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Oligosaccharide Production from Agricultural Residues by Non-Starch Polysaccharide Degrading Enzymes and Their Prebiotic Properties

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Abstract

Oligosaccharides were obtained from different agriculture residues by using non-starch polysaccharides (NSPs) degrading enzymes (Pentozyme®) hydrolysis method. Pentozyme consist of mixture of xylanase, amylase, β-glucanase, cellulase, mannanase, and pectinase. Agricultural residues including sugar palm peel, pine apple peel, spent tea leaves, spent coffee grounds, brewer's spent grain, copra meal, and rice straw were used as a source for oligosaccharide production. At the end of hydrolysis, reducing sugar and oligosaccharides content of all samples were measured. The results showed that reducing sugar content was significantly different ($P<0.05$) among the samples with spent tea leaves produced the highest reducing sugar and oligosaccharide after hydrolysis. Therefore, spent tea leaves were used in the subsequent experiment to evaluate its prebiotic properties. Results showed that the extract were able to inhibits growth of pathogen and support the growth of beneficial bacteria.

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Keywords: oligosaccharide; agricultural residues; NSPs-degrading enzymes; prebiotic

1. Introduction

Agriculture sector played a significant role in economic development in Thailand whereby more than 65% of the total area of 520 million square kilometers, is occupied by agriculture related activities. The main crop components are rice, sugarcane and oil palm.

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Agriculture expansion leads to increase in quantities of livestock waste, agricultural crop residues and agro-industrial by-products. Large quantities of crop residues are produced annually in Thailand (Visvanathan and Chiemchaisri). Plant biomass is the most abundant material in the world. Its sources range from trees to agricultural residues. The most abundant and renewable biomass on earth is lignocellulose or non-starch polysaccharide, which contains three major polymer groups: cellulose, hemicellulose and lignin. Cellulose and hemicellulose comprise 40-60% of NSP (McKendry, 2002). In addition, Bailey proposed a clearer classification of NSP into three main groups, namely cellulose, non-cellulosic polymers and pectic polysaccharides (Bailey, 1973). The hydrolysis of NSP can be used to produce oligosaccharides (OS) and several report have shown that oligosaccharides can be used as prebiotic (Gibson, 2004). Biological methods for NSP degradation relies on the use of enzymes whereby various kinds of NSP-degrading enzymes which include cellulase, hemicellulase, xylanase, pectinase,β-glucanase and α-galactosidase were used (Sinha *et al.*, 2011). Pentozyme® is a commercial NSP-degrading enzymes that consisted xylanase, amylase, β-glucanase, cellulase, mannanase, and pectinase activities. The aims of this study were to determine the OS production from agricultural residues by using NSP-degrading enzymes and to evaluate the prebiotic potential of these hydrolysed products.

2. Materials and Methods

2.1 Materials

Agricultural residues and wastes including sugar palm peel, pine apple peel, spent tea leaves, spent coffee grounds, brewer's spent grain, copra meal, and rice straw were used for oligosaccharide production. All samples were ground (40 mesh) and wash several times in warm distilled water to remove any reducing sugars remaining in these residues. Residual moisture in these samples, with three replicates per sample was determined by drying to constant weight at 105 ºC in an oven.

2.2 Agricultural residues component analysis

Chemical composition of each agricultural residues were analyzed. Lignin, acid detergent fiber (ADF), and neutral detergent fiber (NDF), were determined using the AOAC standard method. The cellulose percentage was calculated indirectly from ADF and lignin, whereas the hemicellulose percentage was calculated indirectly from NDF and ADF (AOAC., 1997)

2.3 Hydrolysis of agricultural residues

Each material was hydrolyzed with the commercial enzyme (Pentozyme®) at level of 0.8% w/w, pH 7.0 (50 mM phosphate buffer) and 50 °C for 18 hours. After incubation, the samples were centrifuged at 10,000 rpm after which the supernatant of sample was collected to be used for reducing sugar and oligosaccharides analysis.

2.4 Analysis of carbohydrates

The amount of reducing sugars was determined using the dinitrosalicylic colorimetric method (DNS method) (Miller, 1959). Oligosaccharides were analyzed by thin layer chromatography using aluminum sheet silica gel 60, F254 (Merck, Darmstadt, Germany). A mixture of n-butanol/acetic acid/distilled water (5:2:3 by volume) was used as a developing agent. The spray agent contained 1 g α-diphenylamine dissolved in a solution of aniline/phosphoric acid/acetone (1.0:7.5:50.0 by volume) (Ratanakhanokchai *et al.*, 1999; Khuwijitjaru *et al.*, 2012).

Oligosaccharide analyses were performed using a high performance liquid chromatography (HPLC)(Gosling *et al.*, 2009). Oligosaccharides were separated using a Shimadzu Prominence HPLC with a 300 x 7.8 mm Rezex RNM-Carbohydrate column (Phenomenex). Milli Q water was use as mobile phase at a flow rate of 0.6 mL/min and oligosaccharides were detected with a RID-10A refractive index detector. The column and detector cell were maintained at 75 and 40 °C, respectively