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Improvement of enzymatic xylooligosaccharides production by the co-utilization of xylans from different origins

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ABSTRACT

This study aimed to improve XOs production by enzymatic hydrolysis of xylans from various lignocellulosic waste biomasses namely corn cob, cotton and sunflower stalks, rice hull, wheat straw by using two commercial xylanase preparations, Shearzyme 500L and Veron 191. Shearzyme 500L showed better xylan hydrolysis capacity with high amount of xylose liberation. Xylobiose was the main hydrolysis product in each case. Even though the enzymatic hydrolyses using Shearzyme 500L resulted higher reducing sugar production compared to those of Veron 191, the hydrolysis of complex xylan structures was improved and the production of undesirable xylose was lowered by the co-utilization of xylanase preparations. By the co-utilization of xylanase preparations, the reducing sugar production from wheat straw, corn cob and sunflower stalk originated xylans was increased by 36%, 33% and 13%, respectively, compared to the expected reducing sugar yields. The highest reducing sugar production was obtained from complex corn cob xylan. The depolymerization of cotton and sunflower stalk xylan was poorest even though they have simple structures. Poor utilization of these xylans might be related to their high residual lignin content which might hinder the accessibility of xylan by the xylanases. However, the utilization of sunflower and cotton stalk xylan was improved when they were hydrolyzed within a xylan mixture containing equal amounts of each of five different xylans. In short, XOs production efficiency from agricultural waste materials was improved by the co-utilization of suitable xylanase and/or xylan mixtures considering the heterogeneous structures of xylan and different substrate specificities of xylanases.

Keywords: Xylan; Xylooligosaccharides; Xylanase; Agricultural waste biomass; Cooperativity of xylanases

1. Introduction

Lignocellulosic waste materials are produced at an annual volume of more than 50 million tons in Turkey (Bascetincelik et al., 2006). They are generally used as animal feed or they are left to rot or burned in the soil (Agrupis and Maekawa, 1999). However, they can be converted into a variety of chemicals, such as alcohols, enzymes, organic acids, vitamins, polymers etc. thanks to their lignocellulosic structures (cellulose (35–50%, db), hemicellulose (20–35%, db) and lignin (5–30%, db)) (Saha, 2003). The utilization of these inexpensive and widely available wastes does not only solve improper disposal problem, but also creates additional value.

Hemicellulose, the second most abundant polysaccharide in the world and is mainly composed of xylans (Ebringerová and Heinze, 2000). Xylan is a heteropolysaccharide which is composed of β-1,4-linked D-xylose backbone with substitution on different side chains with L-arabinose, D-galactose, acetyl, feruloyl and glucuronic acids residues. This heteropolymer has been converted into many different value added products such as xylose (Howard et al., 2003; Gírio et al., 2010; Akpinar et al., 2011), xylitol (Parajó et al., 1998; Tran

Abbreviations: db, dry weight basis; DNSA, dinitrosalicylic acid assay; DP, degree of polymerization; HPLC, high pressure liquid chromatography; S, Shearzyme 500L; XM, xylan mixture; XO, xylooligosaccharide; V, Veron 191.

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et al., 2004; Prakasham et al., 2009), edible or biodegradable films (Kayserilioğlu et al., 2003; Hansen and Plackett, 2008; Bahcegul et al., 2011), antioxidants (Ebringerová et al., 2008; Hromádková et al., 2010) and xylooligosaccharides (Vázquez et al., 2001; Garrote et al., 2002; Parajó et al., 2004; Moure et al., 2006; Akpinar et al., 2007).

Xylooligosaccharides (XOs) are value-added oligosaccharides that can be used as functional foods and prebiotics. Since they are indigestible, they maintain gastrointestinal health by enhancing the growth of probiotic bacteria such as *Bifidobacterium* sp. (Kabel et al., 2002). XOs with degree of polymerization (DP) of 2–5 are preferred in functional food production owing to the utilization of these compounds by probiotic microorganisms (Bailey et al., 1992; Yamada et al., 1993; Chen et al., 1997). The production of XOs with shorter average degree of polymerization (DP of (2–3)) especially xylobiose gains importance because they present faster fermentation kinetics of the probiotic microorganisms and promote a favorable intestinal environment (Gullón et al., 2011). The sweetness of xylobiose is equivalent to 30% of that of sucrose, thus XOs are non-cariogenic, low-calorie, sugarless compounds, allowing their utilization also in anti-obesity diets (Goldman, 2009). L-Arabinose is also a value-added product that can be produced from xylan during XO production. It is an important intermediate for anti-virus drug synthesis and it has also been used as food additives for diet-controlling like XOs (Du et al., 2009).

Food and other industries spend considerable amounts of time and money to produce XOs. The fast growth of the functional food market and the increasing number of other industrial applications force researchers to explore different sources and technologies for producing XOs in high yields (Moure et al., 2006). XOs are generally produced by enzymatic (Pellerin et al., 1991; Yoon et al., 2006) or by partial acid hydrolysis (Sun et al., 2002; Akpinar et al., 2009) of the xylan fragments. Enzymatic hydrolysis is preferable because it does not produce undesirable by-products or high amount of monosaccharides (Akpinar et al., 2007). There are many reports in the literature describing the XOs production by enzymatic hydrolysis of xylan from oat spelt (Chen et al., 1997; De Menezes et al., 2010), bamboo grass (Yoshida et al., 1998), corn cob (Pellerin et al., 1991; Yoon et al., 2006; Aachary and Prapulla, 2009), wheat straw (Zilliox and Debeire, 1998; Swennen et al., 2005), almond shell (Rehman et al., 2008) and cotton stalk (Akpinar et al., 2007).

Due to the complex structure of xylans, microorganisms generally produce multiple forms of xylanases simultaneously to get extra assimilable monosaccharides for their growth and maintenance. Commercial xylanase preparations contain a mixture of various xylanolytic enzymes such as endo-1,4-β-xylanases, 1,4-β-D-xylosidases, α-L-arabinofuranosidase, α-D-glucuronidase, galactosidase and acetyl xylan esterases at different levels and they have wide applications in different industries. Traditionally, xylanases have been used in paper industry for improving paper quality by treating the pulp, in baking industry to improve the quality of dough and in brewing industry to increase wort filterability and to reduce haze in the final product. Developments in biotechnology have opened avenues for xylanases to be used in applications with broader spectrum such as enzymatic treatment of animal feeds to release pentose sugars, protoplasting of plant cells, coffee mucilage liquefaction, recovery of oils from subterranean mines, extracting pigments and plant oils, and production of laundry detergents and fabric care compositions (Kulkarni et al., 1999; Collins et al., 2005; Juturu and Wu, 2012).

Xylanases are also used in the production of valuable chemicals such as xylose, xylitol and xylooligosaccharides in biorefineries (Kulkarni et al., 1999; Collins et al., 2005; Juturu and Wu, 2012). For the enzymatic production of XOs, xylanase preparations with low exo-xylanase and/or β-xylosidase activities are preferred to avoid the production of the undesirable monosaccharide, xylose (Vázquez et al., 2001). Various xylanase preparations yield different XO concentrations and degrees of polymerization (DP) due to the differences in their specificities and activities.

The hydrolysis of xylans might be improved by using different xylanases simultaneously (Wong and Maringer, 1999; Sorensen et al., 2003; Raweesri et al., 2008). In a study of Wong and Maringer (1999), significant synergism was observed between xylanases during the hydrolysis of acetylated pine hemicellulose. In a study of Sorensen et al. (2003), xylose production was improved by using hemicellulolytic and cellulolytic enzymes together. In another study, a significant synergistic effect between α-L-arabinofuranosidase, endo-1,4-β-xylanase, β-xylosidase and acetyl esterase to produce reducing sugars was shown (Raweesri et al., 2008). Enzymatic hydrolysis might also be improved by co-utilization of different substrates. In a study of Beukes et al. (2008), hemicellulases and endoglucanase showed synergistic associations to produce reducing sugars when a substrate mixture containing birchwood xylan, carboxymethylcellulose and locust bean gum was utilized.

Considering the beneficial effect of the co-utilization of different xylanases on xylan hydrolysis, the present study investigates the effect of xylanase co-utilization on the production of XOs. For this purpose, agricultural waste materials which are readily available and abundant in Turkey namely corn cob, cotton and sunflower stalks, rice hull and wheat straw have been selected for XOs production. Two different commercial xylanase preparations, Veron 191 and Shearzyme 500L, have been used to produce XOs from alkaline extracted xylan from these biomasses. The effects of xylanase preparations and the co-utilization of these xylanase preparations on XOs production from alkaline extracted xylans were monitored. The xylans obtained from different lignocellulosic wastes were also co-utilized as the substrate during the hydrolysis in order to monitor the effect of using different xylans together on the XOs yield.

2. Materials and methods

2.1. *Lignocellulosic waste biomasses*

Cotton stalk, rice hull, wheat straw, corn cob and sunflower stalk were obtained from local producers in Adıyaman, Çorum, Konya, Hatay and Tekirdağ, respectively. These agricultural waste biomasses were dried at 50 °C in a convection oven overnight and then ground into smaller particles. The particle size of ground biomasses was between 100 and 1000 μm. The ground biomasses were stored in air tight containers at room temperature before use.

2.2. *Chemicals*

XOs standards, xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentose (X5), xylohexose (X6) were purchased from Megazyme (Bray, Ireland). All other chemicals used in this