0.0116	3033.57	0.0137	9526.47	0.0477	4411.92	0.0272
Residual	390.86		1029.42	–	1977.56	–	732.56	–
Pure Error	3.06	–	30.21	–	80.29	–	6.63	–
Lack of Fit	387.81	0.0194	998.91	0.1124	1897.27	0.0984	725.93	0.0225
R^2	0.8913		0.7467	–	0.8281	–	0.8576	–
Adjusted R^2	0.7515	–	0.5947	–	0.6071	–	0.6745	–

[a] β_0 represents intercept and β_1, β_2 and β_3 represent hydrolysis pH, time and xylanase dosage, respectively.
[b,c,d,e] The binding amount of sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and sodium taurocholate, respectively.
* Coefficients with *p*-values greater than 0.10, indicating they are not significant.
** Coefficients with *p*-value greater than 0.05 but less than 0.10.

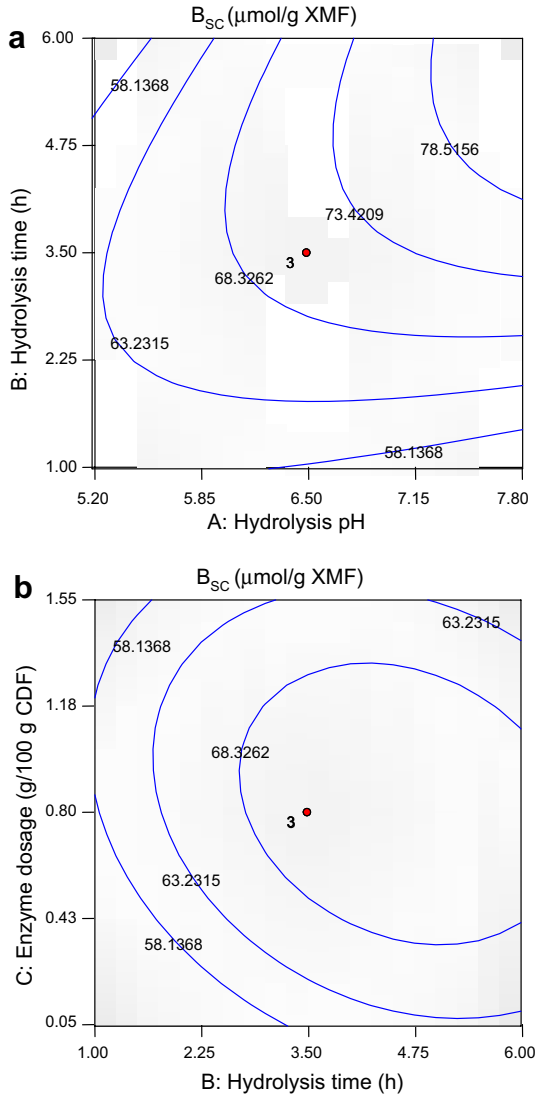

Fig. 1. Response surface plots of binding of sodium cholate expressed as a function of: (a) A: hydrolysis pH and B: hydrolysis time, (b) B: hydrolysis time and C: enzyme dosage. The numbers inside plots present μmol sodium cholate bound by 1 g xylanase modified fiber (XMF).

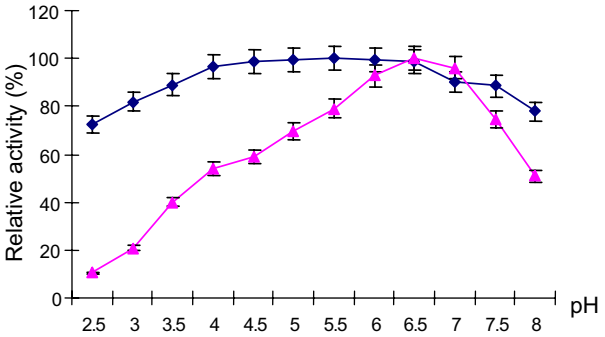

Fig. 2. Effect of pH on the stability and activity of xylanase. For stability, the enzyme solutions in 50 mmol/L citrate–phosphate buffer, at various pH, were incubated for 1 h at 40 °C. After adjustment of pH, the residual activity was assayed by the standard method. The enzyme activity was assayed by the standard method by changing the buffer to the desired pH. (◆ Stability; ▲ Activity).

ler the segments might be produced with persistent longer time. However, only a definite molecular size or structure of the hydrolysate segments has the best B_{SC}. Secondly, when the enzyme dosage was less than 0.8 g/100 g CDF, the amount of enzyme was not enough to moderately hydrolyze the heteroxylan in CDF, however, an excess of enzyme might lead to extensive hydrolysis with resultant decrease in B_{SC}.

3.3. Binding of sodium chenodeoxycholate (B_{SCDC})

$$B_{SCDC} = 35.42 - 6.27A + 5.94B + 13.35AB + 7.91BC \quad (3)$$

The determined values for B_{SCDC} ranged from 12.9–75.5 μmol/g XMF. In the center runs, values ranged over 26.15–33.65 μmol/g XMF with the mean of 29.31 μmol/g XMF and a relative standard deviation of mean (RSD) of 3.89% (Table 2).

Eq. (3) including only significant coefficients ($p < 0.1$) from Table 3 describes the behaviour of B_{SCDC}. The linear coefficients showed that hydrolysis pH affected the binding of chenodeoxycholate by XMF significantly ($p < 0.05$), hydrolysis time influenced significantly at 10% level, while the influence of enzyme dosage was non-significant (Table 3). The contour Fig. 3a shows the 2F1 effects of the hydrolysis pH and time on the response. When hydrolysis time was shorter than 4.7 h, B_{SCDC} decreased with the increase of pH, while when hydrolysis time was longer than 4.7 h, B_{SCDC} increased with the increase of pH. B_{SCDC} increased with the increase of hydrolysis time when pH was above 5.9, and acted oppositely at the range of pH from 5.2–5.9. B_{SCDC} decreased with the increase of dosage and hydrolysis time slightly when hydrolysis time and dosage were lower than 3.2 h and 0.24 g/100 g CDF, respectively. While B_{SCDC} increased rapidly with the increase of dosage when time was higher than 3.2 h and also increased rapidly with the increase of time when dosage was higher than 0.24 g/100 g CDF (Fig. 3b). The main mechanisms for the influences of pH, time and dosage should be the same as those discussed above for B_{SC}. While the ideal point of pH, time and dosage for B_{SCDC} were different from which for B_{SC}, and B_{SCDC} was not so sensitive to hydrolysis pH ($p = 0.0456$) and time ($p = 0.0559$) as B_{SC} ($p = 0.0171$ and 0.0176, respectively), which might be due to the fact that the binding parts in XMF to chenodeoxycholate and the binding interaction between XMF molecules and chenodeoxycholate are not the same as B_{SC}, because of the differences between the molecule structure of chenodeoxycholate and cholate, furthermore chenodeoxycholate is much more hydrophobic than cholate (Story & Kritchevskv, 1976; Zhao, 1998).

3.4. Binding of sodium deoxycholate (B_{SDC})

$$B_{SDC} = 83.97 - 12.97A - 10.06C - 18.85A^2 - 11.71B^2 - 14.19C^2 \quad (4)$$

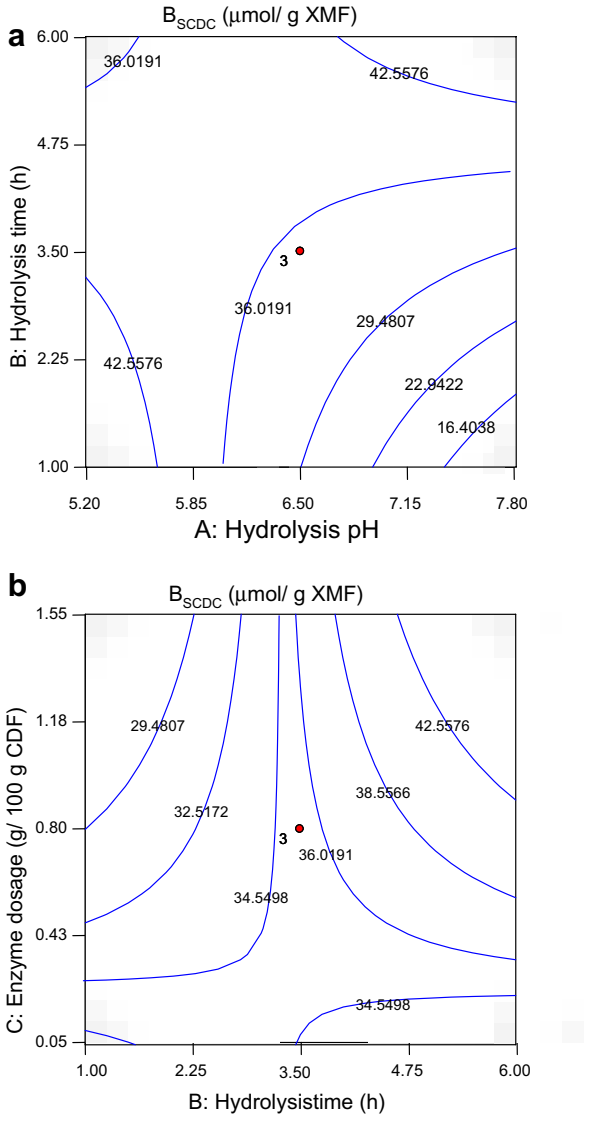

Fig. 3. Response surface plots of binding of sodium chenodeoxycholate expressed as a function of: (a) A: hydrolysis pH and B: hydrolysis time, (b) B: hydrolysis time and C: enzyme dosage. The numbers inside plots present μmol sodium chenodeoxycholate bound by 1 g XMF.

B_{SDC} is best described by Eq. (4), after elimination of the non-significant parameters ($p > 0.1$) from Table 3, which shows that all of the quadratic terms significantly influenced B_{SDC}. The most relevant variable for B_{SDC} was pH ($p = 0.0246$), then xylanase dosage influenced significantly at 10% level, while hydrolysis time did not influence significantly, which was quite not similar to that for B_{SCDC}, although deoxycholic acid and chenodeoxycholic acid are of hydrophobic bile acids (Story & Kritchevsky, 1976; Zhou, Xia, Zhang, & Yu, 2006).

The range of B_{SDC} in this study was between 7.78 and 90.98 μmol/g XMF (Table 2). The lowest B_{SDC} was recorded in the experiment run of pH 8.7, 3.5 h and 0.8 g/100 g CDF xylanase dosage, while the highest was recorded in the center run with pH 6.5, 3.5 h and 0.8 g/100 g CDF xylanase dosage. Fig. 4 indicates that the behaviour of B_{SDC} improved with the increase of hydrolysis pH and time till the point near pH 6.1 at 4 h, thereafter decreased rapidly. The effects of the hydrolysis time and xylanase dosage were quadratic with an optimal point of near 4.2 h and 0.53 g/100 g CDF enzyme dosage (Figure not shown).

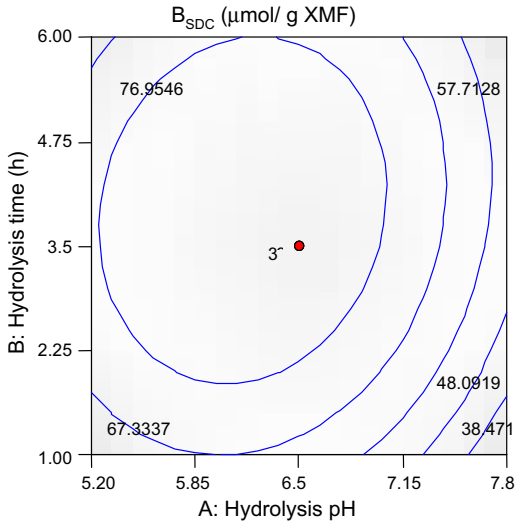

Fig. 4. Response surface plot of binding of sodium deoxycholate expressed as a function of: A: hydrolysis pH and B: hydrolysis time. The numbers inside plot present μmol sodium deoxycholate bound by 1 g XMF.

3.5. Binding of sodium taurocholate (B_{STC})

$$B_{STC} = 73.46 + 7.03A - 5.26B - 12.77B^2 - 10.26C^2 + 7.46BC \quad (5)$$

Eq. (5) obtained from Table 3 describes the behaviour of the chosen variables on B_{STC}. For B_{STC}, pH was the most relevant variable ($p = 0.0387$), followed by the hydrolysis time ($p = 0.0992$) while enzyme dosage was not significant ($p = 0.1460$). Generally, the binding properties of XMF were at least affected by enzyme dosage (except B_{SDC}), this may due to that pH sensitively influences the activity and stability of the xylanase used so much that conceals the significance of enzyme amount which we observed under a constant pH condition in our preparatory study (data not shown).

The range of B_{STC} in this study was from 19.68 to 81.70 μmol/g XMF (Table 2). The effects of hydrolysis pH and enzyme dosage are revealed by the contour plot in Fig. 5a, which shows that there was a significant linear increase in B_{STC} with the increase in pH, while the effect of dosage was quadratic with an ideal point of near 0.85 g/100 g CDF. Similar effects between the hydrolysis pH and time were observed (Figure not shown). Fig. 5b demonstrates that B_{STC} could reach a maximum value of 74 μmol/g XMF near a hydrolysis time of 3 h and enzyme dosage of 0.92 g/100 g CDF.

The influence trend of pH on B_{STC} was quite similar as that on B_{SC}, this might because that they are both highly

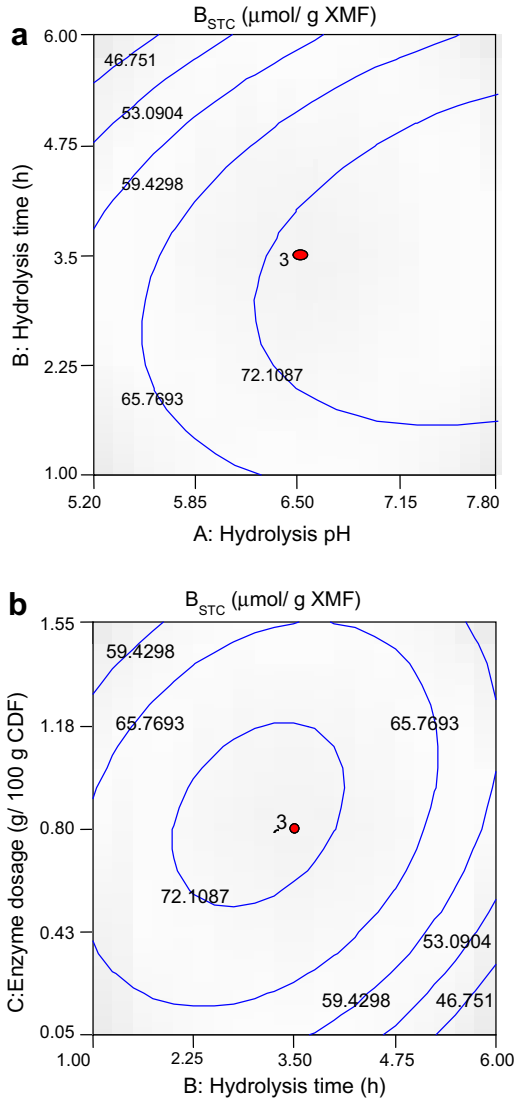

Fig. 5. Response surface plots of binding of Sodium taurocholate expressed as a function of: (a) A: hydrolysis pH and B: hydrolysis time, (b) B: hydrolysis time and C: enzyme dosage. The numbers inside plots present μmol sodium taurocholate bound by 1 g XMF.

hydrophilic, although sodium taurocholate molecule is comparatively larger than sodium cholate with a aminoethanesulfonic group.

3.6. Correlation between the bindings of every two bile salts by XMF

Hydrolysis pH was found to be the most significant factor to the four responses. Figs. 1a and 5a show that the influence trend of pH on B_{STC} was quite similar as that on B_{SC}. There was similar influence trend for enzyme dosage and hydrolysis time on B_{SC}, B_{SCDC} and B_{STC}, although the significances were different. In order to observe whether there is actual linear correlation between the binding capacities of every two bile salts, one way linear regression analysis was performed on the RSM-CCRD experimental data, *p*-values were presented in Table 4. The correlation

Table 4
Linear correlation between the bindings of every two bile salts by XMF

	B_{SC}	B_{SCDC}	B_{SDC}
B_{SC}[a]	–	–	–
B_{SCDC}	0.427	–	–
B_{SDC}	0.468	0.309	–
B_{STC}	0.0651	0.624	0.776

[a] B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} represent the binding amount of sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and sodium taurocholate, respectively.

between B_{STC} and B_{SC} was the largest with the smallest *p*-value ($p = 0.0651$, Table 4), while the significance was not enough. The *p*-values of B_{SC} and B_{SCDC}, B_{SCDC} and B_{SDC} were 0.427 and 0.309, respectively, and those of B_{SDC} and B_{SC}, B_{STC} and B_{SCDC}, B_{STC} and B_{SDC} were 0.468, 0.624 and 0.776, respectively. Therefore, there was not linear correlation between the binding of any two bile salts by XMF under the experimental conditions. There is no possibility to rapidly screen XMF samples for their bile salt-binding capacities using only one of the bile salts studied here. Zhou et al. (2006) found that the cholic acid-binding capacity of chitosan was linearly correlated to the binding capacities against both deoxycholic and chenodeoxycholic acids under their experiment conditions. The binding mechanisms of cholate, chenodeoxycholate, deoxycholate and taurocholate by XMF might be different and the binding of any two bile salts might be not competitive.

3.7. Optimum conditions and some properties of optimized XMF

The method of ANOVA numerical analysis estimated the optimized conditions for xylanase hydrolysis, which were pH 5.28, time 1.73 h and enzyme dosage 0.70 g/100 g CDF. Model verification was performed by additional independent trials at the conditions of pH 5.3, time 1.75 h and enzyme dosage 0.70 g/100 g CDF. Values obtained were 64.60, 48.34, 75.79 and 60.68 μmol/g XMF for B_{SC}, B_{SCDC}, B_{SDC}, and B_{STC}, respectively (Table 5). There was no significant difference between the predicted and actual values ($p < 0.05$). Therefore, RSM analysis is valid.

Among the four kinds of bile salts studied here, sodium deoxycholate is a secondary cholate with the most hydrophobicity, while sodium taurocholate was reported by Story and Kritchevsky (1976) to be the most difficult conjunct bile salt to be bound by several fibers. Lots of samples such as chitosan, raisin fiber and CDF in Table 5 among others, having strong binding capacity against a selected bile acid, did not necessarily exhibit the same strong binding capacity against other bile acids (Zhou et al., 2006). While compared with CDF, the B_{SDC} by XMF was increased to 167%; furthermore, the B_{SC}, B_{SCDC}, and B_{STC} were 1.88, 2.34 and 2.08 fold of CDF (Table 5). Thus through the optimized hydrolysis of xylanase on CDF, XMF can bind sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and

Table 5
Predicted and experimental values of responses by XMF at optimized conditions and those by CDF

Responses	XMF (μmol/g XMF)		CDF (μmol/g CDF)	XMF/CDF
	Predicted[A]	Experimental[B]		
B_{SC}[C]	60.00 ± 0.00^{b}	64.60 ± 5.63^{b}	34.31 ± 2.11^{a}	1.88
B_{SCDC}	47.07 ± 0.00^{b}	48.34 ± 3.41^{b}	20.69 ± 2.01^{a}	2.34
B_{SDC}	74.46 ± 0.00^{b}	75.79 ± 6.37^{b}	45.41 ± 1.47^{a}	1.67
B_{STC}	62.75 ± 0.00^{b}	60.68 ± 4.18^{b}	29.23 ± 1.81^{a}	2.08

[A] Predicted optimized hydrolysis conditions: pH 5.28, time 1.73 h and enzyme dosage 0.70 g/100 g CDF.
[B] Actual experimental hydrolysis conditions for verification: pH 5.30, time 1.75 h and enzyme dosage 0.70 g/100 g CDF.
[C] B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} represent the binding amount of sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and sodium taurocholate, respectively, in μmol/g xylanase modified fiber (XMF).
[a,b] Same letters superscripts in a row show values that do not differ significantly ($p < 0.05$, $n = 3$).

Table 6
Some properties of CDF and optimized XMF

Sample	TDF (%)[A]	IDF (%)	SDF (%)	WHC (g water/g dry fiber)	SW (mL/g dry fiber)	OBC (g oil/g dry fiber)
XMF	93.04	92.66	2.39	5.49 ± 0.07^{b}	2.51 ± 0.46^{b}	4.43 ± 0.38^{b}
CDF	82.76	82.90	0.62	4.96 ± 0.17^{a}	1.87 ± 0.47^{a}	2.37 ± 0.10^{a}

[A] TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; WHC: water holding capacity; SW: swollen capacity; OBC: oil binding capacity.
[a,b] Different letters superscripts in a column show values that differ significantly ($p < 0.05$, $n = 3$).

sodium taurocholate at much higher level, especially the binding of conjunct bile salts, the main components of animal bile salts. Bile acids, especially chenodeoxycholate and deoxycholate, are thought to be involved in the etiology and development of colorectal cancer (Liu, Yu, Hong, & Xu, 1993). While chenodeoxycholate can dissolve gall-stone, and the synthesis of bile acids from cholesterol is adjusted by their concentration in the liver (Zhao, 1998). The presence of XMF in intestine can decrease not only enterohepatic circulation of bile acids, but also their contact with colorectal mucosa through the binding effect. Therefore, it's possible for human to ingest some of XMF to prevent hypercholesterolemia, gall-stone and colorectal cancer at the same time. A kind of lupin protein isolate F digested by proteolytic ferment also showed a much higher deoxycholate binding capacity (Yoshie-Stark & Wasche, 2004). Furthermore, chenodeoxycholate and deoxycholate are reported as the special ligands of FXR, a kind of orphan nuclear receptor regulating the expression of cholesterol 7 Alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid biosynthesis, and the intestinal bile acid-binding protein (I-BABP), a cytosolic protein that serves as a component of the bile acid transport system in the ileal enterocyte (Parks et al., 1999). Thus the decrease of the concentration of deoxycholate in the intestine and chenodeoxycholate in the liver and intestine by the ingestion of XMF might influence the activation or even the gene expression of FXR, and then regulate the homeostasis of cholesterol consequently. Further studies are needed to verify these physiological functions.

The main dietary fiber composition and some functional physical properties of CDF and optimized XMF were shown in Table 6. TDF, IDF and SDF of XMF were increased to 1.12, 1.12 and 3.85 fold of CDF, respectively. The SDF content of CDF was very low, while lots of biofunction of dietary fiber was executed by SDF, it delays gastric emptying, slows glucose absorption, enhance immune function and lowers serum cholesterol levels, and is to a large degree fermented in the colon into short-chain fatty acids, which may inhibit hepatic cholesterol synthesis (Dreher, 2001). The WHC, SW and OBC of XMF were increased to 1.11, 1.34 and 1.87 fold of CDF, respectively, with significance of $p < 0.05$.

The positive effects of optimized xylanase hydrolysis could be elucidated as below. One is that the cell wall fiber of XMF becomes more swollen and looser than that of CDF through moderate xylanase solubilization, thus leading to the exposure of more polar and non-polar groups, which can bind bile salts efficiently. The other is that some capillaries forming with the hydrolysis of xylanase can improve the water/solution holding capacity and oil binding capacity as shown in Table 6, and as a result advance the chances for bile salts to touch the original cell wall fiber and the hydrolysate segments of heteroxylan.

4. Conclusions

Moderate xylanase hydrolysis significantly influenced the binding of bile salts by corn bran dietary fiber (CDF). Hydrolysis pH was found to be the most important factor, because it affected the capacity of xylanase modified fiber (XMF) to bind sodium cholate, sodium chenodeoxycholate, sodium deoxycholate and sodium taurocholate at 5% level. The reaction time significantly affected B_{SC} at 5% level, B_{SCDC} and B_{STC} at 10% level. In addition, the binding properties of XMF were at least affected by enzyme amount except B_{SDC}.

There was not linear correlation between the bindings of any two bile salts by XMF, which indicates that the binding mechanisms of different bile salts by XMF studied here might be different and the binding of any two bile salts might be not competitive.

Hydrolysis conditions were optimized through the RSM analysis, which were pH 5.28, time 1.73 h and enzyme dosage 0.70 g/100 g CDF with the predicted values for B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} being 60.00, 47.07, 74.46 and 62.75 μmol/g XMF, respectively. The actual verification experimental conditions were pH 5.3, time 1.75 h and enzyme dosage 0.70 g/100 g CDF, and results with B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} equivalent to 64.60, 48.34, 75.79 and 60.68 μmol/g XMF, respectively, were obtained. The verification experimental values were not significantly ($p < 0.05$) different from those predicted, implying that the RSM model was valid. After optimization, the B_{SC}, B_{SCDC}, B_{SDC} and B_{STC} of XMF were increased to 188%, 234%, 167% and 208% when compared with CDF, respectively; TDF, IDF and SDF of XMF were increased to 1.12, 1.12 and 3.85 fold of CDF, respectively; the WHC, SW and OBC of XMF were increased to 1.11, 1.34 and 1.87 fold of CDF, respectively, with significance of $p < 0.05$. Further investigations on the composition and structure characters of CDF and XMF are being undertaken to understand the action of the enzyme and possible modification of heteroxylan structure.

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