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Green and chemical-free process of enzymatic xylooligosaccharide production from corncob: Enhancement of the yields using a strategy of lignocellulosic destructuration by ultra-high pressure pretreatment

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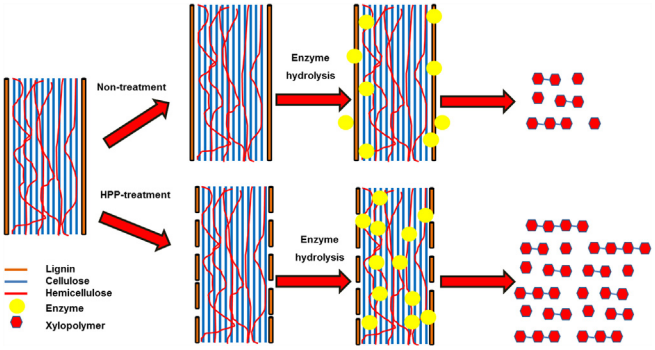
HIGHLIGHTS

- UHP enhances lignocellulosic destructuration and enzymatic hydrolysis of corncob.
- UHP caused significant differences in XOS yields between native and pretreated corncob.
- UHP at 100 MPa significantly improved the accessibility of endo-xylanase.
- UHP pretreatment at 100 MPa relatively did not affect composition of corncob.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the pressures at 50–500 MPa were evaluated at different time to pretreat and further enzyme hydrolysis. The ultra-high pressure (UHP) pretreatment at 100 MPa for 10 min led to improved accessibility of enzyme for conversion of xylan to xylooligosaccharide (XOS). The maximum XOS yield of 35.6 mg/g substrate was achieved and firstly reported at 10% (w/v) of substrate, 100 U of endo-xylanase/g corncobs and incubation time of 18 h. The enzymatic hydrolysis efficiency was increased by 180.3% and released a high amount of xylobiose. The UHP pretreatment relatively did not affect to the composition of corncob, but decreased 34.3% of lignin. Interestingly, antioxidant activities of XOS using UHP pretreatment were higher than untreated corncob. The UHP pretreatment improved lignocellulosic destructuration and XOS yields in a shorter time without the need of chemicals, implying that UHP could be an effective pretreatment of biomass with a chemical-free process.

1. Introduction

Corncob is a lignocellulosic biomass resource, which contains a variety of polymers, including 30–40% hemicellulose, 35–45% cellulose, 35–40% xylan-based hemicellulose and 5–20% lignin (Boonchuay et al., 2014; Kawee-ai et al., 2016; Yang et al., 2005). Since the corncob is a relatively low cost and readily available biomass, it can be used in a wide variety of industrial processes such as bioethanol, furfural, and xylooligosaccharides (XOS) (Kawee-ai et al., 2016). Lignocellulosic biomass represents a major component of different wastes from various industries including agriculture, forestry and municipalities. It mainly consists of three different types of polymers: cellulose, hemicellulose and lignin, which are associated with each other and are resistant to

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enzymatic attack (Taherzadeh and Karimi, 2008). The pretreatment of lignocellulosic biomass prior to enzymatic hydrolysis is an essential step for overwhelming the structural and steric barriers to enzyme access for more efficient sugar production. In order to improve enzyme accessibility and digestibility, several pretreatment methodologies have been applied, including biological, chemical, mechanical, thermal and physico-chemical methods (Taherzadeh and Karimi, 2008). However, each pretreatment has its own effects on the cellulose, hemicellulose and lignin (Hendriks and Zeeman, 2009). The choices of the pretreatment used for lignocellulosic biomass are cost effective with minimal chemical, heat and power requirements, and are also environment friendly (Kumar et al., 2009). Furthermore, an effective pretreatment can reduce downstream pressure by making lignocellulosic biomass more enzymatic accessibility and minimizing the formation of degradation byproducts (Yu et al., 2016, 2015).

High pressure processing (HPP), also called high hydrostatic pressure or high isostatic pressure, is an emerging technology that has already been applied in food industry and related sectors and has the potential to improve the balance between safety and quality of foods (Knorr et al., 2011). HPP is a non-thermal technology with pressure usually ranging from 100 to 800 MPa (Balasubramaniam et al., 2015). In the case of the treatment method, some research suggests that applying HPP as a pretreatment before enzymatic hydrolysis might be beneficial to promote the accessibility of the enzyme. Previous studies showed that HPP treatment of 300–400 MPa improved enzymatic hydrolysis of cellulase (Ferreira et al., 2011) and xylanase (Oliveira et al., 2012) from bleached Kraft *Eucalyptus globulus* pulp. The use of HPP assist-alkali pretreatment increased cellulose content and conversion from cotton stalk (Du et al., 2013) and sugarcane bagasse (Castañón-Rodríguez et al., 2015). However, the effect of HPP on corncob pretreatment and XOS production has not been reported.

XOS are non-digestible oligosaccharides and are usually produced from xylan, a major component of the plant hemicellulose, by chemical and enzymatic hydrolysis. XOS have a great prebiotic properties and are useful for a variety of purposes, including uses in pharmaceutical, food and agricultural products (Vázquez et al., 2000). However, the enzymatic production and a degree of polymerization (DP) in the range of 2–6 for XOS is preferred for use as food ingredients in the food industry. In terms of food applications, xylobiose (X2; DP = 2) is considered a XOS because the sweetness of X2 is equal to 30% of that of sucrose and has no off-taste (Vázquez et al., 2000). In order to increase the X2 yield and apply green technology with the chemical-free process, the objectives of this study were to investigate the effect of UHP as the pretreatment method under mild conditions on the destructuration of corncob and to determine the changes of XOS production and DP yields by xylanase hydrolysis from corncob after pretreatment with different UHP levels. The effect of UHP-pretreatment on the enzymatic hydrolysis of corncob under different conditions was also investigated and firstly reported. Under the UHP conditions, the composition, physical characteristics and structure of the UHP-pretreated corncob were analyzed and assessed by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR), respectively, as well as the antioxidant activities obtained after subsequent enzymatic hydrolysis.

2. Materials and methods

2.1. Raw material

Corncob used in this study was obtained from local farmers in Chiang Mai province, Thailand. After sun-drying for 5 days, the corncob was cut into pieces, ground with a hammer mill (Munson, Utica, NY, USA) and filtered through a sieve with a 100-mesh size, and stored at 4 °C until use. The composition of corncob was determined according to the National Renewable Energy Laboratory methods (Sluiter et al., 2010).

2.2. Endo-xylanase strain and growth conditions

The endo-xylanase produced from *Streptomyces thermovulgaris* TISTR1948 was used in the enzymatic hydrolysis. The strain was cultivated in a basal medium (yeast extract 5.42 g/L, K_2HPO_4 1.0 g/L, KH_2PO_4 0.5 g/L, $(NH_4)_2SO_4$ 1.0 g/L, NaCl 0.2 g/L, $MgSO_4{\cdot}7H_2O$ 0.1 g/L, $CaCl_2{\cdot}2H_2O$ 0.1 g/L, Tween 80 0.1 g/L and rice straw (mesh size <1 mm) 27.45 g/L) and incubated in a shaking incubator (Labtech, Daihan Labtech, Seoul, Korea) with a stirring speed of 250 rpm at 50 °C for 96 h (Chaiyaso et al., 2011). The xylanase activity (crude enzyme form) was measured using a 1.0% (w/v) beechwood xylan (Sigma-Aldrich, St. Louis, MO, USA) in a 0.1 M potassium phosphate buffer (pH 6.5) as the substrate. The crude enzyme was diluted with 0.1 M potassium phosphate buffer (pH 6.5) and incubated at 55 °C with 1.0% (w/v) beechwood xylan for 10 min (Boonchuay et al., 2014). One unit of xylanase activity (U) was defined as the amount of enzyme liberating 1 μmol of reducing sugar per min under assay conditions.

2.3. UHP pretreatment

The corncob (10%, w/v) was first immersed in water for 2 h at 35 °C, as previously described (Kawee-ai et al., 2016), and stuffed into a double polyethylene bag (230 mm long and 150 mm wide) without entrapped air. The samples were pretreated with the UHP apparatus TC10H-350-1212SP-STD (Stansted Fluid Power Ltd, Stansted, UK) at 50 °C, and at 50–500 MPa for 10, 20 and 30 min. After treatment, the corncob was washed with distilled water for 20 min and dried at 80 °C for 24 h, and then kept at 4 °C for further enzymatic hydrolysis.

2.4. Enzymatic hydrolysis

The corncob pretreated with UHP was used as substrate for XOS production. The substrate was subjected to enzymatic hydrolysis by mixing with 0.01 M potassium phosphate buffer at pH 6.5 (15% w/v). Then, 100 U/g of substrate of crude endo-xylanase from *Streptomyces thermovulgaris* TISTR1984 was added and the reaction was carried out at 55 °C for 24 h (Boonchuay et al., 2014). Samples were taken out and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected and analyzed for XOS using HPLC.

2.5. Analytical methods

2.5.1. HPLC analysis

Hydrolysis products were filtered through a cellulose acetate membrane (0.2 μm; Sartorius, Göttingen, Germany) and injected into the HPLC (Shimadzu, Tokyo, Japan) with an Aminex-HPX 87H column (300 × 7.8 mm; Bio-Rad, Hercules, CA, USA). The mobile phase consisted of 5.0 mM H_2SO_4 as an eluent at a flow rate of 0.75 mL/min at 40 °C. Xylose (X1, DP = 1), xylobiose (DP = 2), xylotriose (X3, DP = 3), xylotetraose (X4, DP = 4) and xylopentose (X5, DP = 5) were detected using a refractive index detector in a linear gradient over 25 min and glycerol was used as an internal standard (Boonchuay et al., 2014).The percent recovery and percent removal of each component were calculated by the following formula:

$$\%Recovery = \frac{W_{as\ received}}{W_{raw}} \times 100$$