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High monomeric sugar yields from enzymatic hydrolysis of soybean meal and effects of mild heat pretreatments with chelators

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ABSTRACT

Defatted soybean meal has 30–35% oligo-/polymeric carbohydrates and approximately 50% proteins. Enzymatic carbohydrate monomerization enables easy separation to enrich protein content, reduces indigestibility concerns, and facilitates use of carbohydrate as fermentation feedstock. Among soybean carbohydrates, pectin and glucan are more recalcitrant to hydrolyze. To destabilize Ca^{2+}-bridged junctures in pectin, effects of 3 chelators ethylenediaminetetraacetic acid (EDTA), sodium hexametaphosphate (HMP) and citric acid under 2-h 90 °C pretreatments were investigated here. Citric acid was the most effective while EDTA decreased enzymatic hydrolysis. In a 3-factor 2-level factorial study, heat (90 °C, 2 h) and citric acid (10 g/L) pretreatments and cellulase supplementation (10 FPU/g) were found to increase yields of all monosaccharides, to 86.8 ± 5.2% glucose, 98.1 ± 1.6% xylose, 87.5 ± 5.2% galactose, 83.6 ± 1.6% arabinose, and 91.4 ± 3.1% fructose + mannose. The largest percentage improvements were for arabinose (382%), mannose (113%) and glucose (51%). Achieving high monosaccharide yields greatly increases value of soybean carbohydrate as fermentation feedstock.

1. Introduction

Defatted soybean meal is produced from the oil extracted beans. It has high protein content (around 50%) and good amino acid profile (Medic et al., 2014) and is typically used in animal feed (Baker et al., 2010). Soybean meal also has 30–35% carbohydrate (Medic et al., 2014), which is currently undervalued but has been proposed as biorefinery feedstock, after hydrolysis to fermentable sugars, to produce chemical and biofuels (Loman et al., 2017a; Siqueira et al., 2008; Thakker et al., 2013). The carbohydrate comprises roughly equal amounts of non-structural and structural components (Bach Knudsen and Hansen, 1991; Ouhida et al., 2002). The nonstructural carbohydrates are predominantly sucrose and galacto-oligosaccharides, with very small amounts of monosaccharides and storage polysaccharides (Choct et al., 2010). The structural carbohydrates, also known as non-starch polysaccharides (NSPs), include dietary fiber components such as cellulose, hemicellulose and pectin (Refstie, 1999; Sinha et al., 2011).

Hydrolyzing these carbohydrates enzymatically to fermentable sugars requires a complex enzyme system with at least pectinase, xylanase, cellulase, α-galactosidase and sucrase activities (Li et al., 2017; Loman et al., 2017b). *Trichoderma reesei* and *Aspergillus niger* are among the most extensively studied fungal species for production of these carbohydrases (Castilho et al., 2000; Kolasa et al., 2014; Li et al., 2017). Li et al. (2017) examined 15 *Aspergillus* species and *T. reesei* Rut C30 for production of these enzymes. Loman and Ju (Loman and Ju, 2016) developed models for effects of individual enzyme activities on hydrolytic conversions of soybean meal carbohydrate to soluble reducing sugar (by dinitrosalicylic acid, DNS, analysis) and carbohydrate (by phenol-sulfuric acid analysis). The enzymatic hydrolysis process will be more economical if all types of soybean carbohydrate can be hydrolyzed.

The cellulose content in soybean meal reported in the literature varied from 1–2% (Karr-Lilienthal et al., 2005) to 4.4–6.2% (Bach Knudsen and Hansen, 1991; Irish and Balnave, 1993), presumably depending on processing methods (particularly the dehulling completeness) and different product requirements (Blasi et al., 2000; Stein et al., 2008). Typical *A. niger* fermentation broths had relatively low cellulase activities (~0.6 FPU/mL) (Li et al., 2017; Loman and Ju, 2016). This could limit glucose conversion from the soybean meals with higher cellulose content. Also, soybean meal has rather high (around 8–10%) pectin content. While *A. niger* strains were screened in previous work for higher pectinase production (Li et al., 2017), pectinase still tended to be a more limiting activity for soybean meal carbohydrate hydrolysis (Loman and Ju, 2016). In addition, pectin can be a protective layer that covers cellulosic microfibrils and hinders the cellulase access (Cheng et al., 2013). Enzymatic hydrolysis of soybean meal carbohydrate can

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benefit from improved pectin and cellulose hydrolysis.

Pretreatment has been shown effective to improve enzymatic hydrolysis of soybean meal (Fischer et al., 2001; Jung and Mahfuz, 2009), hulls (Islam et al., 2017; Yoo et al., 2011), and fibers (Karki et al., 2011). Fischer et al. (2001) pretreated (autoclaved) soybean meal at 125 °C for 15 min, at two moisture levels. At the high moisture level, i.e., with 200 g meal in 1800 mL water, the heat pretreatment improved the enzymatic extractability (into hydrolysate) of carbohydrate to 85%, from 76% in the control without pretreatment. The improvement was, however, insignificant (78% versus 76%) on soybean meal containing only 15% moisture. The heat pretreatment in presence of sufficient water was thought to loosen the intertwined structures between protein and carbohydrate and, accordingly, increase the accessibility of carbohydrate to hydrolytic enzymes. Other pretreatment methods such as toasting and extrusion have also been investigated (Jung and Mahfuz, 2009).

The objective of this study is to improve the sugar yields in enzymatic hydrolysis of soybean meal by different treatment methods. The carbohydrate composition of soybean meal used in this study was first analyzed using the standard National Renewable Energy Laboratory (NREL) procedure (Sluiter et al., 2008). An *A. niger* enzyme broth produced in this laboratory was used for the hydrolysis. For potential enhancements in enzymatic hydrolysis, three chelating agents, i.e., ethylenediaminetetraacetic acid (EDTA), sodium hexametaphosphate (HMP), and citric acid were examined at a heated condition (90 °C, 2 h). Multivalent cations such as Ca^{2+} are known to be able to form ionic bonding/crosslinking with the polygalacturonate backbones of pectin (Liu et al., 2010; Nakamura et al., 2002). Use of chelators (EDTA and HMP) has been shown to improve extraction of soybean okara pectin into aqueous media (Yamaguchi et al., 1996; Yoshii et al., 1996). In addition to chelating, citric acid may act as a dilute acid pretreatment agent. Organic acids such as fumaric acid and maleic acid were found to have similar dilute acid pretreatment effects as sulfuric acid (all acids at 50 mM) on wheat straw (at 10% solid loading), giving comparable sugar (glucose and xylose) yields in the subsequent enzymatic hydrolysis (Kootstra et al., 2009). Next, using a three factor-two level factorial design, the effects of heat treatment, citric acid, and cellulase supplementation on improving enzymatic hydrolysis were studied. Here, the rather mild heat treatment was done at 90 °C; at this temperature, sugar degradation and generation of fermentation inhibitors such as furfural and hydroxymethylfurfural (HMF) are expected to be very low (Islam et al., 2018; Jönsson and Martín, 2016) and there is no need for use of expensive pressure vessels. Yields of total reducing sugar and individual monomeric sugars were determined and compared to evaluate pre/treatment effects. This is the first report on citric acid-based biomass pretreatment, using its chelating and dilute acid effects, to improve enzymatic carbohydrate hydrolysis.

2. Materials and methods

2.1. Materials

The soybean meal used in this study was provided by Archer Daniels Midland Company (Decatur, IL). The enzyme broth used for enzymatic hydrolysis was produced in this laboratory by submerged *A. niger* NRRL 341 fermentation using soybean hull as carbon source (Li et al., 2017). The broth was clarified by centrifugation (12,000*g* for 15 min) and had the following measured enzyme activities: cellulase, 0.31 ± 0.02 FPU/mL; xylanase, 82.3 ± 4.2 U/mL; pectinase, 2.2 ± 0.5 U/mL; α-galactosidase, 4.1 ± 0.1 U/mL; and sucrase, 5.4 ± 0.1 U/mL. The commercial enzyme SPEZYME®CP (Genencor, Finland) was also used. It had the following measured activities: cellulase, 147 FPU/mL; xylanase, 349 U/mL; pectinase, 7.3 U/mL; and α-galactosidase, 2.2 U/mL. The chemicals used were all purchased from Fisher Scientific Inc.

2.2. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were done with 250 mL Erlenmeyer flasks in a shaker (Thermo Scientific MaxQ 5000 Incubating/Refrigerating floor shaker, Ashville, NC) at 50 °C and 250 rpm. In each flask 1.5 g soybean meal (at 50 g/L) was added to a 30 mL aqueous solution composed of 3 mL enzyme broth and 27 mL deionized water (i.e., with an enzyme loading of 2 mL per g soybean meal). Initial pH was adjusted to 4.8 and sodium azide (0.05%) was added to prevent microbial contamination during the hydrolysis. Samples taken from the experiments were centrifuged at 10,000 rpm (9300*g*, Eppendorf 5415D) for 10 min to separately collect the wet solids and the supernatant for analyses.

2.3. Effect of soybean meal particle size on enzymatic hydrolysis

Soybean meal particles were separated by sieving into 4 groups of different particle sizes: 23.7–75 μm, 150–212 μm, 300–425 μm and 425–1180 μm. Duplicate systems for each particle size group were then subjected to 48-h enzymatic hydrolysis for comparison. Samples were taken from all systems at 0, 2, 4, 9, 24 and 48 h, and the hydrolysates (supernatants) collected by centrifugation were analyzed for reducing sugar concentrations by the standard dinitrosalicylic acid (DNS) method (described in the analytical methods section). All the subsequent studies were done using soybean meal powders of 23.7–75 μm sizes.

2.4. Effects of heat pretreatment with chelating agents EDTA, HMP and citric acid on enzymatic hydrolysis

EDTA, HMP and citric acid were added, respectively, at different concentrations (2, 5 and 10 g/L) to the hydrolysis systems of 1.5 g soybean meal in 27 mL deionized water (without addition of the 3 mL enzyme broth). All systems were placed in a shaking water bath (ORS 200, Boekel Scientific Inc., Feasterville, PA) at 60 rpm, 90 °C for 2 h. After being cooled to room temperature, each system was adjusted to pH 4.8 and then added with 3 mL enzyme broth to start the enzymatic hydrolysis, following the same procedures described.

2.5. Factorial design for effects of heat and citric acid pretreatments and SPEZYME CP supplementation on enzymatic hydrolysis

Three factors were studied: heat pretreatment, citric acid pretreatment, and SPEZYME CP supplementation, at two levels: with and without each pretreatment or supplementation. Following the 3-factor 2-level factorial design, 8 (= 2^3) systems, each in duplicate, were compared. These systems are summarized in Table 1. Heat pretreatment

Table 1
Matrix for the factorial design for studying the effects of heat pretreatment, citric acid pretreatment, and cellulase (FPU) supplementation, and the code values of variables.

System	Heat	Citric Acid	FPU
Control	−1	−1	−1
Heat	1	−1	−1
Citric acid	−1	1	−1
FPU	−1	−1	1
Citric acid + Heat	1	1	−1
Heat + FPU	1	−1	1
Citric acid + FPU	−1	1	1
Citric acid + Heat + FPU	1	1	1

Factors	Lower level (−1)	Upper level (1)
Heat	No heat treatment	90 °C, 2 h
Citric acid	No citric acid addition	10 g/L (52 mM)
FPU	No FPU supplementation	Additional 10 FPU/g