ID030

A pilot-scale Production of Isomalto-oligosaccharide from Tapioca Starch

Pitchayanin Chanowan[1*], Wirote Youravong[2] and Santad Wichienchot[2]
[1]Functional Food and Nutrition Program, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[2]Center of Excellence in Functional Foods and Nutraceuticals, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[*]E-mail: jha_5561_@hotmail.com

Abstract

Isomalto-oligosaccharides are branch-chain oligosaccharides (α-(1,6) linkages) with or without α-(1,4) linkages with prebiotic property. The aim of this study was to optimize conditions for production of IMO in lab scale (3L and 30L) and pilot scale (70-300L fermentor). The production of IMO by enzymatic synthesis using 3-step approach. The first step was liquefaction by the action of α-amylase, pH 6, temperature of 90˚C and incubation time of 2 hours. For second step was saccharification by action of β-amylase at pH 7, temperature of 55˚C and incubation time of 6 hours. The last step was transglucosylation by action of transglucosidase at pH 5, incubated at 60˚C for 3 hours. The production volumes at 3L and 30L showed significant difference in IMO component. The pilot-scale production between 2-step and 3-step production was compared. The result found that 2-step production at 12 h gave the highest yield of IMO (43.09%) and total oligosaccharide of 89.23%. In addition, purification of IMO was achieved by yeast fermentation. The purified IMO contained glucose (1.53%) less than before purification (3.86%). Total IMO content was slightly different between tIMO before (63.84%) and after (62.4%) purification. The molecular weight distributions of tIMO were IDP2 (15.72%), panose (13.92%), IDP4 (8.94%), IDP5 (5.65%), IDP6 (6.5%) and IDP7 (11.61%).

Keywords: Isomalto-oligosaccharide, pilot-scale, tapioca starch, prebiotic

1. Introduction

At present time, the demand of functional ingredients is increasing annually. The reason is alternative and healthy food raising due to people awareness on personal health and well- being. Isomalto-oligosaccharides (IMO) are food ingredient with a prebiotic property, food additive that stimulates the growth of probiotics. IMO is widely accepted as food ingredient and sweetener. IMO generally consists of 2– 5 glucose moieties linked together by α-(1,6) linkage, and sometimes there can be a possible presence of α-(1,4) linkage along with the α-(1,6) linkage (Chockchaisawasdee and Poosaran, 2013). The most common IMOs are isomaltose, panose, isopanose, isomaltotriose, nigerose, kojibiose, and larger branched malto-oligosaccharides (Sorndech *et al.*, 2017). They are generally associated with specific health benefits and classified as prebiotic (Guo, 2013). They commercially produced from corn starch. The

synthesis process of IMOs from starch is to firstly hydrolyze starch into α-(1,4)-linked α-D-gluco-oligosaccharides using α-amylase, pullulanase and α-amylase, and then converted to α-(1,6)-linked oligosaccharides using α-transglucosidase (Takaku, 1988). Tapioca is one of the most important agricultural products of the world. In Thailand, tapioca is one of commercially economic crops that it is produced over 20 million tons per year. The tapioca flour consists of 75-80% starch and 1.5-2.0% protein (Santisopasri *et al*., 1998). IMO production in Thailand can produce from tapioca due to tapioca is source of starch and it is a low-cost raw material. This might increase economic and nutritive value of this agricultural product of Thailand. In current industrial scale they are using the 2 or 3- step process (liquefaction, saccharification and tranglucosylation). However, it takes long time and high production cost. This study aims to optimize the conditions for production of IMO from tapioca starch in lab (3-30L) and pilot scale (70-300L).

2. Materials & Methods

2.1 Materials

Tapioca starch was supplied by Thai Flour Industry Co., Ltd. (Nakhon Pathom, Thailand). α-amylase, β- amylase and transglucosidase were supplied by Amano Enzyme Co., Ltd. (Nagoya, Japan). Chemicals including HCl, NaOH, acetonitrile and isopropanol were purchased from Sigma-Aldrich, (St. Louis, Missouri, USA). The standards of IMO including maltose, glucose, isomaltose, panose and isomaltotriose were supplied by Megazyme Co., Ltd. (Illinois, USA).

2.2 IMO production by 3-step method

2.2.1 Liquefaction

Starch slurry was prepared at 30% (w/v). pH was adjusted to 6 with 0.1M NaOH. The slurry was added α-amylase 0.05% of tapioca starch. The slurry was placed in a water bath at 90°C for 2 hours with continuous agitation at 200 rpm. The activity of the enzyme was terminated by heating the slurry at 100°C for 10 minutes.

2.2.2 Saccharification

The liquefied starch slurry was adjusted to pH 5.5 by 0.1 M HCl. The enzyme β-amylase 0.1% was added into the slurry. The slurry was placed in water bath at 60°C for 6 hours with continuous agitation at 200 rpm. The activity of the enzyme was terminated by heating the slurry at 100°C for 10 minutes.

2.2.3 Transglucosylation

The saccharified slurry was adjusted to pH 5 by 0.1 M NaOH or 0.1 M HCl. The enzyme transglucosidase was added into the slurry at 0.1%. The slurry was placed in shaking incubator at 55°C for 24 hours with continuous agitation at 200 rpm. The sample (3 ml) was taken every 3 hours and activity of enzyme was terminated by heating the slurry at 100°C for 10 minutes. The sample was determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Hemmaratchirakul *et al.*, 2015).

2.3 IMO production by 2-step method

The liquified slurry was adjusted to pH 5.5 by 0.1M NaOH or 0.1 M HCl. The enzyme β-amylase and transglucosidase was added into the slurry at 0.08% and 0.1%, respectively. The slurry was continuous agitation at 200 rpm with 55°C for 24 hours. The sample was taken every 6 hours and

activity of enzyme was terminated by heating the slurry at 100°C for 10 minutes.

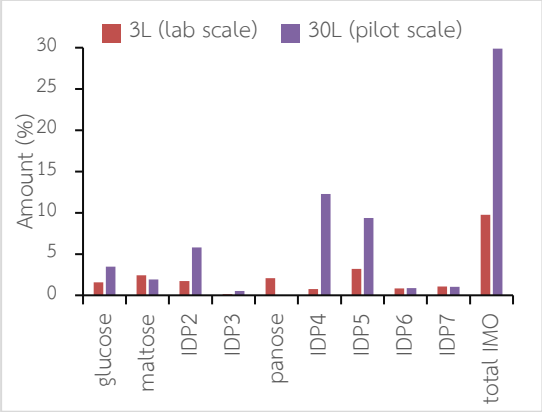

Figure 1. The composition of tapioca IMO produced by 3-step of lab scale and pilot scale.

2.4 IMO purification by yeast fermentation

The IMO syrup was heated to 100°C for 15 minutes and cooled down to 40°C then inoculated with the yeast *Saccharomyces cerevisiae* of 0.4% (w/v) and fermentation for 6 hours. The sample was determined IMO content with HPAEC-PAD.

2.5 IMO compositions analyzed by HPAEC-PAD

The sample was centrifuged at 10,000 xg for 15 minutes. Samples were analyzed by HPAEC- PAD (Dionex ICS 3000) using CarboPac PA- 200 column and an electrical detector. Two mobile phases, A (150 mM sodium hydroxide) and B (150 mM sodium hydroxide + 500 mM sodium acetate) at flow rate of 0.2 ml/min. Twenty-five microliters of sample were injected into the column. Glucose and oligosaccharide standards (maltose, panose and IDP2-7) were used for creating of calibration curves.

3.1 Results and discussion

3.1 IMO production by 3-step method

The optimal conditions for production of tapioca IMO (tIMO) in lab scale was selected to compare with a pilot-scale production. The slurry of tapioca starch (30%, w/v) was used for production of tIMO with 3-step method. The first step started with liquefaction using α-amylase followed by a second step of saccharification using β-amylase and the third step was transglucosylation by transglucosidase. The content of tIMO analyzed by HPAEC-PAD consisted of isomaltotriose (IDP2), panose, isomaltotriose (IDP3), isomaltotetraose (IDP4), isomaltopentaose (IDP5), isomaltohexose (IDP6) and isomaltoheptaose (IDP7) as showed in Figure 1. The molecular weight distribution of IMO produced in lab scale and pilot scale was significantly difference ($p < 0.05$). The amount of IDP2, IDP4 and IDP5 were increased sharply in pilot scale. The total yield of IMO from tapioca produced from 3L was 9.75% whereas the total IMO obtained from pilot scale (29.87%) was significantly elevated ($p < 0.05$). This result is accordance with previous study about scaling up process of glucose production from lab scale to pilot scale. The results showed percentage of glucose obtained for the pilot scale (47 %) was higher than the laboratory scale (36%) (Herath, 2017). This result is accordance with previous study about scaling up process of glucose production from lab scale to pilot scale. The results showed percentage of glucose obtained for the pilot scale (47 %) was higher than the laboratory scale (36%) (Herath, 2017). Other study of Prajummuang (2003) that produced glucose from cassava pulp in lab scale and pilot scale. The result indicated that the enzyme used in pilot-scale production was lower amount than lab scale. In addition, higher yield of glucose was obtained in a pilot-scale production. The reason that pilot scale has ability to produce high yield of product due to

the increase of efficiency of mixing and operation control in pilot-scale or industrial instruments.

3.2 IMO production by 3-step and 2- step method in pilot scale

The 2-step method started with liquefaction by α- amylase followed by saccharification by β-amylase combined with transglucosylation using transglucosidase. The 3-step method started with liquefaction by α- amylase followed by saccharification by β-amylase and the last step was transglucosylation by transglucosidase. The results showed total IMO from 2-step production gave the highest yield (43.09%).

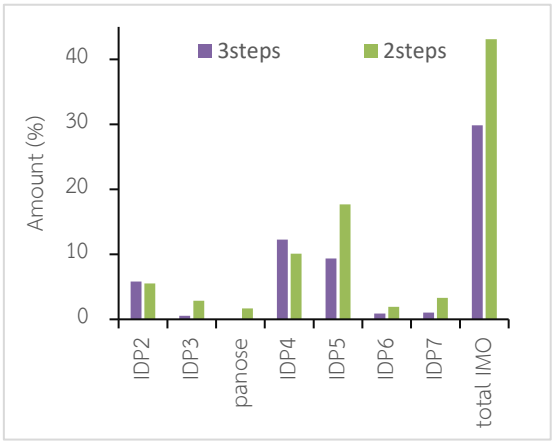

Figure 2. The component of IMO produced by 3-step and 2-step method in pilot-scale production.

3.3 IMO purification

Results from the yeast fermentation for the removal of digestible sugars to produce high purity IMO starting from the tapioca IMO was mixed with yeast *Saccharomyces cerevisiae* of 0.4% and fermented for 6 h. Digestible sugars including glucose, maltose, and maltotriose were utilized by yeast cells. But none of the IMO component was utilized by yeast. Total IMO of tIMO before purified by yeast was 63.84% and after purified was 62.4%. It was slightly difference between before and after purification. The difference between tIMO before and after purification was the reduction in concentration of glucose and maltose. Glucose before purification was 3.86% and after purification was 1.57%. In addition, maltose was decreased from 6.79% to 4.44%. The purified tIMO (ptIMO) and commercial IMO from tapioca (cIMO) were not significant different in IMO total yield. Glucose, IDP2 and IDP4 showed slightly difference ($p < 0.05$) between cIMO and tIMO after purification while maltose, IDP3, panose, IDP5, IDP6 and IDP7 were totally difference ($p < 0.05$). Normally, glucose is by-product from IMO production. The reason that remove glucose from IMO due to high purity of IMO resulted in higher prebiotic property thus less dose of consumption. Previous study of IMO production from rice crumbs and tapioca flour by two strains of yeast. The most effective to remove glucose in IMO was done by *Saccharomyces cerevisiae* fermentation for 24 h followed by *Saccharomyces carlsbergensis* for 3 d. In addition, the death rate of yeast cells in IMO from tapioca flour was higher than IMO from rice crumbs because rice crumbs contain nutrients higher than tapioca flour (Pan and Lee, 2005). Dasaesamoh *et al.* (2016) reported that pectinase from dragon fruit purification by yeast. The result showed glucose, fructose, and sucrose completely removed with no effect to oligosaccharide content by yeast fermentation at 30°C for 4 d with addition of urea as nitrogen source.

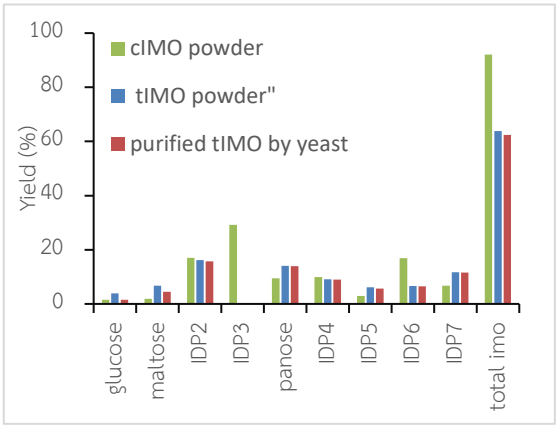

Figure 3. The content of IMO in tapioca IMO before and after purification compared to commercial IMO.

4. Conclusion

The IMO yield produced in a pilot scale (29.87%) was higher than lab scale (9.74%). The 2-step method gave the highest yield of 43.09% at 12 h fermentation. The purification of tapioca IMO was successful by yeast fermentation to remove glucose from 3.86% to 1.57% at 35°C, for 6h. Total IMO was slightly different between tIMO before (63.84%) and after (62.4%) purification. The results obtained from this study showed that tapioca starch could be a potential raw material for IMO production as a prebiotic ingredient.

Acknowledgement

This work was supported by grants from Thailand Research Fund (TRF) and Green Innovative Biotechnology Co. Ltd., Thailand.

References

[1] Suwimol Chockchaisawasdee and Naiyatat Poosaran. 2013. Production of isomalto oligosaccharides from banana flour. Journal of The Science of Food and Agriculture, Volume 93, p. 180-186.

[2] Waraporn Sorndech, Domenico Sagnelli, Andreas Blennow, and Sunanta Tongta. 2017. Combination of amylase and transferase catalysis to improve IMO compositions and productivity. LWT Food Science and Technology, Volume 79, p. 479-486.

[3] Guo, M. 2013. Functional Foods: Principles and Technology: Elsevier Science.

[4] Takaku, H. 1988. Anomalously linked oligosaccharides mixture. In: handbook of amylases and related enzymes: their sources, isolation methods, properties and applications. The Amylase Research Society of Japan. Pergamon Press, New York, p. 215.

[5] Vilai Santisopasri, Klanarong Sriroth, and Kanjana Kurotjanawong. 1998. Biochemical changes of cassava roots in different varieties harvesting dates and physiochemical properties of starch. National science and technology development agency.

[6] Hemmaratchirakul J, Jaturapiree P, Prueksasri S, and Wichienchot S. 2015. Production of galactooligosaccharide by β-galactosidase from *Lactobacillus pentosus* var. *plantarum* BFP32. International Food Research Journal, Volume 22 No.6, p. 2550-2557.

[7] Madara D.W. Samaranayake, Aruma B.G.C.J. De Silva, Warnakulasuriya R.D. Fernando, Katudeni V.T. Gunawardhane and Herath Mudiyanselage T. Herath. 2017. Optimization of liquefaction and saccharification times for laboratory scale production of glucose syrup from cassava starch and scaling up process of optimized conditions at pilot scale. Research Journal of Chemical Science. Volume 7 No.7, p.16-25.

[8] Prajummuang, P. 2003. Production of glucose syrup from cassava pulp by enzymatic hydrolysis in pilot scale. Master of Science in Biotechnology. Khon Kaen University.

[9] Pan Yen Chung and Lee Wen chien. C. 2005. Production of high purity isomalto oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells. Biotechnology and Bioengineering. Volume 89, p. 797-804.

[10] Dasaesamoh R, Wirote Yourawong, and Santad Wichienchot. 2016. Optimization on pectinase extraction and purification by yeast fermentation of oligosaccharides from dragon fruit (*Hyloceus undatus*). International Food Research Journal. Volume 23 No.6, p. 2601-2607.