Effect of Enzymatic Pretreatment on the Preparation and Properties of Soy-Based Adhesive for Plywood

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Response surface methodology was employed to determine the effects of enzymatic pretreatment temperature, time, and pH on the reducing sugar content and bonding strength of soy-based adhesives (SBAs). Plywood specimens bonded by the SBAs with *Pinus massoniana* veneer were then produced. A significant positive correlation was observed between reducing sugar content and the bonding strength of SBAs. The effects of pretreatment temperature and time on bonding strength were also significant, but insignificant with respect to reducing sugar content; the effects of enzymatic pretreatment time on response values were the smallest. The optimal enzymatic pretreatment conditions of SBA were a pretreatment temperature of 54 °C, a pretreatment time of 20.0 min, and a pretreatment pH of 5.1. Under these conditions, the reducing sugar content and bonding strength (boiling-water test) of SBAs were 2.93% and 0.62 MPa, which were higher than the control by 113.9% and 30.6%, respectively. X-ray diffraction (XRD) indicated that the ordered degree of soy protein decreased, but the ordered structure had no variation when defatted soy flour was treated by enzymes with combination of acid, salt, and alkali. The SBAs contain more active functional groups and have better water resistance after curing.

Keywords: Soy-based adhesive; Enzyme; Viscozyme® L; Response surface methodology; Plywood; XRD

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INTRODUCTION

Adhesion is a necessary step for preparing wood-based panels (*e.g.*, plywood). The wide variety of adhesion agents (or adhesives) can be divided into petro-based and biomass-based adhesives. Recently, petro-based adhesives (*e.g.*, phenolic resins) have been widely used. However, most of them are non-biodegradable and generate carcinogenic gases (*e.g.*, formaldehyde) when used, and this has led to serious environmental pollution and caused damage to the health of workers and consumers (Lin *et al.* 2012; Rahman *et al.* 2014). Biomass-based adhesives derived from natural sustainable resources have attracted much attention in recent years and are considered potential substitutes for existing petro-based adhesives because of their low cost, easy availability from renewable resources, and significant environmental benefits, *etc.* (Zhang *et al.* 2014a).

Defatted soy flour (DSF), a sustainable resource derived from soybean and the primary raw material of soy-based adhesives (SBAs), is characterized by its low cost and high protein content. However, applications of SBAs are significantly restricted because of their poor water resistance. Many attempts, such as physical (Sun and Bian 1999; Apichartsrangkoon 2003; Zhang *et al.* 2012) and chemical (Li *et al.* 2004; Gao *et al.* 2012; Lin *et al*. 2012; Zhang *et al.* 2014b) treatment processes, have been made to

modify DSF soy protein to improve the water resistance of SBAs, and positive results have been obtained. Improved water resistance has also been observed in SBAs prepared from enzymatically treated soy protein. It has been demonstrated that SBAs developed from trypsin (Hettiarachchy *et al.* 1995; Kalapathy *et al.* 1995), papain, and urease (Kumar *et al.* 2004) resulted in treated soy protein with higher hydrophobicity, lower viscosity, and better water resistance compared with untreated soy protein. Moreover, several studies have reported that the water resistances of SBAs are also influenced by carbohydrates in DSF (Hettiarachchy *et al*. 1995; Hunt *et al.* 2009; Chen *et al.* 2013) because DSF is a complicated mixture consisting of approximately 50% protein, 40% carbohydrates, and other minor components (Bainy *et al.* 2008; Chen *et al*. 2013). Previous studies by the authors have shown that enzymatic (Viscozyme® L) pretreatment of the carbohydrates (*e.g.*, polysaccharides) in DSF improved the water resistance of the SBAs as a result of the Maillard reaction that occurred between proteins and monosaccharides (Chen *et al.* 2014). However, the preparation conditions and properties of the SBAs, which were prepared from a combination of enzymatic and chemical approaches, have not been studied further. In most preparation processes, the properties of the products involve balancing the preparation conditions to obtain the desired output characteristics. It has been reported that enzymatic treatment conditions of polysaccharides in DSF, including temperature, time, and pH, affect the content of monosaccharides significantly (Guan and Yao 2008; Meyer *et al.* 2009; Rosset *et al.* 2014); thus, these conditions were investigated in this study.

The present work was designed to determine the preparation conditions and properties of SBAs *via* a combination of enzymatic and chemical treatment. In this study, a SBA treated by a combination of enzyme, acid, salt, and alkali was prepared, and this adhesive was then used to manufacture plywood. A standard response surface methodology (RSM) design called a central composite design (CCD) was used to evaluate the interactive effects and optimize the preparation conditions of SBA.

EXPERIMENTAL

Materials

Hydrochloric acid, ferric chloride, and sodium hydroxide of analytical grade were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (China) and were used as received. DSF with 53.4% crude protein (dry basis), 36.3% carbohydrate, and 7.5% water was obtained from Shandong Wonderful Industrial Group Co., Ltd. According to the supplier's instructions, 98% of the DSF was passed through a 200-mesh screen. Viscozyme® L (from *Aspergillus aculeatus*) was donated by Novozymes (Denmark). The activity of this enzyme was 100 fungal beta-glucanase units (FBG) per gram (FBG/g). *Pinus massoniana* veneers 300 mm × 300 mm in size, 1.2 to 1.3 mm in thickness, and with moisture contents of 10 to 12 (wt.%) were supplied by Jianyang Luban Wood Industry Co. Ltd (China).

Methods

SBA preparation

SBA was prepared according to our previous work (Lin *et al*. 2012; Chen *et al*. 2014) with minor modifications. Forty grams of DSF was dissolved in 160 mL of distilled water in a three-necked flask and stirred for 40 min in a 35 °C water bath. The

DSF slurry was then adjusted with hydrochloric acid solution to the required pretreatment pH (Table 1). After 50 FBG units of enzyme were added to the DSF slurry, it was enzymolyzed at a set pretreatment temperature and time (Table 1) and the enzyme-treated DSF slurry was then immediately cooled to room temperature. This enzyme-treated DSF slurry was then adjusted with acid-salt solutions (hydrochloric acid solution containing 0.5% ferric chloride) to pH 1.1 and stirred for 30.0 min. Finally, the slurry was adjusted to pH 11 with sodium hydroxide solution (30%) to obtain the SBA.

Plywood preparation

The SBA was used to prepare three-ply wood by coating 140 g/m^2 of the adhesive on each veneer layer. The assembly time, pressing temperature, pressure, and time were set at 10 min, 160 °C, 1.0 MPa, and 3.6 min, respectively. The results are reported as the average of duplicate values.

Experimental design

The main effects of parameter variables (pretreatment temperature (X_1), pretreatment time (X_2), and pretreatment pH (X_3)) on reducing sugar content (Y_1) and bonding strength (Y_2) were investigated using RSM. A CCD was used, and 20 different formulations with six center points, six axial points, and eight full factorial designs were produced. The range and center point value of three independent variables are summarized in Table 1. The experimental design matrix by the CCD is tabulated in Table 2, and corresponding experiments were performed.

The experimental data obtained by CCD procedures were analyzed by RSM using the following second-order polynomial regression model, developed to describe the relationship between the predicted response variable and the parameter variable of the SBA preparation process,

$$Y = \alpha_0 + \sum \alpha_i X_i + \sum \alpha_{ii} X_{i2} + \sum \alpha_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the response variable, α_0 is a constant, α_i, α_{ij} and α_{ii} are the linear, quadratic and interactive coefficients, respectively, X_i and X_j are the levels of the parameter variables, and ε is the random error. The responses obtained from each set in the experimental design were subjected to multiple non-linear regressions using Design Expert version 8.0.6 (STAT-EASE Inc., USA) software. The quality of the fit of the polynomial model equation was evaluated by the coefficient of determination (R^2), adjusted coefficients of correlation (R^2_{adj}), adequate precision (AP), standard deviation (SD), and analysis of variance (ANOVA).

Table 1. Levels of Factors in Experiment and Code Number

Factors	Levels				
	-1.682	-1	0	1	+1.682
Pretreatment temperature (X_1, °C)	33.2	40.0	50.0	60.0	66.8
Pretreatment time (X_2, min)	13.2	20.0	30.0	40.0	46.8
Pretreatment pH (X_3)	4.2	4.5	5.0	5.5	5.8

Characterization

The reducing sugar (glucose equivalents) content of the SBA was determined according to the method described by the Chinese national standard GB/T 5009.7 (2008).

The bonding strength of the developed SBA was evaluated from the wet strength of plywood according to the methods described by the Chinese national standard GB/T 9846 (2004). A piece of plywood was cut into ten 100 mm × 25 mm specimens. The plywood specimens were soaked in boiling water for 3 h and then cooled to room temperature for 10 min. A tensile testing machine (MTS, USA) with a cross-head speed of 10 mm/min was used to test the wet strength. The number of test specimens for each combination was 20 (10 × 2), of which the average wet strength was calculated.

The SBA samples were freeze-dried at -48 °C and 6.5 Pa for 48 h and then were ground to powder. The XRD spectra of the SBA samples were recorded using an X-ray diffractometer (X/Pert Pro MPD, Holland) with a Cu Kα radiation source at 40 kV and 30 mA from a 2θ of 5° to 60° (step size of 0.02° and acquisition time of 40 s).

RESULTS AND DISCUSSION

Model Fitting

The combined effects of pretreatment temperature (X_1), pretreatment time (X_2), and pretreatment pH (X_3) on reducing sugar content (Y_1) and bonding strength (Y_2) are presented in Table 2. Linear correlation analysis showed that the correlation coefficient between Y_1 and Y_2 ($r = 0.581$) was higher than the critical value ($r_{(0.01, 20)} = 0.561$) (Field 2009; Coolidge 2012), suggesting that there was a significantly positive relationship between reducing sugar content and bonding strength of SBA. This result implied that the bonding strength of SBA can be effectively enhanced *via* enzymolyzed polysaccharides in DSF (Chen *et al*. 2013, 2014).

Table 2. Response Surface Experimental Design Results

Run	Pretreatment temperature (X_1, °C)	Pretreatment time (X_2, min)	Pretreatment pH (X_3)	Reducing sugar content (Y_1, %)	Bonding strength (Y_2, MPa)
1	-1(40.0)	-1(20.0)	-1(4.5)	3.37	0.55
2	1(60.0)	-1	-1	2.21	0.49
3	-1	1(40.0)	-1	2.14	0.40
4	1	1	-1	3.08	0.53
5	-1	-1	1(5.5)	2.83	0.58
6	1	-1	1	2.77	0.49
7	-1	1	1	2.81	0.53
8	1	1	1	2.43	0.48
9	-1.682(33.2)	0(30.0)	0(5.0)	2.05	0.53
10	1.682(66.8)	0	0	2.87	0.60
11	0(50.0)	-1.682(13.2)	0	2.45	0.52
12	0	1.682(46.8)	0	2.41	0.46
13	0	0	-1.682(4.2)	2.12	0.37
14	0	0	1.682(5.8)	2.80	0.62
15	0	0	0	2.67	0.46
16	0	0	0	2.78	0.50
17	0	0	0	3.02	0.57
18	0	0	0	3.14	0.59
19	0	0	0	2.45	0.48
20	0	0	0	1.98	0.54

To further determine the coefficients of the parameter variables (X_1, X_2, and X_3) for the response variables (Y_1 and Y_2), a quadratic model was selected as suggested by the Design Expert software, and the following second-order polynomial equation, as shown below (in terms of the code factors):

$$Y_1 = 2.94 + 0.071\ X_1 + 0.056\ X_2 - 5.292 \times 10^{-3}\ X_3 - 0.13\ X_1 X_2 + 0.12\ X_1 X_3 + 0.056\ X_2 X_3 - 0.28\ X_1^2 + 0.038\ X_2^2 - 0.23\ X_3^2 \quad (2)$$

$$Y_2 = 0.57 + 0.026\ X_1 - 0.018\ X_2 + 0.022\ X_3 + 1.250 \times 10^{-3}\ X_1 X_2 - 3.750 \times 10^{-3}\ X_1 X_3 - 8.750 \times 10^{-3}\ X_2 X_3 - 0.039\ X_1^2 + 3.250 \times 10^{-3}\ X_2^2 - 0.043\ X_3^2 \quad (3)$$

R^2 is the multiple correlation coefficient (also known as the coefficient of determination), which is calculated by regressing the factor in question on all other factors. R^2_{adj} is the correlation measure for testing the goodness-of-fit of the regression equation with a higher value being more favorable. AP measures the signal-to-noise ratio, where a ratio greater than four is desirable. SD is associated with the experimental error. For Eq. (2), the R^2, R^2_{adj}, AP and SD were 0.7471, 0.5159, 5.751 and 0.27, respectively; and for Eq. (3), the R^2, R^2_{adj}, AP and SD were 0.8556, 0.7256, 8.299, and 0.034, respectively. These indicate that the models can be used to navigate the design space.

Statistical Analysis

Results from the ANOVA for the quadratic model for reducing sugar content and bonding strength are listed in Table 3. Larger *F*- and smaller *p*-values suggest more significant corresponding variables (Amini *et al.* 2008; Kalavathy *et al.* 2009). The lack of fit measures the failure of the model to represent data in the experimental domain at points that are not included in the regression. For Y_1, a model *F*-value of 3.28 suggests that the model is significant, while a *p*-value less than 0.05 indicates that the model terms are significant. The *p*-value of the lack of fit was 0.7492, which implies it was not significant relative to the pure error and the model equation was adequate for predicting the reducing sugar content under any combination of values of the variables (Zhong and Wang 2010). The two quadratic terms (X_1^2, X_3^2) affected the reducing sugar content significantly, whereas the pretreatment temperature (X_1), pretreatment time (X_2), pretreatment pH (X_3), interaction term (X_1X_2, X_1X_3, X_2X_3), and X_2^2 were all insignificant to the response. It indicates that enzymatic pretreatment does not significantly affect the final reducing sugar content of SBA. This might result from the reducing sugar that was released from acid-salt treatment process of DSF; such released sugar may have obscured the influence of the enzymatic pretreatment process. According to the sum of squares of the parameter variables (Table 3), the effect of the parameters on the reducing sugar content were as follows: pretreatment temperature (X_1) > pretreatment time (X_2) > pretreatment pH (X_3). For Y_2, the model terms were highly significant ($p < 0.01$) and the lack of fit was not significant ($p > 0.05$). The pretreatment temperature (X_1), pretreatment pH (X_3) and two quadratic terms (X_1^2, X_3^2) affected the bonding strength significantly, whereas the pretreatment time (X_2), interaction term (X_1X_2, X_1X_3, X_2X_3) and X_2^2 were all insignificant to the response. This indicates a significant effect of enzymatic pretreatment temperature (X_1) and pH (X_3) on bonding strength of SBA. The effect of the parameters on the bonding strength were as follows: pretreatment temperature (X_1) > pretreatment pH (X_3) > pretreatment time (X_2).

Table 3. Analysis of Variance for Regression Model for Reducing Sugar Content (Y_1) and Bonding Strength (Y_2)

Source	Y_1			Y_2		
	Sum of squares	*F*-value	*p*-value	Sum of squares	*F*-value	*p*-value [a]
Model	2.20	3.28	0.0390	0.067	6.58	0.0034
X_1	0.070	0.93	0.3572	9.275×10^{-3}	8.24	0.0166
X_2	0.042	0.57	0.4684	4.266×10^{-3}	3.79	0.0801
X_3	3.825×10^{-4}	5.125×10^{-3}	0.9443	6.650×10^{-3}	5.91	0.0354
X_1X_2	0.13	1.78	0.2121	1.250×10^{-5}	0.011	0.9181
X_1X_3	0.12	1.58	0.2379	1.125×10^{-4}	0.10	0.7583
X_2X_3	0.025	0.34	0.5732	6.125×10^{-4}	0.54	0.4776
X_1^2	1.10	14.77	0.0033	0.022	19.66	0.0013
X_2^2	0.021	0.28	0.6079	1.523×10^{-4}	0.14	0.7206
X_3^2	0.78	10.42	0.0090	0.026	23.37	0.0007
Residual	0.75			0.011		
Lack of fit	0.26	0.53	0.7492	6.966×10^{-3}	1.63	0.3033
Pure error	0.49			4.283×10^{-3}		
Correlation total	2.95			0.078		

[a] $p < 0.01$ highly significant; $0.01 < p < 0.05$ significant; $p > 0.05$ insignificant

Analysis of Response Surface

The 3D representation of the response surfaces generated by the model for response variables (Y_1 and Y_2) are given in Figs. 1 through 3. These 3D surfaces can reflect the effects of two parameter variables on the response variables at a time, while the other parameter variables are maintained at zero level.

As shown in Fig. 1, the reducing sugar content and bonding strength improved initially and then decreased with an increase in pretreatment temperature for the constant pretreatment time. The maximum reducing sugar content and bonding strength of SBA were obtained when the pretreatment temperature ranged from 50 to 55 °C, which represents the suitable enzymatic pretreatment temperature of DSF. This was also in agreement with the previous studies, which showed that the suitable temperature is 55 °C when DSF was pretreated by Viscozyme® L to prepare silken tofu (Rosset *et al.* 2012).

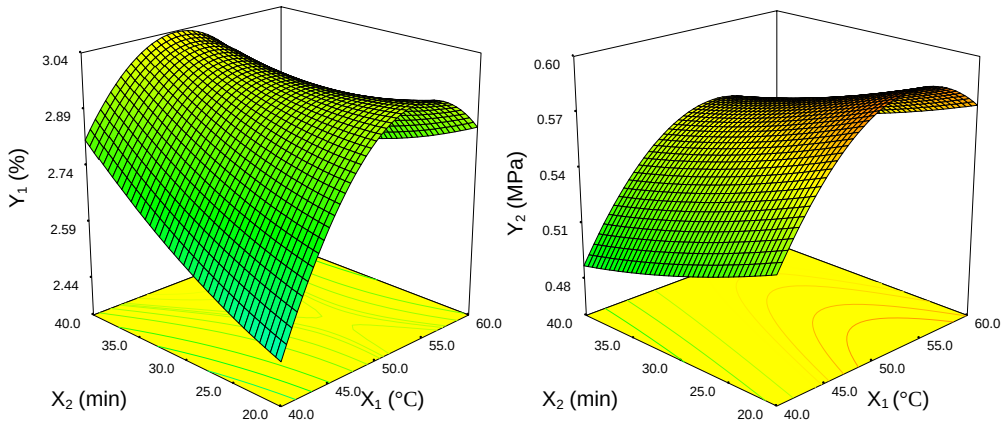

Fig. 1. The effect of enzymatic pretreatment temperature (X_1) and time (X_2) on reducing sugar content (Y_1) and bonding strength (Y_2) of SBAs

At the constant pretreatment temperature, the pretreatment time slightly affected the bonding strength, but it significantly affected the reducing sugar content. At low pretreatment temperatures (*e.g.*, < 50 °C), the reducing sugar content increased as pretreatment temperature increased. At the suitable pretreatment temperature (50 to 55 °C), the reducing sugar content was almost constant. This suggests that the levels of enzymatic pretreatment time can be decreased while the pretreatment temperature is maintained at 50 to 55 °C.

As shown in Fig. 2, there were some interaction effects of enzymatic pretreatment temperature and pH on reducing sugar content and bonding strength of SBA. At a constant pretreatment pH, the reducing sugar content and bonding strength of the SBA increased first and then decreased as pretreatment temperature was increased; at the constant pretreatment temperature, the effect of pH on reducing sugar content and bonding strength followed a similar trend to that of the pretreatment temperature. The maximum reducing sugar content and bonding strength of SBA were obtained when the pretreatment pH ranged from 5.0 to 5.3, which indicates the suitable activity pH of the enzyme is located at a pH range of 5.0 to 5.3.

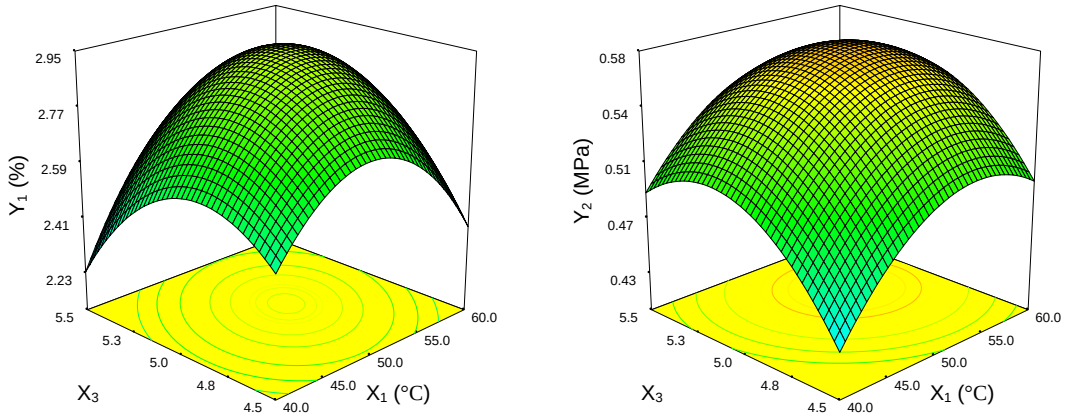

Fig. 2. The effect of enzymatic pretreatment temperature (X_1) and pH (X_3) on reducing sugar content (Y_1) and bonding strength (Y_2) of SBAs

As shown in Fig. 3, at constant enzymatic pretreatment time, the effect of pretreatment pH on reducing sugar content and bonding strength also followed a similar trend to that of the pretreatment temperature in Fig. 2. The reducing sugar content increased and bonding strength decreased as pretreatment time was increased when pH ranged from 4.8 to 5.3, while the reducing sugar content and bonding strength were affected slightly by pretreatment time when pH < 4.8 or > 5.3. It is assumed that the denaturation of soy protein increased with pretreatment time at 50 °C (zero level), leading to a decrease of the Maillard reaction.

Optimization of Enzymatic Pretreatment Conditions

The optimum enzymatic pretreatment conditions for making SBA were achieved by the desirability function method and determined to obtain the maximum reducing sugar content and bonding strength using Eq. (2), derived from the surface response experiments *via* Design Expert software.

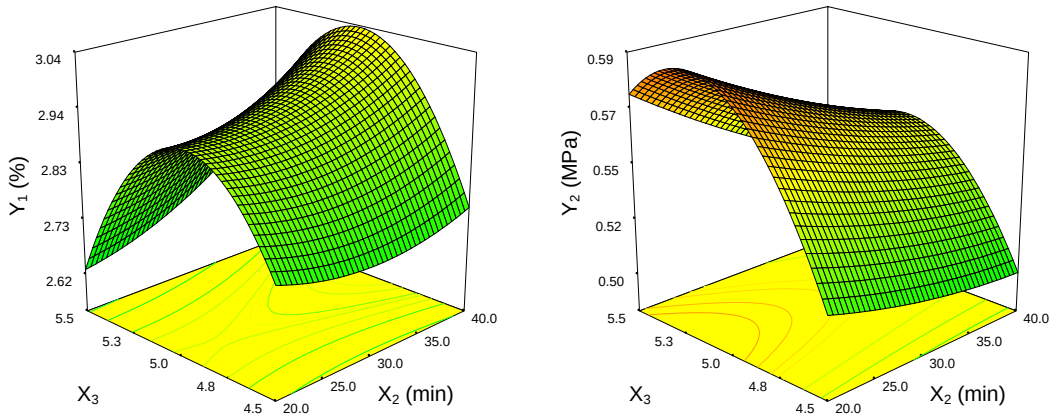

Fig. 3. The effect of enzymatic pretreatment time (X_2) and pH (X_3) on reducing sugar content (Y_1) and bonding strength (Y_2) of SBAs

The predicted optimal conditions for preparing SBA were as follows: pretreatment temperature of 53.6 °C, pretreatment time of 20.0 min, and pretreatment pH of 5.1. To verify the results, experiment rechecking was performed using these modified optimal conditions: pretreatment temperature of 54.0 °C, pretreatment time of 20.0 min, and pretreatment pH of 5.1. Moreover, the reducing sugar content and bonding strength of the control (using modified optimal conditions, only the enzyme was replaced by an inactivated enzyme) and DSF slurry were also investigated. The results are given in Table 4.

Table 4. Results of Verification and Control Tests

	Reducing sugar content (%)	Bonding strength (MPa)
DSF slurry	0.21 (±0.07)	0.19 (±0.04)
Control	1.37 (±0.13)	0.43 (±0.05)
Modified optimal conditions	2.93 (±0.11)	0.62 (±0.11)
Predicted optimal conditions	2.95	0.60

The reducing sugar content of DSF, control, and SBA increased in the order SBA > control > DSF (Table 4), implying that some of the polysaccharides in DSF can be hydrolyzed by acid treatment, and more enzymatic pretreatment can hydrolyze the polysaccharides further. The bonding strength of DSF, control, and SBA followed a similar trend to that of the reducing sugar content. This was in agreement with the linear correlation analysis of reducing sugar content and bonding strength, which showed a significantly positive relationship between reducing sugar content and bonding strength of SBA. Compared to the SBA prepared only using the enzyme (Chen *et al*. 2014), the bonding strength of SBA prepared from the modified optimal conditions increased by 16.1%, indicating that the bonding strength of SBA can be improved further by a combination of acid, salt, and alkali treatment. The results agree with previously published studies that showed increased water resistance of bio-adhesives, which was

ascribed to the reaction that occurred between monosaccharides and protein (He *et al.* 2014). The reducing sugar content and bonding strength of SBA obtained from real experiments, which were in significant agreement with the predicted value ($p > 0.05$), demonstrated the validity of the RSM model. Viscosity, solid content, and pH of the adhesive were 753 mPa•s, 19.2%, and 11, respectively.

XRD Analysis

The X-ray diffraction patterns of DSF, enzyme-treated DSF, and SBA in Fig. 4 show peaks at diffraction angles 2θ of 22.0°, which is in agreement with the characteristic X-ray diffraction pattern of soy protein (Su *et al.* 2010a,b; Garrido *et al.* 2014), suggesting that the three samples contain the ordered structure of soy protein. The characteristic X-ray diffraction peaks of cellulose (angles 2θ of 15.3°, 21.3°, and 34.0° (Ofomaja *et al.* 2013)) were not obvious in the X-ray spectra of DSF, which displayed the lower content of the ordered structure of cellulose in DSF (Karr-Lilienthal *et al.* 2005). The angles 2θ of 32.9°, 36.9° and 53.3° in the X-ray spectra of SBA were the characteristic X-ray diffraction peaks of sodium chloride, which was derived from the neutralization reaction of sodium hydroxide and hydrochloric acid. A comparison of the spectra of DSF and enzyme-treated DSF shows that the shape and strength of X-ray diffraction peaks did not change at angles 2θ of 22.0°, implying that structure of soy protein in DSF was not hydrolyzed during the hydrolysis process of polysaccharides because of the catalytic selectivity of the enzyme. A comparison of the spectra of enzyme-treated DSF and SBA shows that the shape and strength of X-ray diffraction peaks decreased at angles 2θ of 22.0°, indicating that the ordered structure of soy protein decreased after treatment with a combination of acid, salt and alkali, as a result of the acid- and salt-catalyzed hydrolysis of soy protein (Réat *et al.* 2000; Lin *et al*. 2012). The results also indicate that there are more activated functional groups (*e.g.*, -NH_2, -COOH, -OH) in SBA than in the enzyme-treated DSF, which may be the main cause of the enhanced water resistance of the cured SBA.

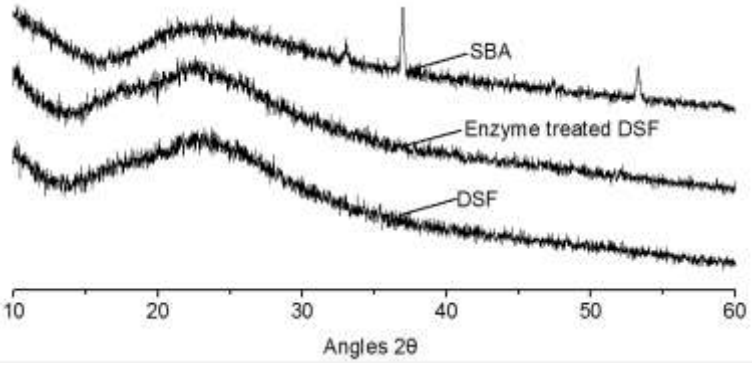

Fig. 4. X-ray diffraction spectra of SBAs

CONCLUSIONS

1. The reducing sugar content and bonding strength of SBA had a significantly positive relationship. The regression model for the enzymatic pretreatment conditions of SBA was satisfactory and accurate and could be used to navigate the experimental design space.

2. The interaction between enzymatic pretreatment temperature and pH did impact the bonding strength of SBA significantly, whereas the reducing sugar content was insignificant. The reducing sugar content and bonding strength of SBA increased initially and then decreased as enzymatic pretreatment temperature and pH increased; they were not obviously affected by pretreatment time. The optimal enzymatic pretreatment conditions are a pretreatment temperature of 54 °C, a pretreatment time of 20.0 min, and a pretreatment pH of 5.1.
3. The soy protein had a decreased ordered degree, but the ordered structure was unchanged after DSF was treated by a combination of enzyme, acid, salt, and alkali. The increased numbers of activated functional groups of SBA were derived from the increased reducing sugar and hydrolyzed soy protein, resulting in the improved water resistance of cured SBA.

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