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Optimization of bamboo autohydrolysis for the production of xylo-oligosaccharides using response surface methodology

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ABSTRACT

Bamboo powder (10.0 g) was subjected to autohydrolysis with a solid to liquid ratio of 1:10 under non-isothermal conditions to produce xylo-oligosaccharides (XOS) with a degree of polymerization of 2 to 6. The experiment was performed with two independent variables (reaction temperature 152 - 208 ºC and reaction time 1.72 - 58.28 min) to optimize the reaction condition by central composite design of response surface methodology. The analysis of variance of the regression model of XOS yield was in good agreement with the experimental results, and the predicted optimal condition for the production of xylo-oligosaccharides was observed at 182 ºC for 31 min with the yield of 36.4%. Under the optimal reaction condition for the production of XOS, relatively low concentrations of monosaccharides and byproducts were obtained. The investigation of antioxidant activity revealed that XOS produced from autohydrolysis exhibited a comparable scavenging activity with commercial antioxidant in superoxide and hydroxyl radicals.

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1. Introduction

With the growing emphasis on human health, the production of healthy and economically affordable foods for the world's population has been one of the greatest challenges. Prebiotics are nutrients that have the potential to considerably influence the physiology of human body by promoting the growth of beneficial bifidobacteria in colon, and consequently influence the health.[1] In recent years, more attentions have been paid on the new prebiotics especially xylo-oligosaccharides (XOS). XOS are sugar oligomers made up of 2 to 10 D-xylose units with β-1, 4 bonds, which naturally present in bamboo shoots, fruits, vegetables, milk, and honey.[2] However, few works are available on the extraction of XOS present in these sources due to their low contents of XOS.

Currently, the main process to obtain XOS is to degrade the hemicellulose fraction from carbohydrate-rich lignocellulose by chemical, physical or biological methods. The XOS derived from xylan show various applications in chemical, food, nutraceutical and pharmaceutical industries, which could act as a prebiotics to promote the growth of beneficial bifidobacteria in colon as well as low-calorie sweeteners and antioxidant additives.[3] Actually, recent reports show that ingestion of 4 g of XOS per day for 3 weeks improves the intestinal microbiota among the elderly who are above 65 years old.[4, 5] Besides, it also has the potential application in agricultural field as fodder additives to enhance the growth of fish, livestock and pets.[6-8]

The main procedure to generate XOS from biomass including enzymatic hydrolysis, dilute acid hydrolysis, and autohydrolysis. The commercial XOS is mainly

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produced by enzymatic hydrolysis of xylan extracted from biomass by alkaline, which caused the entire deacelation, and exhibits a low solubility in water.[3] However, some reports determined that acidic/acetyl XOS (XOS with glucuronic acid/acetyl substitution) shows a high prebiotics benefit as compared to neutral XOS.[2, 9] Due to the formation of too many monosaccharides as compared with XOS, and the large amount of toxic byproducts such as furfural and 5-hydroxymethylfurfural (HMF), diluted acid hydrolysis has limited application.[5] Autohydrolysis, an effective procedure to produce XOS, is well known as an environmentally friendly and economical technology. In autohydrolysis, hydronium ions derived from the autoionization of water act as a catalyst cause the depolymerization of hemicelluloses to XOS, xylose and other sugar degradation products. The partial cleavage of acetyl groups to acetic acids during the autohydrolysis process results in the increase of hydronium ions concentration in the reaction media, which also promote the hydrolysis efficiency. As an effective method to produce oligosaccharides, autohydrolysis has the advantages of no chemical regents and high selectivity separation. There are a lot of reports about XOS produced by autohydrolysis from corncob wheat straw, rye straw, etc.[10-12] The biological activity of XOS is mainly influenced by the degree of polymerization and substitutions.[13, 14] Some recent studies show that the XOS with low DP is of particular interest for prebiotic applications. XOS with DP from 2 to 6 are non-digestible oligosaccharides (NDOs), which show high value in food additives industries.[15] However, the XOS produced in the above studies exhibited a wide range of degree of polymerization of 2 to 20.[10-12] Previous study investigated the production of XOS by autohydrolysis, which contains a

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large portion of high DP XOS (>6).[3] Bamboo powder was employed as the raw material for autohydrolysis in the present work, since it has advantages of easy propagation, rapid growth and high productivity. Furthermore, some reports show that XOS derived from bamboo have a cytotoxic effect on human leukemia cells.[16] In this study, the autohydrolysis process was optimized to find the best reaction condition for the production of XOS (DP 2-6) by using response surface methodology (RSM), and the influence of reaction temperature and time on the yield of XOS was investigated.[3] The monosaccharides, byproducts and the antioxidant activity of XOS were also detected.[3]

2. Materials and methods

2.1. Raw materials

Bamboo culms were collected in July, 2014 from Yunnan province, China. The raw material was dried in an oven at 60 ºC for 24 h and then ground in a mill to obtain a fraction of 40 to 80 mesh. After that, the obtained bamboo powder was dewaxed with toluene-ethanol (2:1 v/v) in a Soxhlet extractor for 6 h and destarched with water at 80 ºC for 6 h. The extractive-free sample was dried in an oven at 60 ºC for 12 h and stored for further use.[3] The standards of xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose were purchased from Megazyme (Ireland), and other chemicals were provided by Sigma-Aldrich.

2.2. Autohydrolysis process

Bamboo samples (10.0 g, oven-dried basis) and deionized water were mixed in a 1 L

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stainless steel autoclave with mechanical stirring (Parr, USA) at a solid to liquor ratio of 1:10 g/g. The autoclave was heated by an external electrical furnace with the agitation of 150 rpm, and the reaction temperature was measured with a type-J thermocouple for different reaction times. A Proportional-Integral-Derivative (PID) controller was employed to dominate the temperature, and cooling of the mixture was accomplished by using circulating cold water.

2.3. Analysis of autohydrolysis liquors

The liquors obtained by the autohydrolysis were filtered and stored to determine monosaccharides, XOS and sugar degradation products. A high-performance anion-exchange chromatography (HPAEC, Dionex ICS-3000, USA) system equipped with a Carbopac PA-20 column (4×250 mm, Dionex, USA), and a Carbopac PA-100 column (4×250 mm, Dionex, USA) was employed to quantify the monosaccharides and oligosaccharides in the liquor. The eluent of 100 mM NaAc in a 150 mM NaOH at a flow rate of 0.4 mL/min and a column temperature of 30 ºC was used to separate the XOS. The degradation products were quantified and analyzed by an high-performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies, USA) equipped with a refractive index detector using a HPX-87H ion exclusion column (7.8×300 mm, Bio-Rad Laboratories, USA). The mobile phase was 0.005M sulfuric acid at flow rate of 0.6 mL/min with the column temperature of 50 ºC. More details of the analysis method were described in the previous literatures.[3, 17]

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133 **2.4. Experimental design**

134 The autohydrolysis variables were studied to determine the optimal conditions for the
135 maximum xylo-oligosaccharides yield. The influences of temperature and reaction time
136 were determined through a response surface methodology, and central composite design
137 (CCD) with a total of 11 experiments was employed to determine the best combination
138 of parameters for the autohydrolysis process. Four axial points, four fractional points
139 and three center points were carried out with the alpha factor of 1.414 for rotatable
140 design. The yield of XOS was determined as the response variable Y. The variables
141 were coded according to the equation:

142 $x_i = (X_i - X_{i,0})/\Delta X_i$ (i=1, 2) (1)

143 where x_i is the coded value of the variable X_i, $X_{i,0}$ is the real value of X_i at the center
144 point, and ΔX_i is the step change.

145 The relationship of the variables and response was calculated by the quadratic
146 regression equation:[18, 19]

147 $$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} X_i X_j \quad (2)$$

148 where k is the number of variables, X_i and X_j are independent variables, which influence
149 the response variable Y, β_0 is the constant term, β_i represents the coefficients of the
150 linear parameters, β_{ii} is the quadratic coefficient and β_{ij} estimates the interaction
151 parameters.

152 The conditions of each run are shown in Table 1. The results were summarized and
153 statistically analyzed by using Design-Expert software (version 8.0.6, StatEase Inc.,
154 USA). Analysis of variance (ANOVA) was employed to estimate the regression model.

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155

156 **2.5. Antioxidant activities**

157 The phenazine-methosulfate (PMS)-NADH method was employed for the generation of
158 O_2^- to investigate the superoxide radical scavenging assay.[20] The mixture containing
159 varying concentrations of XOS samples (1 mL, 0.05-2 mg/mL), PMS (1 mL, 60 μM),
160 NADH (1 mL, 676 μM), and NBT (1mL, 144 μM) in phosphate buffer (0.1 M, pH 7.4)
161 was incubated at room temperature for 5 min. The mixture was shaken for 1 h, and then
162 absorption was measured at 560 nm. The capability of scavenging to superoxide radical
163 was calculated using the following formula:

164 Superoxide radical scavenging effect (%) = $(1 - A_1/A_0) \times 100\%$ (3)

165 where A_1 is the absorbance of the sample mixed with reaction solution, and A_0 is the
166 absorbance of control. Superoxide radical scavenging assay was plotted as a function of
167 XOS concentration. From this graph, the XOS concentration needed to achieve
168 superoxide radical scavenging activity of 50% was defined as IC_{50}. Radical scavenging
169 index (RSI) was defined as the inverse of IC_{50}. The analysis was performed in triplicate.

170 The Fenton's system ($Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+OH^-+OH\cdot$) was employed to investigate
171 the hydroxyl radical scavenging assay of the XOS samples. The method was conducted
172 according to the previous literature reported by Smirnoff and Cumbes, and hydroxyl
173 radical was produced by the solution containing 2 mM EDTA-Fe^{2+} (0.5 mL), 3% H_2O_2
174 (1 mL), 360 μg/mL crocus in 4.5 mL sodium phosphate buffer (150 mM, pH7.4).[21, 22]
175 Subsequently, the XOS samples with different concentrations were added, and the
176 mixtures were incubated at room temperature for 30 min. The capabilities of scavenging

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hydroxyl radical of the mixtures were measured at 520 nm using a UV 2300 spectrometer (Thermal Scientific, USA), and calculated using the following formula:

$$\text{Hydroxyl radical scavenging effect (\%)} = (1 - A_1/A_0) \times 100\% \quad (4)$$

where A_1 is the absorbance of the samples, and A_0 is the absorbance of control. The concentration required to quench 50% of the initial hydroxyl radical was defined as IC_{50}, and the analysis was performed in triplicate.

The free radical-scavenging activity was measured by the effect of scavenging 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) according to Li et al.[23] 0.2 mM DPPH solution in 50% methanol was prepared before UV measurements, and then different concentrations of XOS samples (1 mL) were thoroughly mixed with 2 mL of freshly prepared DPPH and 2 mL of methanol. The mixture was shaken vigorously and kept in the dark at room temperature for 30 min. The capabilities of the samples to scavenge the DPPH radicals were calculated using the following equation:

$$\text{DPPH radical scavenging effect (\%)} = (1 - A_1/A_0) \times 100\% \quad (5)$$

where A_0 is the absorbance of control, and A_1 is the absorbance of the sample. The concentration of the samples needed to achieve DPPH radical-scavenging activity of 50% was defined as IC_{50}, and the analysis was performed in triplicate.

3. Results and discussion

3.1. Composition of the raw materials

The composition of the extractive-free bamboo powder was determined by the NREL laboratory analytical procedure.[24-26] As described in the precious literature, the

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199 composition of destarched sample was ash 2.8%, moisture 9.3%, and lignin 25.9%
200 (Klason lignin 24.1% and acid-soluble lignin 1.8%). Cellulose (40.4%) was the most
201 significant portion of biomass, and hemicelluloses (21.6%) were measured as xylan
202 substituted with arabinose (0.8%), galactose (0.2%), mannose (0.1%), glucuronic acid
203 (0.1%) and acetyl groups (2.2%). The ratio of acetyl group to xylose was 0.27 mol/mol.[3]
204

205 **3.2. Central Composite Design Model Fitting**

206 Response surface methodology is an effective statistical method using quantitative data
207 from an appropriate experiment design to investigate and to simultaneously solve
208 multivariate equations, which has the advantages of reducing set of experiments to
209 determine the interactions of a mathematical model and the optimum conditions.[27] Thus,
210 the reaction temperature and time were examined as factors to investigate the
211 correlation between the autohydrolysis variables to the XOS yield by using CCD. The
212 complete experiment variables design matrix together with the values of experimental
213 responses is presented in Table 1, and the ANOVA was carried out to select a suitable
214 model, to detect the significances of the model equation, and to investigate the model
215 terms. The following equation represents the description of XOS (2-6) yields from the
216 experimental responses:

217 $$Y=35.98+2.88X_i+1.63X_j-6.65X_iX_j-13.89X_i^2-14.26X_j^2$$

218 where X_i and X_j are represent reaction temperature and time, respectively.

219 In order to acquire a best fit model, ANOVA was employed to identify the adequacy
220 of the models. The ANOVA data are summarized in Tables 2 and 3, in which the

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coefficient of determination R^2 was defined to explain variation to the total variation and to demonstrate the agreement between the observed and predicted results. It was suggested that R^2 value should be at least 0.80 for a good fit of a model. A high R^2 represents that predicted values for XOS yield are more accurate and closer to the actual values. The R^2 (0.9823) in the present work (Table 2) indicated that 98.23% total variation in XOS yield was attributed to the experimental variables. This illustrated that a high correlation between the experimental values and theoretical predicts could be observed by the model and expressed good enough fit (Fig. 1). Additionally, the Pred. R^2 of 0.8741 was in reasonable agreement with the Adj. R^2 of 0.9646, exhibited a good adjustment between the experiment and predicted values. As shown in Table 2, standard deviation and coefficient of variation percent (CV %) in the present study were reasonably low and acceptable, indicated a high reliability of the experiment.

The probability value (*P*-value) was employed to check the significance of the coefficient, indicating the interaction between each independent variable.[18] *P*-values <0.05 for the regression model are statistically significant. In the present work the *P*-value of the model (0.0002) was significant enough with significance in lack of fit of 0.0016. The analysis also showed that *P*-values of X_i, X_j, X_iX_j, X_i^2 and X_j^2 were 0.0289, 0.1455, 0.0043, <0.0001 and <0.0001, respectively, demonstrating that the variables X_i, and quadratic variables X_iX_j, X_i^2 and X_j^2 affect XOS yield outstandingly. This illustrated that the reaction temperature and time significantly affected the production of XOS, and as compared with reaction time, the reaction temperature has a smaller *P*-value. The variables with smaller *P*-value showed a higher significance of the corresponding

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coefficient, indicated that the effect of reaction temperature (0.0289) on the production of XOS was greater than that of reaction time (0.1455). Additionally, *F*-value is another factor implies that the response surface model is significant.[28] In the current study, *F*-value of 55.42 (Table 3) indicated that there was only a 0.02% chance that a "Model *F*-value" this large could occur due to noise.

3.3. Optimization of the production of xylo-oligosaccharides

Response surface plots were built up by plotting the response (XOS production) against two independent variables to identify the optimal conditions of each variable for maximum XOS yield. Two-dimensional contour plots and three-dimensional response surface plots in the present work were generated by Design-Expert as shown in Fig. 2. In Figs. 2a and b, a quadratic effect of both reaction temperature and time was observed, although temperature had a greater influence on the response. The benefit of reaction temperature on XOS yield was observed in the range of 152-180 ºC, and a further increase in reaction temperature resulted in a rapid decline. On the other hand, similar phenomenon could be found in reaction time, which positively affected the XOS yield from 0 to 30 min, but led to the decrease after 30 min reaction. The decrease trend of the yield was mainly due to the excessive degradation of xylan with both the raise of the temperature and prolongation of time. The longer reaction time and/or higher reaction temperature would generate more xylose and sugar degradation products, leading to the decline of the XOS yield. The result illustrated that both variables significantly affected the production of XOS, and the optimal reaction condition was obtained. In Fig. 2b, the

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265 red zone represents the optimal reaction condition for the production of XOS, and the
266 predicted optimal point was observed (182 ºC and 31 min). This indicated that the
267 production of XOS achieved the highest yield (36.1 %) at 182 ºC for 31 min reaction,
268 which was a relatively high yield as compared with the result of enzymatic hydrolysis
269 (31.8%) and diluted acid (13%) hydrolysis.[18, 29]

270 An additional experiment run was conducted under the predicted condition to verify
271 the present suggested model. Experimentally, the XOS yield of the autohydrolysis
272 reaction at 182 ºC after 31 min was 36.4%, which showed no remarkable difference to
273 the predicted value, indicated that the model can mathematically represent XOS
274 production by autohydrolysis with the coefficient of 0.98. Furthermore, a relatively low
275 yield of monosaccharides and sugar degradation products were observed under the
276 optimal reaction condition, which was benefit for the further purification and utilization
277 of XOS (Table 1 and Fig. 3, the details will be discussed in the following parts).

278

279 **3.4. Formation of monosaccharides and degradation products during**
280 **autohydrolysis process**

281 The liquors after autohydrolysis reaction contained not only XOS, but also a number of
282 monosaccharides and sugar degradation products (acetic acid, formic acid, furfural and
283 5-hydroxymethylfurfural, etc.). In the present study, the monosaccharides were detected
284 by HPAEC and the sugar degradation products were determined by HPLC. As the data
285 presented in Table 1 and Fig. 3, a lot of monosaccharides and sugar degradation
286 products were generated during the autohydrolysis process apart from XOS, especially

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at a high temperature with a long reaction time. The concentration of monosaccharides increased with the raise of reaction temperature and prolongation of reaction time, and the highest values were achieved at 180 ºC for 58.28 min (arabinose 0.43 g/L, galactose 0.25 g/L, glucose 2.58 g/L and xylose 6.34 g/L). However, a further increase in reaction temperature and time caused a rapid decline of the concentration of monosaccharides, which could be interpreted by the fact that a part of pentose and hexose were degraded into furfural and HMF under the intensive reaction conditions. Moreover, the result also revealed that the formation rate of monosaccharides was much lower than that of the degradation rate, which led to the rapid decline of the concentration, could be certified by the data of sugar degradation products. On the other hand, the concentration variation of monosaccharides indicated that α-1, 2 and α-1, 3 bond cleaved in the early stage due to the relatively high concentration of arabinose at a low reaction temperature. In Fig. 3, the concentration of arabinose at a low reaction temperature was higher as compared with others, demonstrated that arabinose was easier cleaved from xylan backbone. The variation trends also revealed that arabinose was easier to be degraded into furfural since the concentration decreased at 180 ºC after 30 min (0.56 to 0.42 g/L from 30 to 58.28 min).

Besides oligosaccharides and monosaccharides, some byproducts would be performed during autohydrolysis process, especially under an intensive reaction condition. Table 1 presents the main byproducts formatted during the reaction period, which were furfural, HMF, acetic acid and formic acid. Furfural and HMF were the degradation products of pentose and hexose, respectively, whose yield were remarkably

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affected by the reaction temperature and time. As seen in Table 1, the maximum concentrations of furfural and HMF in the present work were observed when the reaction was conducted at an axial point (208.28 ºC, 30 min; furfural 4.12 g/L, HMF 1.80g/L). The result revealed that the production of furfural and HMF exhibited a positive correlation with the reaction temperature. On the other hand, as the data shown in Table 1, the concentrations of furfural and HMF increased (the concentration of furfural and HMF raised from 0 to 1.63 g/L and 0 to 0.36 g/l, respectively) with the prolongation of reaction time (from 1.72 to 58.28 min) when the temperature was 180 ºC, which indicated that a longer reaction time led to a higher concentration of furfural and HMF. These results demonstrated that large amounts of the sugar degradation product would be generated under the intensive reaction conditions. Since furfural and HMF have negative effects on the further utilization of XOS, the formation of sugar degradation products should be avoided during the XOS production process, which is beneficial to the prospective purification and utilization. The concentrations of furfural and HMF at the optimal reaction point (182 ºC and 31 min) in the present work were 0.59 and 0.12 g/L, respectively, which were comparatively low to the XOS yield of 36.4% (equivalent to 7.85 g/L). In comparison to others, the result of furfural and HMF in present study was lower than previous literatures of autohydrolysis and diluted acid hydrolysis, which was positive for the further utilization of XOS.[30, 31]

Acetic acid is one of the main byproducts generated during autohydrolysis process, which is produced by the release of acetyl groups attached on the xylan chain. Additionally, it could be obtained by the degradation of furfural and HMF. The ester

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linkage was cleaved when the autohydrolysis was conducted at a high temperature, and led to the release of acetyl groups and the formation of acetic acid in the reaction media. The acetic acid generated during the autohydrolysis process would be a diluted acid catalyst and caused the decline of pH, resulted in the promotion of the reaction. The concentration of acetic acid produced in the present study is shown in Table 1, where the concentration was increased with the raise of reaction temperature and/or prolongation of reaction time. Similar situation could be found in pH, which declined with the raise of reaction temperature and/or prolongation of reaction time, indicating a negative linear correlation with acetic acid concentration. Furthermore, the maximum acetic acid concentration of 1.68 g/L was obtained at an axial point (208.28 ºC, 30 min), which also have the lowest pH (2.66) in the present work. However, under the optimal reaction condition for XOS production (182 ºC, 31 min), the detected acetic acid concentration was only 0.61 g/L, equivalent to 18.9% of the initial acetyl groups. It means that large amounts of acetyl groups were still attached on the chain of XOS in the liquor and xylan in the residue. The previous findings in biology activity of XOS revealed that the in vitro fermentation of acetylated XOS was much slower than that of neutral XOS, which produced more organic acids, resulted in the promotion in the prebiotic effect.[14, 32] The current result of acetic acid indicated that a lot of XOS obtained in the present work was substituted with acetyl groups, which was positive for the prospective utilization. Formic acid is another weak acid which could be presented, and it is formed by the degradation of furfural and HMF during autohydrolysis process.[33] Thus, formic acid could only be observed at a high reaction temperature, and

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it also played a role in diluted acid catalyst in the reaction. Similarly, the concentration of formic acid was increased with the raise of reaction temperature and prolonging of time, and the maximum formic acid concentration of 0.71 g/L was obtained at 200 ºC for 50 min. In addition, only 0.17 g/L formic acid was observed under the predicted optimal reaction condition (182 ºC, 31 min) in the present study, which was fairly low as compared with the XOS yield.

In autohydrolysis process, hemicelluloses were degraded first at a low temperature followed by lignin decomposed at intermediate temperature. Previous research about the wheat bran showed that the lignin started to degrade at 170 °C and achieved to the highest degradation at 220 °C, in which 30% of the initial ferulic acid was released into the reaction media.[34] In the present study, the degradation was occurred, which 9.7% of the initial lignin was released under the optimal reaction condition (182 ºC, 31 min). The degradation compounds might be the ferulic acid, p-coumaric acid, and lignin degradation products, which would be exactly investigated in our following studies.[35]

3.5. Antioxidant activities of XOS

The antioxidant activity of XOS samples was investigated in comparison with commercial antioxidants by three different methods. BHA and BHT are the two commercial antioxidants control group, and the curves of the inhibitory effects of these specimens are shown in Fig. 4. The XOS sample was generated under the optimal reaction conditions (182 ºC and 31 min).

Superoxide radical is the one of the oxygen radicals, which leads to H_2O_2 formation

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by indirectly initiate lipid peroxidation, creating precursors of hydroxyl radicals.[36] The superoxide radical scavenging ability is obviously significant for the antioxidant research, and Fig. 4a shows the superoxide radical scavenging activity of XOS samples in the present work, and the radical-scavenging index (RSI) values of BHA, BHT and XOS were 0.21, 0.79 and 0.95, respectively. Obviously, the result indicated that the superoxide radical scavenging activity of all samples showed a concentration-dependent, and BHA exhibited highest scavenging ability. The scavenging activity of XOS was close to that of BHT, which demonstrated that the XOS obtained by autohydrolysis exhibited an acceptable superoxide radical scavenging activity.

Hydroxyl radical is the most active oxygen radical, which induces severe damage to the adjacent molecule.[37] The superoxide radical scavenging activity of XOS samples in the present work is shown Fig. 4b. It could be observed that the superoxide radical scavenging activity of the samples raised with the increase of the concentration. Moreover, the RSI value of XOS was 0.65, as compared to 0.17 for BHA and 0.57 for BHT, respectively. This suggested that the XOS presented a comparable scavenging activity with BHT, whereas the scavenging activity of BHA was much better than that of BHT and XOS.

The investigation of scavenging activity of DPPH radical is a common procedure for the evaluation of the free radical scavenging ability of natural products. The scavenging ability of XOS obtained by autohydrolysis at optimal predicted reaction condition is shown in Fig. 4c. The result suggested that all samples (BHA, BHT and XOS) exhibited concentration-dependent antioxidant activity, and the RSI values of

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BHA, BHT and XOS were 0.07, 0.34 and 0.84, respectively. The scavenging activity of BHA increased rapidly with the growing concentration, while that of XOS exhibited a relatively flat growth. The percent of inhibition of XOS gradually increased and reached 86.4% at the concentration of 2 mg/mL. Meanwhile, the DPPH radical scavenging activity of XOS obtained in the present work was higher than that of maize XOS and sugarcane bagasse XOS produced by enzymatic hydrolysis as reported in previous literatures, which may be due to the presence of phenolic compounds degraded from lignin during autohydrolysis process.[38, 39] The phenolic compounds in the obtained liquor by autohydrolysis might enhance the antioxidant activity of XOS. According to the previous literature, the antioxidant activity would decline after the purification of XOS, since the degraded products had a positive effect on antioxidant activity and would be removed.[39] Besides, type of phenolic acids, the effects of degree of polymerization, linkage type, substituted groups and position, ester linked phenolic acids, and the presence of sugars with uronic/acetyl groups cannot be underestimated since they played a very important role in the antioxidant activity of the XOS produced by autohydrolysis.[39]

4. Conclusion

Bamboo powder was autohydrolyzed in a stainless steel autoclave to generate xylo-oligosaccharides with low a degree of polymerizations (DP 2-6). The reaction temperature and time significantly affected the production of XOS, and the optimal reaction condition was observed by using response surface methodology. The analysis

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419 indicated that autohydrolysis conducted at 182 ºC for 31 min achieved the maximum
420 XOS yield of 36.4% with a relatively low concentration of monosaccharides and
421 byproducts. The investigation of antioxidant activity revealed that XOS by
422 autohydrolysis exhibited an acceptable scavenging activity in superoxide radical and
423 hydroxyl radical as compared with the commercial antioxidant BHT.
424

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Figure captions

Fig. 1. The correlation between predicted and actual values.

Fig. 2. Response surface (a) and contour plot (b) of XOS yield.

Fig. 3. Concentration of monosaccharides generated during autohydrolysis.

Fig. 4. Scavenging activity of superoxide radical (a), hydroxyl radical (b) and DPPH free-radical (c) of XOS.

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Table 1 Experimental design (independent variables), pH, response and byproducts of the CCD.

Run	Coded variables		Real variables		pH	Response	Sugar degradation products (g/L)			
	X_i	X_j	Temp. (ºC)	Time (min)		XOS yield (%)	Acetic acid	Formic acid	Furfural	HMF
1	-1	1	160	50	3.11	14.0	0.23	n.d. [a]	0.07	n.d.
2	0	0	180	30	3.02	36.0	0.57	0.16	0.53	0.07
3	0	1.414	180	58.28	2.77	10.1	0.81	0.38	1.63	0.36
4	-1	-1	160	10	3.71	0.1	0.11	n.d.	n.d.	n.d.
5	1.414	0	208.28	30	2.66	11.5	1.68	0.66	4.12	1.80
6	1	1	200	50	2.68	5.4	1.66	0.71	3.80	1.55
7	0	-1.414	180	1.72	3.65	1.7	0.14	n.d.	n.d.	n.d.
8	0	0	180	30	2.92	35.8	0.58	0.17	0.53	0.07
9	0	0	180	30	2.98	36.1	0.61	0.16	0.52	0.09
10	-1.414	0	151.72	30	3.50	1.8	0.09	n.d.	n.d.	n.d.
11	1	-1	200	10	2.86	18.1	1.07	0.23	1.02	0.23

[a] n.d., not detected

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Table 2 Statistical parameters of ANOVA of the XOS predicted model.

Statistical Parameter	Value	Statistical Parameter	Value
Std. Dev.	2.6819	R^2	0.9823
Mean	15.507	Adj R^2	0.9646
C.V. %	17.2953	Pred R^2	0.8741
PRESS	255.5708	Adeq Precision	19.8443

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Table 3 Analysis of variance of the model for XOS yields.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-Value	*p*-value Prob > F
Model	1993.33	5	398.67	55.42	0.0002
X_i	66.31	1	66.31	9.22	0.0289
X_j	21.35	1	21.35	2.97	0.1455
X_iX_j	176.91	1	176.91	24.59	0.0043
X_i^2	1089.33	1	1089.33	151.44	< 0.0001
X_j^2	1147.58	1	1147.58	159.54	< 0.0001
Residual	35.97	5	7.19		
Lack of Fit	35.93	3	11.98	640.13	0.0016
Pure Error	0.037	2	0.019		
Corrected Total	2029.29	10			

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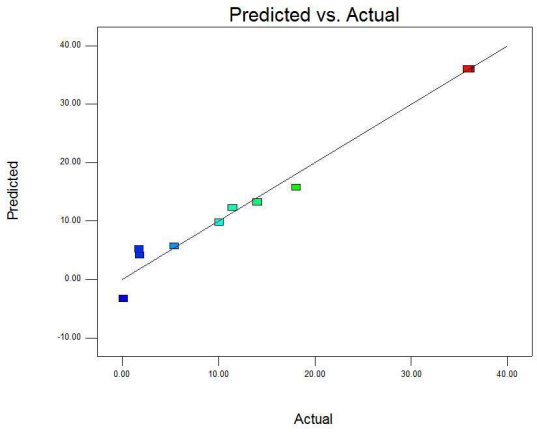

Fig. 1.

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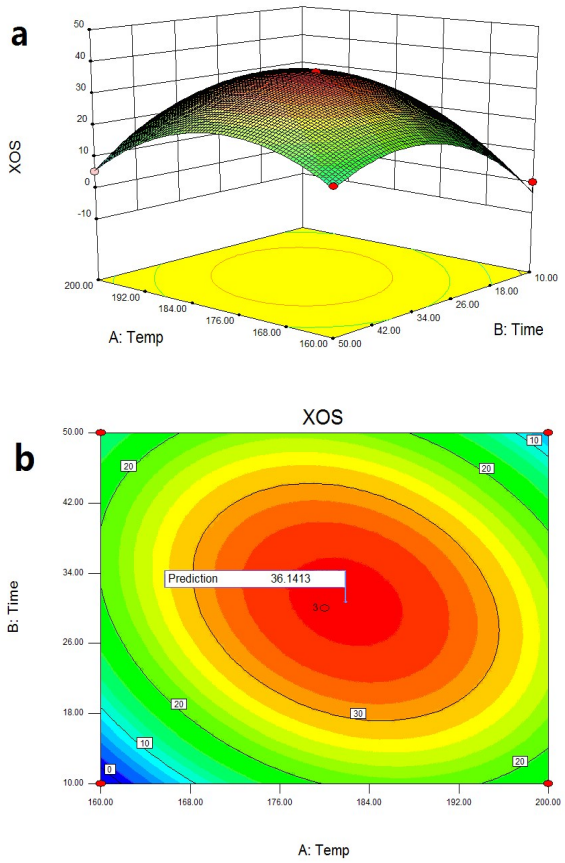

Fig. 2.

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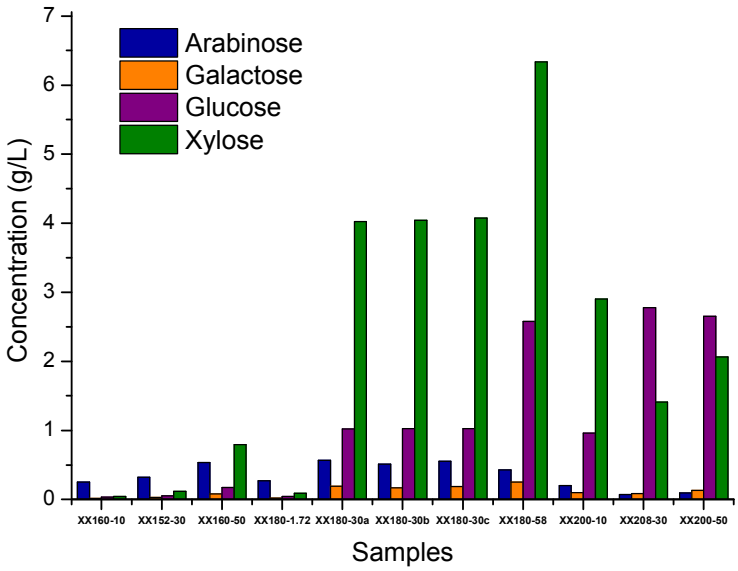

Fig. 3.

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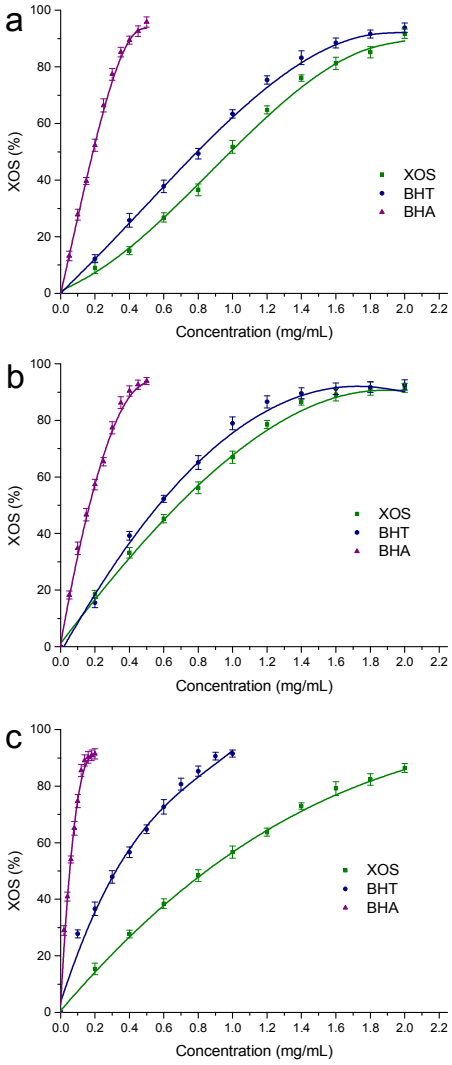

Fig. 4.

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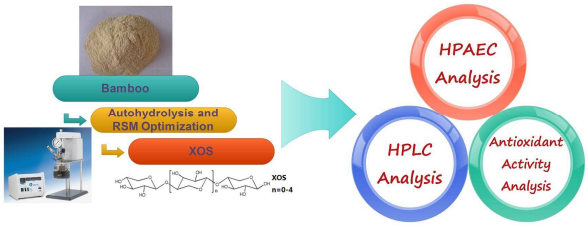

Title: Optimization of bamboo autohydrolysis for the production of xylo-oligosaccharides using response surface methodology

Author(s): Xiao Xiao, Chen-Zhou Wang, Jing Bian* and Run-Cang Sun*

Synopsis: Bamboo was employed to generate xylo-oligosaccharides by using autohydrolysis, and the process was optimized via response surface methodology to achieve the highest yield.

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