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Application of Cassava Peel and Waste as Raw Materials for Xylooligosaccharide Production using Endoxylanase from *Bacillus subtilis* of Soil Termite Abdomen

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Abstract

Xylooligosaccharides (XOS) are the sugars produced from xylan hydrolysis. XOS have a prebiotic characteristic by promoting the growth of probiotic microorganisms. Xylan containing agriculture wastes *e.g.* rice straw, sugarcane bagasse, corncobs, cassava peel and waste can be used to produce XOS by a consecutive process of alkali-pretreatment and enzymatic hydrolysis. In this study, we focused on enzymatic production of XOS from cassava peel and waste, which is a low cost material with a relatively high xylan content. The dried cassava peel and waste were ground and sieved to be <100 mesh size, and were then subjected to pretreatment with 0.5 % (w/v) sodium hypochlorite solution for 5 h to remove the lignin in the sample. In the next stage, the xylan was extracted by soaking in 10% sodium hydroxide (NaOH) for 24 h, followed by adjusting the pH to pH 7 by adding 5% (w/v) hydrochloric acid (HCl). Next, after centrifugation, the obtained filtrate was precipitated with ethanol (ratio 1:3) and dried at 80°C for 48 h. The NaOH pretreatment enabled almost 4.83% and 6.23 % recovery of the xylan that was present in the cassava peel and waste. Next, the xylan from cassava peel and waste was hydrolyzed using endoxylanase (2.21 U/mL) from *Bacillus subtilis* of soil termite abdomen at pH 5 and 50°C for 15 h. Analysis by TLC showed the production of XOS, with especially X5 as the major band. HPLC chromatography confirmed that the most abundant product was indeed X5. X3 and X4 were also found but no X2. The results were not so different from the hydrolysis of xylan from oat spelt xylan, but showed a relatively lower yield.

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Keywords: Xilooligosaccharides; Cassava peel and waste; Endoxylanase

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Nomenclature

h	hour
mM	millimolar
ppm	Parts Per Million
U	Unit Activity
µmol	Micromolar

1. Introduction

Cassava is an abundant product in Indonesia, especially in East Java. According to the Indonesian Statistical Board, in 2013 the production of cassava in Indonesia was 23,824,000 tonnes and in the East Java Province it was 3,600,000 tonnes. Around 16% of the weight of cassava is waste such as peel and fibre. These waste products contain almost 70% water and 30% dry weight. In the dry weight fraction, there is 3.5% protein, 10% crude fibre, 11% lignin, 14%, cellulose, and 27% hemicelluloses[1]. There is also a small amount of poisonous HCN present in cassava waste, which must be reduced to below 10 ppm to make it less poisonous. Cassava wastes are used for bioethanol and compost production, and for cattle feed and food. Hemicelluloses are the second highest component in cassava waste. Bioconversion of hemicelluloses gets high attention because of its benefit in many fields such as the generation of fuel and chemicals, delignification of paper pulp, clarification of juice, digestibility enhancement of animal feedstuffs in addition to the production of emerging prebiotics, i.e., xylooligosaccharides (XOS)[2-4]. Among the available list of prebiotics, only XOS can be produced from the lignocellulosic biomass, which is renewable, abundantly available and does not compete with human foods. XOS have demonstrated physiological benefits along with protection against several chronic and infectious diseases[5]. There is a great need to identify new raw materials for XOS production in accordance with the growing demands for prebiotics in the near future.

Xylooligosaccharides are sugar oligomers composed of 2-10 units of xylose; they are considered as non-digestible food ingredients[6]. XOS exhibit prebiotic effects when consumed as an agent to maintain and improve a balanced intestinal microflora for enhancing health and well-being, such as *Bifidobacteria* and *Lactobacillus*, and hence improves one's health[7-8]. XOS are produced from various xylan-rich agro-residues by physico-chemical and biological processes, or by a combination of processes. XOS are produced from biomass such as wheat straw, rice straw, corncobs, tobacco stalks, sunflower stalks etc. by various methods such as chemical, autohydrolysis, direct enzymatic hydrolysis of susceptible fractions, acid hydrolysis, or by a combination of these methods[9-11]. Generally, xylan exists as a xylan-lignin complex in the lignocellulosic biomass and is resistant to hydrolysis. Therefore, XOS production is carried out in two stages, alkaline extraction of xylan from lignocellulosic biomass followed by enzymatic hydrolysis[10,12].

In our research, we used the endoxylanase enzyme isolated from *Bacillus* sp of soil termite abdomen. Our previous research (unpublished) has tested this enzyme for hydrolysing oat spelt xylan to produce xylooligosacharides. The products of the enzyme are xylobiose X2, xylotriose X3, xylotetraose X4 and xylopentaose X5, with X5 as dominant product. Considering the potential market demand of XOS in the food and pharmaceutical industry, the present study aimed to produce XOS from cassava peel and waste employing an indigenous endoxylanase enzyme from *Bacillus subtilis* of soil termite abdomen. Xylan was extracted from cassava peel and waste by dilute alkali treatment and enzymatically hydrolysed for XOS production.

2. Methods

2.1. Materials

All the reagents, media and chemicals used in this research were of analytical grade (Qualigens, Hi-media, Merck, Sigma). Xylan from oat spelt xylan was procured from Sigma, Germany. Standard xylooligosaccharides (X2, X3, X4 and X5) were purchased from Megazyme, Ireland. TLC plates of silica gel 60 F254 were obtained from Merck, Germany. Agro wastes like cassava peel and waste were procured from local farmers.