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# Combination of amylase and transferase catalysis to improve IMO compositions and productivity

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## ABSTRACT

Isomaltooligosaccharides (IMOs) is a mixture of branched-maltooligosaccharides (MOSs), classified as a functional food ingredient with prebiotic potential. The objective of this study was to investigate alternative enzymatic methods to produce a sweetness/viscosity/degradability balance product containing soluble carbohydrate ranging from glucose, maltose, isomaltose and linear and branched MOS (IMOs), of different digestibility. The modified samples were produced using two main routes: 1. simultaneous catalysis by α-amylase and branching enzyme (BE) followed by α-transglucosidase (ABT) treatment, 2. simultaneous catalysis by α-amylase and BE followed by simultaneous β-amylase and α-transglucosidase (ABbT). For both routes, 30% or 50% (w $v^{-1}$) cassava starch were used and a commercial IMO (comIMO) preparation was used as a positive control. The 30ABbT and 50ABbT showed the highest α-1,6 glucosidic linkages, panose and branched MOS (Degree of Polymerisation (DP) 4–7) compared to all other samples. All ABT and ABbT products showed decreased molecular weight and increased resistance to degradation by amylolytic enzymes as compared to cassava starch. The 50ABbT sample demonstrated the lowest digestion rate constant. Due to their high solubility and structural composition, containing-balanced-glucose, maltose, isomaltose and linear and branched MOS (IMOs), of different digestibility, these compositions can be suitable especially in soft drink- and sport drink formulations.

## 1. Introduction

The consumer attention on personal health and well-being as well as the demand of alternative and healthy foods has increased in the last decade. The production of new ingredients based on starch has been promoted in response to that demand. Isomaltooligosaccharide (IMO) is widely accepted as food ingredient. IMO preparations are composed of low molecular weight α-glucans including isomaltose, panose, isopanose, isomaltotriose, nigerose, kojibiose, and larger branched MOSs. They are generally asscociated with specific health benefits and classified as prebiotic (Guo, 2013). IMOs consist of both α-1, 4 and α-1, 6 linked-glucose units and such configurations have the capability to delay α-amylase and α-glucosidases activity resulting in decreased glucose release rates and increase in indigested α-glucans transferred to the colon. Thus, IMOs are strongly related to the modulation of gut hormones triggered by the rate of glucose release and colonic microbiotal production of short chain fatty acids (Lee et al., 2013; Rivellese, Giacco, & Costabile, 2012).

Different structures of IMO can be adapted for different foods. Specific IMOs have been developed and used as substitute sugars to prevent dental caries for diabetics or to improve the gastrointestinal microbiotal profile. Stimulation of the growth of bifidobacteria and lactobacilli in the colon by IMO has been reported (Guo, 2013). Ingestion of IMO in humans and pigs can reduce glucose and triglyceride concentrations in serum (Hashizume et al., 2012; Li, Zhao, Du, & Zhang, 2009). Enzymatic strategies focused on IMO production and structures diversification have been recently reported. Branching enzyme (BE, EC 2.4.1.18) catalyzes the cleavage of α-1,4 glucosidic linkages followed by transfer of the glucan fragment to the 6-hydroxyl group of a glucose unit to form a branched structure (Roussel et al., 2013; Shinohara et al., 2001). BE was used to produce slowly digestible starch or MOS from various types of starch (Le et al., 2009; Sorndech et al., 2015; Sorndech et al., 2016). Hydrolytic cleavage as well as glucanotransfer reactions can be performed by α-transglucosidase (EC 2.4.1.24) to enrich IMOs from MOS

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preparation using starch as substrate liberated high contents of isomaltose, isomaltotriose, and panose. It has been suggested that cooperative reaction of maltogenic α-amylase and amylomaltase has synergistic effect on the IMO production (Lee et al., 2002). The action of amylomaltase combined with α-transglucosidase on cassava starch resulted in production of relatively long chain (DP $\leq 9$) IMOs with low degree of sweetness (Kaulpiboon, Rudeekulthamrong, Watanasatitarpa, Ito, & Pongsawasdi, 2015; Rudeekulthamrong, Sawasdee, & Kaulpiboon, 2013). Using an enzyme membrane reactor with glucosidases and ultrafiltration membranes was readily converted maltose to IMO (Zhang et al., 2010).

Cassava is an important upcoming global crop with starch functionality that in many aspects resembles that of potato and maize. In this study, we modified 30% and 50% (w $v^{-1}$) cassava starch to generate IMO with various molecular structures. The strategy was based on the combination of α-amylase and BE for starch liquefaction and saccharification yielding low molecular α-glucans with highly branched structures. Subsequent addition of β-amylase and α-transglucosidase resulted in further saccharification and generation of IMOs. Our data demonstrate that combinatorial hydrolase and glucanotransferase catalysis provides a clean strategy to generate a diversity of IMO compositions aimed at retarding dietary amylolytic digestion.

## 2. Materials and methods

### 2.1. Materials

Cassava starch was obtained from SanguanWongse Industries Co., Ltd. (NakhonRatchasima, Thailand). BE was a kind gift from Novozymes (Bagsvaerd, Denmark) and α-transglucosidase (TG-L2000) was kindly supplied by Danisco Singapore Pte Ltd- Dupont Industrial Biosciences (Singapore). Porcine pancreatic α-amylase (PPA, EC 3.2.1.1, specific activity 22 U $mg^{-1}$), glucoamylase (GA, EC 3.2.1.3, specific activity 129 U $mg^{-1}$) from *Aspergillus niger* and PGO (peroxidase and glucose oxidase) enzyme kit for glucose determination were from Sigma-Aldrich (Missouri, USA). Enzyme activity units of isoamylase, PPA and GA are given according to the suppliers. The commercial IMO was from Ingredion Ltd. (Bangkok, Thailand) and termed comIMO which was used as a positive control.

### 2.2. Enzymatic modification

#### 2.2.1. Cassava starch simultaneously treated with α-amylase and BE followed by α-transglucosidase

Cassava starch was suspended in deionised water (30% or 50% (w $v^{-1}$)), adjusted to pH 6.5 with 50 mM Na phosphate buffer. The suspension was heated to 80 °C in a water bath to gelatinize starch and followed by addition of α-amylase (A, 10 U g $starch^{-1}$) and BE (B, 4000 U g $starch^{-1}$). The reaction mixture was incubated at the same temperature for 1 h in order to liquefy the starch and then cooled to 60 °C followed by incubation at this temperature for 24 h with mixing. The reaction was terminated by heating in boiling water bath for 30 min. The pH was adjusted to 4.5 using 50 mM Na acetate buffer, α-transglucosidase (T, 15 U g $starch^{-1}$) was added to the mixture and incubated at 40 °C for 3 h. The reaction was terminated by heating in boiling water bath for 30 min. The α-glucan product was recovered and dried by lyophilisation. The end products from these steps were termed 30ABT and 50ABT based on the starch concentrations used.

#### 2.2.2. Cassava starch simultaneously treated with α-amylase and BE followed by simultaneously treated with β-amylase and α-transglucosidase

A gelatinized starch paste was prepared as described above and the reaction was terminated by heating in boiling water bath for 30 min. The pH was adjusted to 4.5 using 50 mM acetate buffer and α-transglucosidase (T, 15 U g $starch^{-1}$) and β-amylase (b, 30 U g $starch^{-1}$) were added to the mixture, incubating at 40 °C for 9 h (for 30% starch) and 15 h (for 50% starch). The reaction was terminated by boiling, the product was recovered, dried by lyophisation and termed 30ABbT and 50ABbT.

### 2.3. IMO compositions analysed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Enzyme-modified starches were gelatinized by boiling and enzymatically debranched at 40 °C for 48 h by using 0.24 U of isoamylase per 5 mg of sample. The obtained linear α-glucan fragments were analyzed by HPAEC-PAD (Dionex, Sunnyvale, CA, USA). Samples of 20 μL (100 μg of linear α-glucan) were injected on a CarboPac PA-200 column using 0.4 mL $min^{-1}$ flow rate, 150 mM isocratic NaOH and the following NaOAc gradient profile: 0–5 min: 0–110 mM linear gradient and 5–130 min: 110–350 mM convex gradient while for determined the IMO compositions gradient profile was: 0–5 min: 0–110 mM linear gradient and 5–60 min: 110–350 mM convex gradient (Viksø-Nielsen, Blennow, Nielsen, & Møller, 1998).

### 2.4. Molecular weight distribution analysis by size-exclusion chromatography with triple detection array (SEC-TDA)

Molecular composition was evaluated by size exclusion chromatography (SEC) using a Viscotek System (Malvern, UK) equipped with a GS-220HQ column (Shodex) with a size exclusion limit of $3.0 \times 10^3$ g $mol^{-1}$. The column was attached to a TDA302 module (Triple detector array) equipped with a refractive index detector (RI). The glucan size was estimated from authentic glucose, maltose and linear glucan standards (maltotriose, maltotetraose maltopentaose, maltoheptaose, and maltooctaose). Ammonium formate (50 mM) was used as eluent at a flow rate of 0.5 mL $min^{-1}$ and a column temperature of 60 °C. The analysis was performed using the OmniSec Software 4.7 (Malvern Instrument, ltd.).

### 2.5. α-1,6 glucosidic linkage determination by nuclear magnetic resonance spectroscopy (NMR)

The α-glucan samples were dissolved in 500 μL $D_2O$ (Cambridge Isotope Laboratories, Andover, MA, USA) to concentration of 0.3% (w $v^{-1}$) under gentle heating. $^1H$ NMR spectra were recorded on a Bruker (Fällanden, Switzerland) DRX spectrometer equipped with a TCI CryoProbe and an 18.7 T magnet (Oxford Magnet Technology, Oxford, UK) at 37 °C. NMR spectra were processed using Bruker Topspin 2.1 software with zero filling in all dimensions and mild resolution enhancement. The degree of branching was calculated from the anomeric signals of branch point α-1,6 linkages as quantified relative to anomeric signals of α-1,4 linkages (Miller, Klyosov, Platt, & Mayo, 2009). The data was averages of two experimental replications and each of them was measured in duplicate.

### 2.6. In vitro α-glucan digestion analysis

*In vitro* α-glucan degradation was performed by a modification of the Englyst method using gelatinized cassava starch, comIMO and modified glucan products. All samples (2% (w $v^{-1}$) in 250 μL),