Xylo-Oligosaccharide Preparation through Enzyme Hydrolysis of Hemicelluloses Isolated from Press Lye

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Abstract: The emerging food additive, xylo-oligosaccharide (XOSs), was prepared through enzymatic hydrolysis of hemicelluloses isolated from press lye. Two of the three experimental enzyme preparations presented favourable effects, while the other one consisting of the nominal enzyme preparations with high enzyme activity was found to have poor catalytic effects. The conversion of xylan exceeded 40% and the selectivity for XOSs reached 90% when the temperature, hemicellulose concentration, dosage of enzymes, and hydrolysis time were 40 °C, 100 g/L, 1 ku/g, and 4 h, respectively. Xylo-oligosaccharide preparation through enzymatic hydrolysis of hemicelluloses isolated from press lye was proved to be a feasible process to utilize the by-product of the lye regeneration, which will substantially improve the economy of the lye regeneration and recycling.

Keywords: Xylo-oligosaccharide; Hemicelluloses; Enzyme; Hydrolysis; Press lye

1 Introduction

Viscose fibres are chemical fibres and their first production can be dated back over a century. In the production process, pulp is first immersed in NaOH solution to remove the hemicelluloses therein, so as to obtain relatively pure celluloses that are easier to process. The NaOH solution that results from dissolving the hemicelluloses after it has been pressed and separated is called press lye. The press lye not only contains dissolved hemicelluloses but also some degradation products of the celluloses^[1]. When the concentrations of the hemicelluloses and degradation products of celluloses dissolved in the press lye reach certain values, the press lye has to be replaced or quality of the viscose fibres becomes impaired. The processing and recycling of press lye, as well as the separation and value-added exploitation of hemicelluloses, are of great significance. Hemicelluloses have a wide range of applications and can be used to manufacture other products such as furfural, xylose, xylo-oligosaccharides (XOSs), packing materials and additives. In recent years, producing XOSs with hemicelluloses has aroused interest among scholars and shows good market potential^[2-5]. XOSs are prebiotics and can be added to feed, food products, and beverages. XOSs seem to impart nutritional benefits in various animal species and humans and have many functions such as: immunomodulatory activity, anti-cancerous activity, growth regulatory activity, and anti-microbial activity^[6-7]. The methods used for the preparation of XOS can be classified into three groups: auto-hydrolysis^[8-11], acid-hydrolysis^[12-14], and enzymatic hydrolysis^[15-18].

Compared with lignocelluloses feedstock such as crop wastes, wood and grass, the hemicelluloses (Hereinafter "LBC") isolated from press lye have more advantages with regard to the production of XOSs. The precursor, that is, pulps, of the LBC contains hardly any other components except for hemicelluloses and degraded celluloses. This allows the hydrolysis conditions of the LBC to be more easily controlled thus XOSs are more easily separated and purified. Million tons of press lye are discharged from the viscose manufacturing process each year, and the potential amount of LBC is substantial. Therefore, improving the value-added approaches for use of LBC is important. Griebel et al.^[19] studied the preparation of XOSs through hydrothermolysis of LBC. Zhang et al.^[20] investigated the production of XOSs through the hydrolysis of LBC catalysed by acetic acid. No publications are found which involve XOSs preparation with enzymatic hydrolysis of LBC. A lot of types of xylanase, which may be produced by fungi, bacteria, yeast, marine algae, protozoa, snails, crustaceans, insects, and seeds^[21], can hydrolyze xylan^[22-23], and many xylanase preparations are found in market^[24]. The purpose of this work is to evaluate the possibility of XOSs preparation by enzymatic hydrolysis of LBC.

2 Materials and Methods

2.1 Hemicelluloses Preparation

Hemicelluloses were isolated from press lye by means of ethanol precipitation with a 3/1 volume ratio of ethanol/lye. The press lye was provided by Xinxiang Chemical Fibre Cooperation Limited, China, and had been concentrated *via* a membrane process, and contained 50–60 g/L of hemicelluloses (as determined by dichromate oxidation method). The pentosan content of the isolated hemicelluloses was about 30% (dry weight basis), as determined by distillation^[25], hydrolysis^[26], colorimetric methods^[27], and HPLC, all of which are consistent with the results of Mozdyniewicz et al.^[1] Before utilisation, LBCs were neutralised, washed and analysed.

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2.2 Enzyme Preparation

Three types of solid enzyme preparations of different components, enzyme activities, and optimal use conditions were purchased from JD.com and Taobao.com (Table 1). When being used, moderate solid enzyme preparations were weighed according to the needed enzyme activity, then dissolved, and diluted to the required concentration, followed by adjustment to an appropriate pH using dilute sulphuric acid.

2.3 Enzymatic Hydrolysis

The authors purchased another xylanase on-line to prepare XOSs with LBC, however absolutely no XOSs were produced, which indicated that the enzyme is not able to catalyse and degrade LBC. For this reason, a group of experiments were designed through the orthogonal experimental design method to compare the catalytic effects of the three types of enzymes, so as to select the optimal combination for subsequent research and determine the ranges of the values of other variables. According to the data provided by the suppliers of the enzymes, the three types of enzymes differed slightly in terms of the optimal pH values and had wide ranges of suitable temperature, and varying the temperature and pH, in small ranges, exerted little influence on the yield of XOSs. Therefore, the pH and temperature were preliminarily fixed at 5.0 and 40 °C to compare the influences of four factors, *i.e.*, type of enzymes, hydrolysis time, dosage of enzymes (C_E , activity unit per mass of LBC), and LBC concentration (C_S , g of LBC / L of solution) on the yield of XOSs. Three levels were set for each of the four factors (enzyme: E1, E2, E3; hydrolysis time: 2, 8, 16 h; C_E : 1 000, 2 000, 3 000; C_S : 10, 50, 100). The Taguchi OA Design in the professional software Design-Expert and the orthogonal array L_9 (3^4) were used to arrange the experiments. The results are displayed in Table 2.

In compliance with the experimental plan in Table 2, a certain amount of LBC was put into a 100 mL conical glass bottle to which water was then added, as well as the enzyme solution, prepared according to the required enzyme activity. Finally, the solution was adjusted to required volume and pH adjusted with dilute sulphuric acid. Then, the bottle was sealed and placed on a shaker preheated to the set temperature to be shaken at a suitable velocity. After the specified time, the bottle was taken out and transferred to a water bath preheated to, and maintained, at 90 °C where it was allowed to stand for 1 h to inactivate the enzyme. Then the bottle was taken out, cooled to room temperature and centrifuged. The supernatant was extracted and then diluted to a certain volume before analysis of the contents of xylose and XOS therein. The hydrolytic residues were washed and dried to analyse the content of xylan therein. The conversion (C_X) of xylan and the selectivity (S_{XOS}) for XOSs were calculated according to Eq. (1) and Eq. (2).

$$C_X = \frac{\text{mass of xylan in LBC} - \text{mass of xylan in residue}}{\text{mass of xylan in LBC}} \times 100\% \quad (1)$$

$$S_{XOS} = \frac{\text{mass of xylan in LBC} - \text{mass of xylan in residue}}{\text{total mass of XOSs}} \times 100\% \quad (2)$$

Where XOSs refer to xylans with the degree of polymerization from two to nine.

2.4 XOS Quantification

The supernatants resulting from the enzymatic hydrolysis of LBC were analysed by high-performance liquid chromatography to detect the yield of XOSs released. Separation was performed using Shimadzu instrument equipped with an Xbridge Amide analytical column (Waters, 3.5 μm , 4.6 $\times$ 250 mm) coupled to an Evaporative Light-Scattering Detector. The temperature of the column was 40 °C, and the detector temperature was 60 °C. Air was used as carrying gas at a flow rate of 1.5 L/min. The mobile phase consisted of 0.2% triethylamine-acetonitrile (A)/0.2% triethylamine-water (B), and the gradient elution mode was as follows: 26%–43.5% B at 0–25 min, 43.5% B at 25–25.5 min, and 26% B maintained at 25.5–41 min. The flow rate was 1.0 mL/min. The concentrations of each saccharide were quantified based on the construction of a calibration curve with a mixture of xylose and XOS standards.

3 Results and Discussion

Intuitive/range analysis was used to analyse the experimental results. Range analysis is the simplest measurement of variation and is the easiest way to show the significance of each factor. The range is simply the maximum value minus the minimum value. The last four lines of Table 2 show the range analysis from direct observation. K_1 , K_2 and K_3 represent the mean values of S_{XOS} of each level of each factor. R is the range between the maximum and minimum value of the mean value K . The larger the value of R , for any factor, the greater that the influence on the response will be. According to the range analysis, it can be concluded that the effects of the four factors on S_{XOS} are, in descending order: enzyme, time, C_E , and C_S . Owing to the value of R corresponding to C_S being much smaller than those of the other three factors, it can be regarded as a pure error in the variance analysis (Table 3). As shown in the table, the changes of the other three factors significantly influenced the selectivity for XOSs ($P < 0.01$). Fig. 1a illustrates the trends of influences of different brands of enzymes on the selectivity for XOSs. It can be seen that the first two enzymes (E1 and E2) presented similar catalytic effects while E3 showed poor effect. For this reason, E1 and E2 were further compared. Fig. 1b shows the change in effects of different dosages of enzymes on the selectivity for XOSs: the selectivity for XOSs increased with increasing dosage of enzymes, while the enzymes with the dosages at the second and third levels exhibited slight influences on the selectivity for XOSs. Therefore, it is more cost-efficient to apply the dosage of enzymes at the second level. The trends in the influences of different hydrolysis times on the selectivity for XOSs are demonstrated in Fig. 1c. It can be seen that with the extension of the hydrolysis time, the selectivity for XOSs increased. While, whether, or not, it is economical to increase the selectivity for XOSs at the cost of prolonging the hydrolysis time remains to be evaluated.

Based on the above analysis, experiments were carried out to optimise the hydrolysis conditions. Although the concentration of LBC exerted no significant influence on the selectivity for XOSs, the larger the concentration, the greater the concentration of XOSs

Table 1 Source and application conditions of enzymes

Name	Enzyme trade name or producer	Enzyme activity (ku/g)	Application temperature (°C)	Optimal pH
E1	Sunson (Ningxia Sunson Industry Co. Ltd., China)	180	40–60	5.0
E2	Jiangsu Yinong, China	100	40–60	
E3	Ruibio X7411 (Hefei Bomei Bioscientific Co. Ltd., China)	60 000		

Table 2 Experimental plan and results for the first group of experiments

Run	Enzyme	Time	C_E	C_S	S_{XOS} (%)
1	E1	2	1 000	10	73.0
2	E1	8	2 000	50	86.8
3	E1	16	3 000	100	92.4
4	E2	2	2 000	100	85.4
5	E2	8	3 000	10	93.0
6	E2	16	1 000	50	87.0
7	E3	2	3 000	50	36.3
8	E3	8	1 000	100	28.3
9	E3	16	2 000	10	45.1
K1	84.1	64.9	62.8	70.4	
K2	88.5	69.4	72.4	70.0	
K3	36.6	74.8	73.9	68.7	
R	51.9	9.9	11.1	1.7

Fig. 1 Effect plot of single factor for the first group of experiments

in the hydrolysate, the lower the purification cost, and the more economically beneficial the preparation process. Therefore, the concentration of LBC can be further improved. As mentioned above, although the change of hydrolysis time significantly affected the selectivity for XOSs, prolonging the hydrolysis time caused a reduction in production efficiency, so it is probably not cost-efficient. Therefore, secondary experiments were conducted with the hydrolysis time in the range of 2 to 8 h, to study the changes in selectivity for XOSs. Similarly, secondary experiments were carried out with changing the dosages of the enzymes. Two levels were set for the above four factors (enzyme: E1, E2; hydrolysis time: 4, 8 h; C_E : 500, 1 000; C_S : 100, 150) and the experiments were arranged according to orthogonal array L_8 (2^7). The results are given in Table 4.

The last two columns in Table 4 list the rates of hydrolysis of xylan and the selectivity for XOSs. The main effects of the four factors were analysed taking C_X and S_{XOS} as the target response, respectively. Fig. 2 shows the resulting half-normal probability plot, in which there are influence and error columns of the selection. On this basis, the variance analysis was conducted, with the results shown in Table 5 and Table 6. Table 5 indicates that LBC content (C_S) and enzyme dosage (C_E) influence the conversion of xylan significantly, that is, conversion of xylan is different substantially for LBC content 100 g/L from 150 g/L, and for enzyme dosage 500 u/g from 1 000 u/g. The effect plots are displayed in Fig. 3. The levels 1 and 2 in Fig. 3a represent enzyme dosage 1 000 u/g and 500 u/g, respectively. Fig. 3a

illustrates that a higher enzyme dosage helps to improve the conversion of xylan, which is consistent with the results of the first group of experiments and with the expectation. The levels 1 and 2 in Fig. 3b represent LBC content 100 g/L and 150 g/L, respectively. Fig. 3b indicates that a higher LBC concentration is unfavourable to the conversion of xylan. Combining this with that of the first group of experiments, it can be concluded that 100 g/L is the preferred content of LBC. Table 5 indicates that P values of all the factors are over 0.05 which means that the changes of all the factors have no significant effect on the selectivity of XOSs. It seems to be contradictable with the conclusion of the first group of experiments. Actually, it is the change in the levels of the factors that results in lower effects on S_{XOS} .

To verify the reliability of the results of the experimental design, several experiments were carried out, and the experimental conditions and results are reported in Table 7. It was found that a high LBC concentration led to a low conversion of xylan and low selectivity for XOSs, while high conversion and high selectivity for XOSs can be obtained with a low LBC concentration, however, the concentration of XOSs in the obtained hydrolysate did not differ to any significant extent, therefore, it is necessary to carry out the hydrolysis using 100 g/L of LBC. The results attained under two different enzyme activities showed little difference, while in comparison, those under 1 000 u/g of enzyme activity were superior to those at 500 u/g.

Fig. 2 Half normalized probability plots for the secondary experiments

Fig. 3 Effect plot of C_E (a) and C_S (b) on C_X

Table 3 Analysis of variance for the first group of experiments

Source	Sum of squares	df	Mean square	F value	P-value prob > F
A-enzyme	4 969.22	2	2 484.61	1 064.83	0.000 9
B- C_E	148.51	2	74.25	31.82	0.030 5
C-time	219.55	2	109.77	47.05	0.020 8
Residual	4.67	2	2.33		
Cor total	5 341.94	8			

Table 4 The plan and results of the secondary experiments

	A	B	C	D	E	F	G		
Run	Enzyme	Time (h)	C_S (g/L)	C_E (u/g)				C_X (%)	S_{XOS} (%)
1	E1	8	100	1 000				37.8	95.7
2	E1	8	100	500				36.7	91.8
3	E1	4	150	1 000				30.9	94.3
4	E1	4	150	500				20.4	96.8
5	E2	8	150	1 000				28.3	89.7
6	E2	8	150	500				31.9	54.2
7	E2	4	100	1 000				30.1	83.1
8	E2	4	100	500				18.8	90.4

Table 5 Analysis of variance for C_X of the secondary experiments

Source	Sum of squares	df	Mean square	F value	P-value prob > F
A-enzyme	16.56	1	16.56	4.79	0.160 0
B-time	30.15	1	30.15	8.73	0.098 0
C- C_S	162.81	1	162.81	47.14	0.020 6
D- C_E	65.49	1	65.49	18.96	0.048 9
G-G	46.71	1	46.71	13.52	0.066 6
Residual	6.91	2	3.45		
Cor total	328.63	7			

Table 6 Analysis of variance for S_{XOS} of the secondary experiments

Source	Sum of squares	df	Mean square	F value	P-value prob > F
A-enzyme	337.35	1	337.35	4.25	0.175 5
B-time	126.01	1	126.01	1.59	0.335 0
C- C_S	143.23	1	143.23	1.80	0.311 5
F-F	165.17	1	165.17	2.08	0.286 1
G-G	427.05	1	427.05	5.38	0.146 3
Residual	158.89	2	79.45		
Cor total	1 357.69	7			

Table 7 Results of the verification experiments (E1, 4 h)

Run	C_S (g/L)	C_E (u/g)	C_X (%)	S_{XOS} (%)	C_{XOS} (g/L)	C_{XY} (g/L)
1	150	500	36.4	70.7	23.20	7.56
2	100	500	42.9	84.8	21.93	2.03
3	100	1 000	48.6	89.4	25.93	4.05
4	100	1 000	45.3	86.0	23.35	4.69
5	100	1 000	47.3	82.0	24.51	5.96

Note: C_{XOS} : XOSs concentration; C_{XY} : xylose concentration

4 Conclusions

The research investigated the preparation of XOSs through enzymatic hydrolysis of LBC. The hydrolysis conditions and effects of several enzymes were compared by using the orthogonal experimental design method. Two of these enzyme preparations with the activities of 100 ku/g and 180 ku/g showed favourable catalytic effects, while the other, with an activity of 60 ku/mg, was found to have a poor effect. The conversion of xylan exceeded 40 % and the selectivity for XOSs reached 90 % under conditions where LBC concentration, dosage of enzymes, temperature, and hydrolysis time were 100 g/L, 1 ku/g, 40 °C, and 4 h, respectively.

Conflict of Interest

The authors declare that there is no conflict of interest.

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