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Original Research Article

Isomalto-oligosaccharides production from rice flour and cassava starch

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ABSTRACT

This research aimed to evaluate the use of enzyme technology for the production of functional isomalto-oligosaccharides (IMO) from rice flour and cassava starch. Three different samples of non-glutinous rice flour, glutinous rice flour and cassava starch were compared to produce IMO syrup through the enzyme hydrolysis process. The first step was liquefaction of starch using α-amylase. The second step was saccharification using β-amylase and pullulanase to accumulate maltose. The last step was transglycosylation using transglucosidase to convert maltose to IMO. 25% (w/v) sample slurries were processed using 5-litre fermenters. The production process was maintained with appropriate conditions to obtain the maximum yield of IMO. Liquid samples were taken during bioconversion process to measure sugar and oligosaccharide concentration. Results showed that the highest concentration of IMO was obtained from non-glutinous rice flour with the value of 169.1 g/l (isomaltose = 78.2 g/l, isomaltotriose = 38.7 g/l and panose = 52.1 g/l) at the transglycosylation time of 1 h. However, using glutinous rice flour to produce IMO could produce IMO up to 106.4 g/l followed by cassava starch (IMO = 97.9 g/l). These results could indicate that non-glutinous rice flour was the best raw material among 3 samples to produce IMO.

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INTRODUCTION

Functional oligosaccharides are increasing interests as prebiotic functional food ingredients. They can be extracted from a variety of biomass sources or synthesized from carbohydrate raw materials, especially starch by enzymatic reactions. Many types of oligosaccharides are produced from starch such as gentio-oligosaccharides, cyclodextrins, malto-oligosaccharides and isomalto-oligosaccharide (Rastall, 2010 and Patel and Goyal, 2011).

Starch is a polysaccharide carbohydrate consisting of a large number of glucose units linked by glycosidic bonds (Amagliani et al., 2017; Vanier et al., 2017). The major starch sources are tubers, such as potatoes and cassava, and cereals. Starch can be physically, chemically or enzymatically modified and processed into many value-added products (Jane, 1995; Masina et al., 2017; and Zhu, 2015), especially the prebiotic isomalto-oligosaccharides (IMO) (Saman et al., 2012; Sawangwan and Saman, 2016).

IMO are glucosyl saccharides containing one or more α-(1,6)-glycosidic linkages with or without α-(1,4)-glycosidic linkages, that include isomaltose, panose, isomaltotriose, isomaltotetraose, isomaltopentaose, nigerose, kojibiose, and higher branched oligosaccharides (Bharti et al., 2015; Niu et al., 2017). IMO are considered the potential prebiotic capable of promoting the growth of colonic bifidobacterium and lactobacilli in the large intestine of humans and animals (Lin et al., 2011; Basu et al., 2016). IMO may also exert health benefits in the host. As they have the potential to improve bowel function, prevent overgrowth of pathogenic bacteria through selective stimulation of nonpathogenic members of intestinal microbiota and increase production of short-chain fatty acids (SCFA) (Ketabi et al., 2011; Kothari et al., 2014).

The commercial IMO usually include short-chain saccharides like isomaltose (DP2), panose (DP3), isomaltotriose (DP3) and isomaltotetraose (DP4) (Pan and Lee, 2005; Goffin et al., 2011; Ketabi et al., 2011). Niu et al (2017) reported that the commercially scaled starch-as-substrate process included three steps of enzymatic processes. First, starch was liquefied by bacterial or thermotolerant α-amylase to form maltodextrin. Second, resultant maltodextrin was saccharified by fungal α-amylase or β-amylase to generate syrup containing 40–50% maltose and maltotriose. In this step, pullulanase could be used to obtain higher maltose solution. Finally, the saccharified syrup was transglycosylated by transglucosidase to produce IMO.

The aim of this study was to evaluate the use of low cost economic rice flour and cassava starch as a raw material to produce IMO by using enzyme hydrolysis process. The commercial enzymes such as α-amylase, pullulanase and transglucosidase were applied to produce IMO under controlled condition. The concentrations of sugars and oligosaccharides were monitored during bioconversion process.

MATERIALS AND METHODS

Materials

Three samples of glutinous rice flour (0.1 to 0.3% amylose), non-glutinous rice flour (28-30% amylose) and cassava starch (15-18% amylose), were used in this study. Two types of rice flour were manufactured by Chlor Heng Noodle Factory, Thailand. Cassava starch was kindly supported by Chao Khun Agro Products Company Limited, Thailand. Enzyme solutions consisting of α-amylase, β-amylase, pullulanase and transglucoside were kindly supported by Siam Victory Chemicals Co., Ltd, Thailand. Glucose, maltose, maltotriose, isomaltose, isomaltotriose, panose and maltotriose were purchased from Sigma-Aldrich, United States.

Bioconversion of starch samples to IMO product

The process of IMO production was modified from the method of Pan and Lee (2005). One kilogram of each sample was weighed and transferred to 5000 ml-beaker. RO water was added to raise the final concentration of sample suspension to 25% (w/v) with the total volume of 4 liters. Each sample suspension had its pH adjusted to 6 by adding either 1 N HCl or 1 N NaOH followed by the adding of 0.02% (v/v) α-amylase for liquefaction. The sample mixture was then heated at 95°C for 10 minutes. Each sample slurry was transferred into an individual 5-liter fermenter and hydrolyzed further by adding 0.04% (v/v) β-amylase and 0.15% (v/v) pullulanase. All fermenters were maintained at 50°C for 6 h to obtain a saccharified solution. The resultant saccharified solution was then added with 0.1% (w/w) transglucosidase for tranglycosylation and incubated further at 60°C for 24 h. Samples were taken interval during bioconversion process. The concentration of sugars and oligosaccharides in each solution sample was analyzed by Thin layer chromatography (TLC) and High performance anion exchange chromatography (HPAEC).

Sugar analysis by TLC

Sugar and oligosaccharide contents in each samples were monitored by TLC analysis following the method of Joseph and Murrell (1983). All sample were diluted appropriately and applied to the TLC plates. A 6 µl of diluted sample was spotted on a silica gel TLC plate (K5, Merck, Germany) along with 3 µl of standard sugars (40 mg/ml): glucose, maltose, maltotriose, isomaltose, panose and isomaltotriose. After drying with hot air, the plates were developed with a solvent system of n-propanol-ethyl acetate-water, 6:1:3 by volume. The carbohydrates on TLC plates were visualized by dipping the plates into 5% (v/v) sulfuric acid in ethanol containing 0.5% α-naphthol before applying 110°C of heat to those plates for 10 min.

Determination of sugars by HPAEC

Quantitative sugar analysis was carried out using HPAEC (Dionex model ICS 5000, Thermoscientific, USA.) with a Carbo Pac PA-200 column and an electrochemical detector as the pulsed amperometric detector (PAD). For a quantitative and qualitative analysis of peaks, the software Chromeleon 7.2 SR4 (Thermoscientific, USA.) was used. Glucose, maltose, isomaltose, maltotriose, isomaltotriose and panose were identified and quantified base on external standards.

RESULTS AND DISCUSSION

Sugar and oligosaccharide contents during bioconversion process

In this experiment, three types of flour and starch were used as raw materials for IMO production. The enzymes consisting of α-amylase, β-amylase, pullulanase and transglucosidase were applied during bioconversion process. In principle, each enzyme acts differently. The role of α-amylase is partially cleavage of α-1,4-oligosaccharide links to yield predominately dextrins and higher DP oligomers (Pan and Lee, 2005; Chockchaisawasdee and Poosaran, 2013). β-amylase cleavages on α-1,4-glycosidic links. Pullulanase, optionally-debranching enzymes, is used to obtain high maltose solution with residual low DP maltodextrins. IMO are then produced through the action of a fungal α-transglucosidase from *Aspergillus* sp. This enzyme catalyzes both hydrolytic and transfer reactions. The non-reducing D-glycosyl residue of maltose may be transferred to water (hydrolysis), to

D-glycosyl residues released by hydrolysis, or to the non-reducing residue of maltose or every α-glucooligosaccharide present in the solution. The transfer occurs most frequently to the 6-OH group of the non-reducing glucose unit, producing isomaltose from D-glucose, or panose from maltose. Maltose and maltodextrins can actually act as both glycosyl donor and acceptor in the reaction. The transglucosidase from *Aspergillus niger* acts only on oligosaccharides with a low degree of polymerization (Goffin et al., 2011).

Sugar and oligosaccharide contents of three sample slurries were shown on TLC plates. Before enzyme addition, no band of sugar was found. However, some bands of sugar and oligosaccharides occurred after the adding of α-amylase (Figure 1a, 1b and 1c). When adding β-amylase and pullulanase, the content of maltose increased in all starch syrups. In the TLC plates, lane 10 to lane 18 represented the bands of sugars and oligosaccharides during transglycosylation period. Similarly, among three TLC plates the content of glucose increased while the content of maltose declined. Besides, the content of IMO occurred during transglycosylation in all TLC plates.

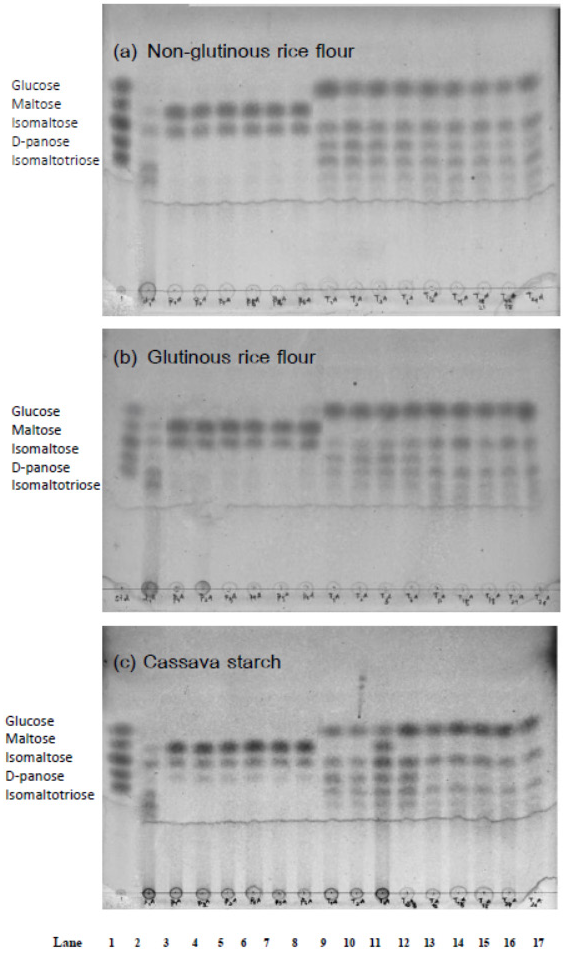

Figure 1. TLC chromatogram of liquid sample from the batches using (a) non-glutinous rice flour, (b) glutinous rice flour and (c) cassava starch as a substrate

Lane 1 = mixed standard sugars (glucose, maltose, isomaltose, panose and isomaltotriose)

Lane 2 = liquid sample added α-amylase and incubated for 10 minutes.

Lane 3-8 = liquid sample after added beta-amylase and pullulanase at 1 to 6 h.

Lane 9-17 liquid sample after added transglucosidase at 1, 2, 3, 6, 12, 15, 18, 21 and 24 h.

Sugar and oligosaccharide measurement by HPAEC during starch hydrolysis

For the first step of IMO productiohn, each sample mixture was added with α-amylase at 95°C for liquefaction. All mixtures were hydrolyzed partially to produce small amount of sugars such as maltose and maltotriose (Figure 2). After 10 min of incubation time, the sample slurry was hydrolyzed further by β-amylase and pullulanase for saccharification. The major product obtained was maltose followed by maltotriose. Among three sample slurries, the highest maltose yield was obtained from non-glutinous rice flour with the value of 433.1 g/l while concentrations of maltose from cassava starch and glutinous rice flour were 323.7 g/l and 318.5 g/l respectively. High concentration of maltose obtained was necessary for IMO production. McCleary and Gibson (1989) reported that for IMO production, glucose could be used as acceptors and potentially as glycosyl-donors although it may not be as efficient as maltose.

In the step of transglycosylation, transglucosidase was applied in the process. This enzyme can catalyze either reverse hydrolysis (thermodynamic control) or transglycosylation (kinetic control) under a retaining mechanism. When maltose was used as substrate, the transglycosylation product was formed via autocondensation. This process must be faster than glycoside hydrolysis, and the enzyme transfers a glycosidic residue from a glycoside donor to an acceptor with retention in the anomeric configuration (Mangas-Sanchez and Adlercreutz, 2015).

After adding transglucosidase, the amount of maltose decreased rapidly due to the bioconversion action of transglucosidase. The maximum yield of oligosaccharides was dependent on two parameters: the concentration of maltose and the intrinsic transferase/hydrolase ratio of the enzyme (Buchholz et al., 2005). However, the concentration of glucose increased in all sample hydrolysis batches (Figure 2a, 2b and 2c). This mechanism corresponded to the report of Takaku (1988). The accumulated glucose in the final reaction mixture is around 40%. Maltotriose also declined rapidly and not found after 2 to 3 hours of transglycosylation. This trisaccharide was quickly hydrolyzed as it only contained α-(1-4) bonds. At the end-time of the reaction, only three transglycosylation products, isomaltose, panose and isomaltotriose, were presented.

The profile of oligosaccharides obtained from non-glutinous rice flour was shown in Figure 2a. The amount of isomaltose, panose and isomaltotriose, increased significantly at 1 h of transglycosylation. The highest concentration of IMO production was isomaltose (78.3 g/l) followed by isomaltotriose (38.7 g/l) and panose (52.2 g/l) respectively. The amounts of isomaltose and isomaltotriose became stable while that of panose gradually decreased until 24 h of transglycosylation.

Figure 2b represented the concentrations of sugar and oligosaccharide derived from glutinous rice flour during hydrolysis process. The maximal value of IMO was found at 2 h of transglycosylation (isomaltose =38.5 g/l, panose =17.6 g/l and isomaltotriose =50.3 g/l). After that the amount of panose gradually decreased until the end of transglycosylation.

In Figure 2c, the profile of sugar and oligosaccharide obtained from cassava starch was represented. The maximal value of IMO was found at 2 h of transglycosylation (isomaltose (38.5 g/l), panose (17.6 g/l) and isomaltotriose (50.3 g/l)). However, the amount of panose gradually declined until the end of the process.

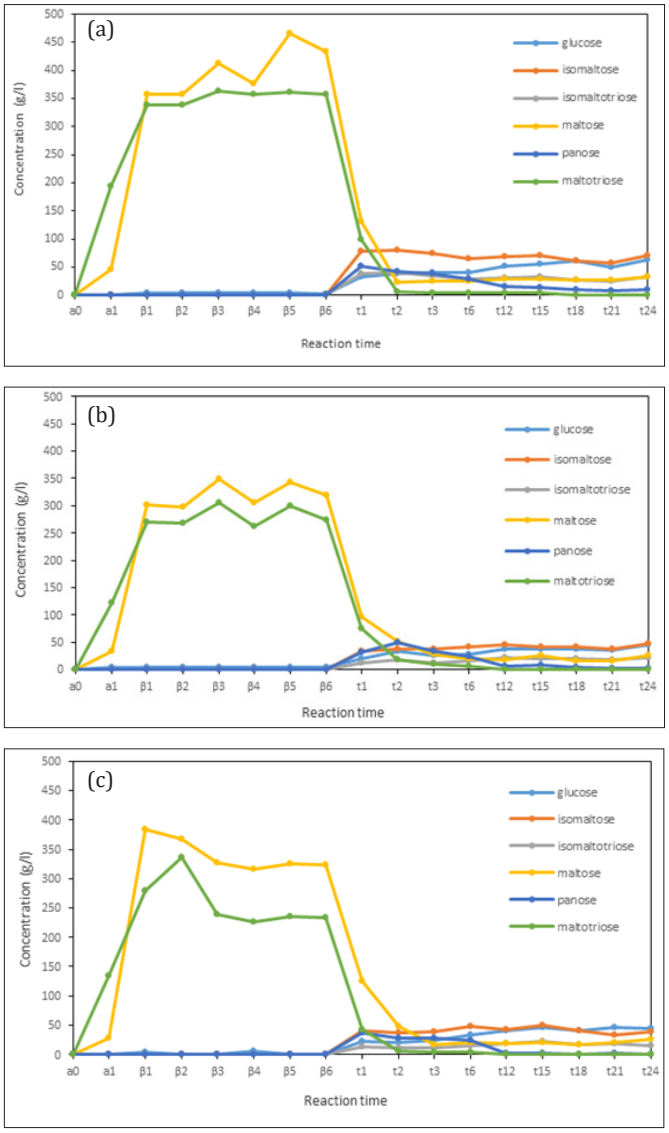

Figure 2. Sugar and oligosaccharide concentration during the production of IMO using (a) non-glutinous rice flour, (b) glutinous rice flour and (c) cassava starch as a substrate

a0 = liquid sample before incubated at 95°C

a1 = liquid sample after incubated at 95ºC for 10 min

β 1-6 = liquid sample after adding α-amylase, β-amylase and pullulanase at 1 to 6 h

t1- 24 = liquid sample after adding transglucosidase at 1 to 24 h

As reported by McCleary and Gibson (1989), the main transglycosylating product using maltose as substrate was isomaltose. Campa et al. (2002) also reported that with this enzyme two trisaccharides were formed, one of them (panose) was especially fast hydrolyzed and the second (isomaltotriose) presented a notably lower concentration than that of isomaltose throughout the process.

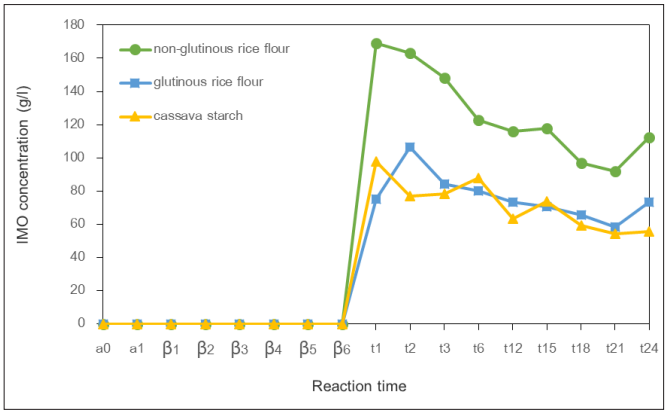

Figure 3 The profile of total concentration of IMO (isomaltose, panose and isomaltotriose) during IMO production using non-glutinous rice flour, glutinous rice flour and cassava starch as a substrate.

a0 = liquid sample before incubated at 95ºC

a1 = liquid sample after incubated at 95ºC for 10 min

β 1-6 = liquid sample after adding α-amylase, β-amylase and pullulanase at 1 to 6 h

t1- 24 = liquid sample after adding transglucosidase at 1 to 24 h

Comparing among three different samples (Figure 3), non-glutinous rice flour could produce the highest concentration of maltose during saccharification resulting in the highest yield of IMO in transglycosylation. This results corresponded to the report of Planas and Faijes (2002), where the increase in maltose concentration caused an improvement in the yield of transglycosylation products. When transglycosylation was conducted, transglucosidase added preferentially catalyzed the formation of α-(1→6) glucosidic linkages in addition to hydrolysis, resulting in the production of isomaltose, panose, and isomaltotriose with α-1,6 linkages from maltose. As expected, the maximum IMO obtained from glutinous rice flour and cassava starch were lower than that obtained from non-glutinous rice flour. Duan et al. (1995) reported that the ratio of transglucosylating to hydrolysing activity for the α-glucosidase from *Aspergillus niger* notably decreases when lowering maltose concentration.

CONCLUSIONS

Thai economic flour especially, non-glutinous rice flour could be digested relatively easily and, therefore, could be well suited as a raw material for producing IMO syrup. The maximum IMO obtained was 169.1 g/l (isomaltose = 78.2 g/l, isomaltotriose = 38.7 g/l and panose = 52.1 g/l) at the transglycosylation time of 1 hour. Furthermore, the shorter reaction time could save the production cost of the syrup. In addition, the purification of IMO should be done for a further study. Besides, the effect of IMO on the growth of microbiota in the gastrointestinal track should also be investigated.

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