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Prebiotic Potential of Xylooligosaccharides Derived from Cassava Dregs in Balb/c Mice Colon

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ABSTRACT

Xylooligosaccharides (XOS) are polymers of the sugar xylose bound by β(1→4) glycoside bonds. XOS have potency as a prebiotic and can be produced from agricultural waste such as cassava dregs. The purpose of this study was to examine prebiotic potential of XOS derived from cassava dregs from hydrolysis reaction catalyzed by endo-β-1,4-D-xylanase. The prebiotic activity of XOS derived from cassava dregs was examined by the number of *Bifidobacterium*, *Lactobacillus*, and *Escherichia coli* in Balb/c mice colon, the fermentation products of *Bifidobacterium* and *Lactobacillus* including changes in pH in the colon and short chain fatty acids (SCFAs) produced by the bacteria as well as the concentration of Ca^{2+} excreted through mice faeces. This study administered XOS derived from cassava dregs at 0.5 and 1.0 g (kg.BW) for 14, 21, and 28 days. The negative control group was Balb/c mice without XOS derived from cassava dregs. The results showed that feeding with XOS derived from cassava dregs at 0.5 and 1.0 g/(kg.BW) could increase the number of both *Bifidobacterium* and *Lactobacillus* and decrease the number of *E.scherichia coli*. The main fermentation products of *Bifidobacterium* and *Lactobacillus* were SCFAs such as acetic acid and butyric acid with respective concentrations of 11.57 and 2.97 mM. The pH in the colon of treatment groups was more acidic than that of in the control groups. The Ca^{2+} absorption

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was increased by 0.27% (w/v) that is characterized by decreased concentration of Ca^{2+} in Balb/c mice faeces of treatment groups. This study has led to a conclusion that XOS derived from cassava dregs of xylan hydrolyzed by endo-β-1,4-D-xylanase has prebiotic effect.

Keywords: Cassava, endo-β-1,4-D-xylanase, prebiotic, xylooligosaccharides

INTRODUCTION

A prebiotic is defined as a nondigestible food ingredient that can stimulate the growth and/or activity of limited number of bacteria in the colon (Saier & Mansour, 2005). Many oligosaccharides have been reported to have prebiotic properties, such as galactooligosaccharides, fructooligosaccharides, and inulin (Merrifield et al., 2010). Moure, Gullon, Dominguez and Parajo (2006) reported that xylooligosaccharides (XOS) derived from a variety of xylan had been proposed as excellent candidates for new generation prebiotics. XOS are polymers of the sugar xylose bound by β(1→4) glycoside bonds. XOS are naturally found in bamboo shoots, fruits, vegetables, milk, and honey (Ebringerova & Heinze, 2000; Gibson & Fuller, 2000; Okazaki, Fujikawa, & Matsumoto, 1990). XOS can be generated from the hydrolysis of xylan by endo-β-1,4-D-xylanase (EC. 3.2.1.8). Endo-β-1,4-D- xylanase cuts backbone of xylan randomly and generates XOS and little of xylose (Polizeli et al., 2005). Ratnadewi, Santoso, Sulistyaningsih and Handayani (2016) has succeeded extracting xylan from cassava dregs as much as 6.23% and yielded hydrolysis products such as xylotriose, xylotetraose, and xylopentaose enzymatically using the endo-β-1,4-D-xylanase from *Bacillus* sp.

XOS have high stability under acidic conditions and high temperatures. They can improve the quality of food by giving a change in taste and physico-chemical characteristics as well as stimulate the activity of *Bifidobacterium* in the intestine (Nakano, 1998; Suwa et al., 1999). In addition, XOS can decrease cholesterol and increase the absorption of Ca^{2+}. Specific XOS can increase the population of good bacteria (probiotics) in the colon of the elderly and pregnant women (Okazaki et al., 1990; Rycroft, Jones, Gibson, & Rastall, 2001). Compared to fructooligosaccharide (FOS), XOS are more effective in improving bowel health (Hsu, Liao, Chung, Hsieh, & Chan, 2004). XOS, composed of two to seven xyloses unit attached by the β(1→4) glycoside bond, caused difficulties in hydrolysis by enzymes in the digestive tract enhancing the growth of *Bifidobacteria* with high selectivity (Fedorak & Madsen, 2004; Guan, Zhou, & Wang, 2011; Manning & Gibson, 2004). According to Gullon, Moura, Esteves, Dominguez and Parajo (2008), the percentage of total consumption of XOS by *Bifidobacterium adolescentis* after 24 h was approximately 77%. The highest percentage of consumed XOS is xylotriose (90%), followed by xylobiose (84%), xylotetraose (83%), and xylopentaose (71%). Studies on human showed that XOS intake could increase

the number of probiotic bacteria in the large intestine, where xylobiose (X2) is kept for 24 h before excreted in faeces and urine. Xylobiose is not hydrolyzed both by enzymes in the saliva and pancreas and by gastric acid, instead it acts as a substrate by the probiotic bacteria (Garcia & Lopez, 2013).

Cassava dregs as a byproduct of cassava processing are easily found in Indonesia, especially in East Java (Indonesian Statistics Board, 2017). Recent studies on the *in vitro* have shown that XOS derived from cassava dregs have potential prebiotic to enhance the growth of *Lactobacillus acidophilus* up to 8.61 log CFU/mL and produce short chain fatty acids (SCFAs) such as acetic acid, propionic acid, isobutyrate acid, n-butyric acid, isovaleric acid, and n-valeric acid (Ratnadewi et al., 2017). In this study, the *in vivo* prebiotic activity of XOS derived from cassava dregs was determined by calculating the number of *Bifidobacterium*, *Lactobacillus,* and *E. Coli* in the Balb/c mice colon, measuring the pH of colon, assessing fermentation products of *Bifidobacterium* and *Lactobacillus* in the form of SCFA such as butyric acid, propionic acid, acetic acid and lactic acid, and calculating the Ca^{2+} ion concentration in Balb/c mice faeces.

MATERIALS AND METHODS

Xylooligosaccharides Derived from Cassava Dregs

This study employed xylooligosaccharides (XOS) from hydrolysis of cassava dregs xylan by endo-β-1,4-D-xylanase as the main material (Ratnadewi et al., 2016). Endo-β-1,4-D-xylanase was purified by precipitation with ammonium sulfate at a concentration corresponding to 50% saturation and by dialysis. The purity level of endo-β-1,4-D-xylanase was 6.3-fold purer than the crude extract of endo-β-1,4-D-xylanase.

Animals and Treatments

As many as 27 male Balb/c mice weighing 29- 31 g were housed for 14, 21, and 28 days after receipt. Every three mice were housed in suspended stainless steel cage under a 12- h cycle of light and darkness. The animal use protocol was reviewed and approved by the Ethical Committee of Faculty of Medicine University of Jember.

Nine groups of Balb/c mice were randomly divided into three control groups, three low-dose groups [0.5 g/(kg. BW)] and three high-dose groups [1.0 g/(kg.BW)]. XOS derived from cassava dregs were given for 14, 21, and 28 days, once a day by intragastric administration**,** while the negative control group did not receive any XOS derived from cassava dregs.

Sample Collection

The different doses of XOS were given for 14, 21, and 28 days. At the end of the experimental period, faeces were collected to analyze Ca^{2+} concentration and then Balb/c mice were euthanized by chloroform 90%. A ventral midline incision was made and the colon was excised. Immediately

after removal, the colon contents were collected, pH was measured, and as much as 0.4 g aliquot was processed for SCFA analysis. The remaining colon contents were immediately placed into a sterile assay tube for bacterial calculation.

Bacterial Enumeration

Samples for enumeration of selected genera of *Bifidobacterium*, *Lactobacillus* and *E. coli* were serially diluted 7-fold with physiologic salt solution immediately after collection. As much as 100 µl of the dilutions were inoculated onto duplicate plates using selective media for the enumeration of different bacteria. Bacteria were counted on BD^{TM} Bifidobacterium Agar, BBL™ Eosin Methylene Blue (EMB) Agar, and BBL™ LBS (*Lactobacillus* Selection). Plates were incubated for 24 or 72 h. After incubation, single colony was counted, and the results were expressed as the log values of the colony forming unit (CFU) per mL of wet of colon content.

SCFA Analysis

Extraction of SCFA was carried out through acidification 0.05 g of colon contents using 0.05 mL of H_2SO_4. Next, SCFAs were extracted by adding 0.6 mL of diethyl ether, and agitated and centrifuged for 30 s at 14,000 rpm. The organic phase was taken for further analysis of SCFA concentrations by gas chromatography (Garcia & Lopez, 2013).

Analysis of Ca^{2+} Concentration in the Faeces

Ca^{2+} concentration in the faeces of Balb/c mice colon was determined by previous method (Coudray et al., 2005). As much as 1.0 g of sample was added to 1 mL $HClO_4$ 60% pure analysis and 5 ml HNO_3 65% pure analysis, and incubated overnight. The next day, the sample was heated by hot plate (Stuart MC 152) at 100°C for 90 min, then the temperature was gradually increased to 130, 150, 170, and 200°C, each for 1 h to form white clouds. Destruction was completed with the formation of a white precipitate or the remainder of the clear solution of about 1 mL. The extract was cooled and then diluted with deionized water to 10 mL, then shaked. Clear extract was measured by Atomic Absorption Spectrometry (AAS) (Perkin-Elmer 420, Norwalk, CT USA) at wavelength of 422 for calcium (Ca).

RESULTS AND DISCUSSION

Bacterial Concentration

Microbiota concentration in Balb/c mice colon of different dose of XOS derived from cassava dregs for 14, 21, and 28 days was shown in Table 1. The results showed that the administration of XOS derived from cassava dregs at 0.5 and 1.0 g/(kg.BW) in Balb/c mice increased the number of *Bifidobacterium* by 1.5%, 4%, and 10% in the feeding for 14, 21, and 28 days, respectively ($P < 0.05$). The number of *Lactobacillus* was also increased by 1.5%, 5%, and 11.5% on feeding at 0.5 and 1.0 g/(kg.BW) XOS derived from cassava

dregs for 14, 21, and 28 days, respectively (P<0.05). According to Wei et al. (2013), feeding a commercial XOS (Shandong factory Long-li Biotechnology Co., Ltd.) 0.5 and 1.0 g/ (kg.BW) for 14 days can promote the growth of *Bifidobacterium* and *Lactobacillus* by 5% ($P < 0.05$) and 9% ($P < 0.05$), respectively. Whereas, in this study to achieve an increase in the number of *Bifidobacterium* and *Lactobacillus* by 5% and 9% ($P < 0.05$) took 7-14 days longer.

The total growth of *E. coli* in the study decreased by 1%, 3%, and 8% ($P < 0.05$) on feeding at 0.5 and 1.0 g/(kg. BW) XOS derived from cassava dregs for 14, 21, and 28 days, respectively, but the difference was not statistically significant. It indicates that XOS derived from cassava dregs is effective in increasing the growth of *Bifidobacterium* and *Lactobacillus* in the Balb/c mice colon.

Table 1
Microbiota concentration in the Balb/c mice colon

Sample	Time	Number of bacteria (log CFU/ ml)		
		Bifidobacterium	*Lactobacillus*	*Escherichia coli*
Control	14th day	7.67±0.02	7.37±0.02	7.77±0.00
XOS 0.5 g /(kg.BW)		7.78±0.00#	7.44±0.00	7.61±0.01
XOS 1.0 g /(kg.BW)		7.83±0.01#	7.56±0.07	7.63±0.00
Control	21th day	7.60±0.02	7.26±0.01	7.82±0.01
XOS 0.5 g /(kg.BW)		7.99±0.00	7.63±0.02	7.54±0.01
XOS 1.0 g /(kg.BW)		8.22±0.01	7.80±0.01	7.58±0.01
Control	28th day	7.36±0.01	7.00±0.00	7.95±0.01
XOS 0.5 g /(kg.BW)		8.33±0.01	8.15±0.01	7.24±0.02
XOS 1.0 g /(kg.BW)		8.39±0.00	8.21±0.01	7.00±0.06

Note: # indicates the data are not significantly different ($P < 0.05$)

pH of Balb/c Mice Colon

The administration of XOS derived from cassava dregs at 0.5 and 1.0 g/ (kg.BW) in Balb/c mice for 14, 21, and 28 days significantly decreased the pH of Balb/c mice colon ($P < 0.05$), compared to control (Table 2). A decrease in the pH of Balb/c mice colon generated the growth of probiotic bacteria (*Bifidobacterium* and *Lactobacillus*) (Hsu et al., 2004). The decline in the colon pH was concomitant with the increase of probiotic bacteria growth, as shown in Table 1. This finding is in accordance with the previous study by Hsu et al. (2004). Probiotic, bacteria such as *Bifidobacterium* and *Lactobacillus*, produces lactic acid and SCFA as metabolic products of carbohydrate fermentation that is not digested in the gut. SCFA can maintain the homeostasis and decrease the intestinal pH (Jan et al., 2002).

Table 2
pH of Balb/c mice colon

Sample	Time	pH of Colon
Control		7.1
XOS 0.5 g/(kg.BW)	14th day	6.34
XOS 1.0 g/(kg.BW)		6.62
Control		7.28
XOS 0.5 g/(kg.BW)	21th day	5.95
XOS 1.0 g/(kg.BW)		6.6
Control		7.24
XOS 0.5 g/(kg.BW)	28th day	5.85
XOS 1.0 g/(kg.BW)		6.57

SCFA Concentrations

Fermentation of prebiotic by probiotic bacteria in the colon produces SCFA. SCFA is a key product that can maintain intestinal health, gut morphology, and function (Roy, Kien, Bouthillier, & Levy, 2006; Scheppach, 1994). Commonly produced SCFA are acetic acid, propionic acid, and butyric acid (Jan et al., 2002). In this study, feeding Balb/c mice with 0.5 and 1.0 g/(kg. BW) XOS derived from cassava dregs for 14 and 21 days resulted in the production of SCFA such as acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric acid and n-valeric acid (Table 3). The dominant SCFA produced were acetic acid and butyric acid resulted from feeding 1.0 g/ (kg.BW) XOS derived from cassava dregs for 21 days with concentrations of 11.57 and 2.97 mM, respectively.

The similar result of acetic acid concentrations was reported by Pan, Chen, Wu, Tang, and Zhao (2009), assessing the fermentation of 90% cytooligosaccharides (COS) (Weikang, Shanghai, China) by probiotics. The concentrations of acetic acid and butyric acid found in Pan's et al. (2009) study were 37.13 and 3.64 μmol g^{-1}, respectively (1 Molar solution = 1×10^{-3} mol g^{-1}). The present study proves that XOS derived from cassava dregs is effective in increasing the growth of probiotic (*Bifidobacterium* and *Lactobacillus*) in the Balb/c mice colon, and cause the increase of fermentation product (acetic acid and butyric acid).

Butyric acid serves as an energy source for the colon by introducing the butyrate that produces strain *Butyrivibrio fibrisolvens* into germ-free mice or by adding butyrate to isolated colonocytes of germ- free mice. They rescued the colonocytes from both the deficit in mitochondrial respiration and autophagy. In the presence of an inhibitor for fatty acid oxidation, butyrate was unable to suppress autophagy. Those phenomena indicate that the rescue was due to butyrate acting as an energy source rather than as a regulator (Donohoe et al., 2011) and decreased the luminal pH. At the molecular level, butyric acid acts as an inhibitor of histone deacetylase promoting epigenetic hyperacetylation of histone proteins and non-histone that regulates the expression of cell cycle regulation CDKN1A, and alter DNA methylation resulting in increased accessibility of transcription factors to nucleosomal DNA (Hamer et al., 2008; Jan et al., 2002; Li et al., 2012; Sanderson, 2004; Scheppach, 1994; Smith, Yokoyama, & German, 1998). Butyric acid can also induce the differentiation of cells, suppress

the proliferation and increase apoptosis to remove the damaged DNA in cells that may develop into malignant cells both *in vitro* and *in vivo* (Leu, Brown, Hu, & Young, 2003; Li & Elasser, 2005; Medina et al., 1997).

Fermentation of XOS derived from cassava dregs by probiotics in the Balb/c mice colon produces branched-short chain fatty acids (BSCFAs) such as isobutyric, and isovaleric acids. Isobutyric and isovaleric acids were produced from the fermentation of branched amino acids, valine, leucine, and isoleucine, derivations of indigestible protein-reaching colon (Lynch & Adams, 2014; Yao, Muir, & Gibson, 2016). According to Heimann, Nyman, Palbrink, Petersson and Degerman (2016), BSCFA such as isobutyric and isovaleric acids have an effect on adipocyte and glucose metabolism that may contribute to improve insulin sensitivity. It implies that the intake of XOS derived from cassava dregs in mice Balb/c colon produce fermentation products such as SCFA and BSCFA that can improve health.

Table 3
SCFA concentrations in the Balb/c mice colon

Samples	Time	SCFA (mM)					
		C_2	C_3	iC_4	nC_4	iC_5	nC_5
Control		8.99	5.00	1.44	3.09	1.67	2.10
XOS 0.5 g/(kg.BW)	14th day	6.79	1.73	0.22	0.56	0.53	0.15
XOS 1.0 g/(kg.BW)		*	*	*	*	*	*
Control		9.93	7.16	1.02	1.92	1.84	0.83
XOS 0.5 g/(kg.BW)	21th day	57.26	4.43	0.80	1.45	0.43	0.26
XOS 1.0 g/(kg.BW)		11.57	4.2	0. 55	2.97	0.86	0.13

Note: (*) The sample is dried
C_2 (acetic acid), C_3 (propionic acid), iC_4 (iso-butyric acid)
nC_4 (n-butyric acid), iC_5 (iso-valeric acid), nC_5 (n-valeric acid)

Concentrations of Ca^{2+} in Balb/c Mice Faeces

The SCFA produced by probiotics in the gut lumen contribute to decrease pH in the colon, which is associated with an increase in dissolved calcium absorption, especially in the caecum. This is because SCFA affects the transcellular absorption of calcium by modifying the exchange of intracellular H^+ for Ca^{2+} in the distal colon (Van den Heuvel et al., 1999). Butyric acid can stimulate the intestinal epithelial cells, promote colon motility, and increase its absorptive capacity (Canani et al., 2011; Zhang et al., 2010). In this study, feeding with XOS derived from cassava dregs at 1.0 g/(kg.BW) for 14 days resulted in an increased concentration of Ca^{2+} absorption in the intestinal epithelial cells indicated by the decreased concentration of Ca^{2+} in

the faeces. The Ca^{2+} concentration in the control group faeces was found higher than that of in the treatment group 1.57% (w/v) to 1.34% (w/v), respectively. Based on the data in Table 4, XOS derived from cassava dregs at 1.0 g/(kg.BW) stimulated better in Ca^{2+} absorption in the intestine than corn bran arabinoxylans (Lopez et al., 1999). A recent study found higher Ca^{2+} released in mice faeces fed with corn bran arabinoxylans than that of from the control group, 79.6 and 52.7 mg/d, respectively. This finding proved that Ca^{2+} absorption was higher in the intestinal epithelial cells of Balb/c mice with XOS derived from cassava dregs intake.

Table 4
Concentrations of Ca^{2+} in Balb/c mice faeces

Sample	Time	Results of analysis % (w/v)
Control		1.57
XOS 0.5 g/(kg.BW)	14th day	1.70
XOS 1.0 g/(kg.BW)		1.34
Control		1.57
XOS 0.5 g/(kg.BW)	21th day	2.42
XOS 1.0 g/(kg.BW)		2,09
Control		1.57
XOS 0.5 g/(kg.BW)	28th day	2.19
XOS 1.0 g/(kg.BW)		2.24

CONCLUSION

XOS derived from cassava dregs could increase the growth of both *Bifidobacterium* and *Lactobacillus* and decrease the growth of *E. coli* in Balb/c mice colon. XOS derived from cassava dregs produced fermentation products such as SCFA and BSCFA resulting in a decreasing pH of the colon. In addition, the resulted butyric acid plays a role in the increased uptake of Ca^{2+} in the intestine characterized by the decrease of Ca^{2+} concentration in Balb/c mice faeces.

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