Electronic Journal of Biotechnology 26 (2017) 46–51

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Highly efficient enzymatic preparation of isomalto-oligosaccharides from starch using an enzyme cocktail

Dandan Niu [a,b,d,*], Jian Qiao [b,d], Pujun Li [b,d], Kangming Tian [c,d,*], Xiaoguang Liu [c,d], Suren Singh [e], Fuping Lu [b,d]

[a] *Fujian Provincial Key Laboratory of Marine Enzyme Engineering, College of Biological Science and Engineering, Fuzhou University, Fuzhou 350116, China*
[b] *The College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China*
[c] *Department of Biological Chemical Engineering, College of Chemical Engineering and Material Sciences, Tianjin University of Science and Technology, Tianjin 300457, China*
[d] *The Key Lab for Fermentation Microbiology, Ministry of Education, Tianjin University of Science and Technology, Tianjin 300457, China*
[e] *Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa*

ARTICLE INFO

Article history:
Received 28 August 2016
Accepted 7 December 2016
Available online 13 December 2016

Keywords:
Aspergillus niger
Bacillus amyloliquefaciens
Bacillus licheniformis
Bacillus naganoensis pullulanase
Barley bran β-amylase
Iisomalto-oligosaccharides production
Saccharification
Ttransglycosylation
α-Amylase
α-Transglucosidase

ABSTRACT

Background: Current commercial production of isomalto-oligosaccharides (IMOs) commonly involves a lengthy multistage process with low yields.
Results: To improve the process efficiency for production of IMOs, we developed a simple and efficient method by using enzyme cocktails composed of the recombinant *Bacillus naganoensis* pullulanase produced by *Bacillus licheniformis*, α-amylase from *Bacillus amyloliquefaciens*, barley bran β-amylase, and α-transglucosidase from *Aspergillus niger* to perform simultaneous saccharification and transglycosylation to process the liquefied starch. After 13 h of reacting time, 49.09% IMOs (calculated from the total amount of isomaltose, isomaltotriose, and panose) were produced.
Conclusions: Our method of using an enzyme cocktail for the efficient production of IMOs offers an attractive alternative to the process presently in use.

© 2017 Pontificia Universidad Cat\u00f3lica de Valpara\u00edso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Isomalto-oligosaccharides (IMOs) are glucose oligomers with α-D-(1,6)-linkages, with or without α-(1 → 4) linkages [1], that include isomaltose, panose, isomaltotriose, isomaltotetraose, isomaltopentaose, nigerose, kojibiose, and higher branched oligosaccharides [2,3]. In addition, this definition has been extended to include glucooligosaccharides linked by α-(1 → 6) linkage and/or a lower proportion of α-(1 → 3) (nigerooligosaccharides) or α-(1 → 2) (kojioligosaccharides) glucosidic linkages found in commercial IMO products [4,5,6,7] and IMO-like products. For example, branched IMOs known as glucooligosaccharides are produced using dextransucrase [8]; oligodextran by controlled-hydrolysis of dextran [9]; nonreducing IMO alditols by dextransucrase-catalyzed glucosylation of alditols such as glucitol, mannitol, maltitol, and Isomalt[R] [10], isomaltulose or palatinose (α-D-Glc *p*-(1 → 6)-α-D-Fru *f*) [11], and cyclic IMOs [12]. Thus, IMO structure is characterized by the IMOs degree of polymerization (DP) (from 2 to ~10), type of linkages (α-1-2, 3, 4, or 6), and the proportion and position of each type of linkage. The most abundant and well-recognized functional components in IMOs are isomaltose/α-D-Glc *p*-(1 → 6)-α-D-Glc *p*, isomaltotriose/α-D-Glc *p*-(1 → 6)-α-D-Glc *p*-(1 → 6)-D-G1c *p*, and panose/α-D-Glc *p*-(1 → 6)-α-D-Glc *p*-(1 → 4)-D-Glc *p*, collectively abbreviated as iMTP [3].

IMOs are of considerable interest in the food, pharmaceutical, and cosmetic industries because of their unique properties such as low viscosity, resistance to crystallization, reduced sweetness, and bifidogenic effects. The most important property of IMOs and the key to their success is their prebiotic property that is responsible for their beneficial effects, including better intestinal health, mineral absorption, cholesterol regulation, and immunity and prevention of and resistance to various diseases such as dental caries. IMOs are also used as substitute sugars for patients with diabetes [13,14]. IMOs are normal components of the human diet and occur naturally in many fermented foods, including rice miso, soy sauce, and sake [15]. Isomaltose has also been identified as a natural constituent of honey [16].

Most commercial IMOs are enzymatically produced from starch through transglycosylation [2,3]. The typical commercially scaled starch-as-substrate process includes the following enzymatic processes: (1) starch is liquefied to form maltodextrin with bacterial

* Corresponding author.
E-mail addresses: ddniu0529@fzu.edu.cn (D. Niu), kangmingtian@tust.edu.cn (K. Tian).

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2016.12.002
0717-3458/© 2017 Pontificia Universidad Cat\u00f3lica de Valpara\u00edso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

or thermotolerant α-amylase (EC 3.2.1.1); (2) fungal α-amylase or β-amylase (EC 3.2.1.2) is used for saccharification to generate syrup with approximately 40–50% maltose and maltotriose [pullulanase (EC 3.2.1.41) may also be used in this step to obtain higher maltose solution with residual maltodextrins with low DP]; and (3) transglycosylase such as *Aspergillus niger* α-transglucosidase (EC 2.4.1.24) is used for IMO synthesis.

Improved efficiencies of IMO preparations have been reported by optimizing the coupled effects of enzymes used in the manufacturing process. Maltogenic amylases (EC 3.2.1.133) from various bacteria have been shown to exhibit both α-(1,6)-transglycosylation and α-(1,4)-hydrolysis activities [17,18,19]. The coupled transglycosylation and hydrolysis activities of maltogenic amylases [20] have been used to produce IMOs from liquefied starch in a more efficient way than the conventional three-step process, resulting in a significant increase in the maximum yields of IMOs. Further improvement of IMO yields from liquefied starch was observed with the combined use of maltogenic amylase from *Bacillus stearothermophilus* ET1 and α-glucanotransferase (EC 2.4.1.25) from *Thermotoga maritima* with α-(1,4)-glycosidic bond transferring ability [21]. Production of long-chain IMOs from maltotriose has been reported using the thermostable amylomaltase and transglucosidase in combination, although, separately, amylomaltase resulted in products containing linear malto-oligosaccharides (MOSs) and transglucosidase resulted in short-chain IMOs [22]. In addition, *Corynebacterium glutamicum* amylomaltase was used to produce palatinose glucosides through the acceptor specificity of its intermolecular transglycosylation activity [23]. NMR analysis showed that the major products palatinose glucoside 1 and 2 were a tri- and tetra-saccharide, respectively, with the structure [O-α-D-glucopyranosyl-(1 → 4)]$_n$-O-α-D-glucopyranosyl-(1 → 6)-D-fructofuranose, where n = 1 or 2 [23]. No commercial application, however, has been reported using these improved processes or enzymes.

Although several new enzymes with desirable activities as described earlier have potential applications in IMO preparation, many aspects of the current IMO manufacturing need to be further improved or innovated because of its low substrate conversion rates, lengthy transglycosylation step, and undesirable enzyme usage/formula. In this study, using an enzyme cocktail composed of *Bacillus amyloliquefaciens* α-amylase (BAA), barley bran β-amylase (BBA), recombinant *Bacillus naganoensis* pullulanase in *Bacillus licheniformis* (PulA), and *A. niger* α-transglucosidase (An-TGase), the optimization of each step of IMO production including starch liquefaction, saccharification, and transglycosylation was investigated to develop novel processes for efficient IMO production.

2. Materials and methods

2.1. Enzymes and activity assay

The enzymes used in this study were BAA [24], thermostable α-amylase from *licheniformis* (BLA) [25], BBA, PulA [26], and An-TGase [27]. BLA (40,000 U/mL), BAA (2000 U/mL), and PulA (2000 U/mL) were obtained from Fujian Fuda Biotech Development Co., Ltd., China; BBA (700°DP/g) and An-TGase (360,000 U/g) were obtained from Jiangsu Ruiyang Biotech Co., Ltd., China.

The respective enzyme preparation activities were measured according to the methods described previously [25]. For BAA, one enzyme unit was defined as the amount of enzyme required to hydrolyze 1 g of starch in 1 h at pH 6.0 and 60°C. For BLA, one enzyme unit was defined as the amount of enzyme required to hydrolyze 1 mg of starch in 1 min at pH 6.0 and 70°C. PulA activity was determined according to the manufacturer instructions under the following conditions: the reaction mixture contained 0.1 mL of 0.5% (*w*/*v*) Red-Pullulan as substrate (MegaZyme, Co. Wicklow, A98 YV29, Ireland), 0.05 mol/L Tris–HCl buffer (pH 5.0), and 0.2 mL of enzyme solution incubated at temperature of 40°C for 30 min. Then the reaction was stopped by adding 0.5 mL of ethanol; after holding for 10 min, the reaction mixture was centrifuged at 12000*g* for 2 min, and the absorbance of the supernatant was measured at 510 nm OD with water as the control. For PulA, one enzyme unit was defined as the amount of enzyme required to release reducing sugar equal to 1 μmol glucose from 1 mL of 0.5% (*w*/*v*) Red-Pullulan in 1 min under the above reaction conditions. BBA activity was determined according to the protocol described in the fifth edition of Food Chemicals Codex [28]. One enzyme unit, expressed as degrees of diastatic power (°DP), was defined as the amount of enzyme in 0.1 mL of a 5% solution of the enzyme preparation that will produce sufficient reducing sugars to reduce 5 mL of Fehling's solution when the sample is incubated with 100 mL of the substrate for 1 h at 20°C. An-TGase activity was determined according to a previously described method [27]. One enzyme unit was defined as the amount of enzyme required to form 1 μg of glucose in 1 h at pH 5.0 and 50°C.

2.2. Determination of pH and temperature optima

To determine the optimum temperature, the activities of the enzymes were measured at various temperatures ranging from 30°C to 80°C. To determine the optimum pH, the enzymatic activity was measured at the above temperatures under different pH conditions: pH 4.0–5.5 using acetate buffer and pH 6.0–7.5 using phosphate buffer.

2.3. Production of IMOs

For enzyme selection and parameter optimization, IMOs were prepared in a 500-mL reactor as follows: (1) Starch liquefaction: this step was performed using 25% (*w*/*v*, dry starch weight) corn starch slurry and 200 U/g (dried starch) of BLA at pH 5.8 and ~100°C for different time periods, and the samples were collected and the dextrose equivalent (DE) values were determined as described in Section 2.4. (2) Saccharification: When the reaction mixture was cooled to 55°C, a combination of enzymes was added to prepare MOS-rich syrup. The reference values of each enzyme (calculated based on dry substrate starch) are BBA 0.1°DP/g and PulA 0.1 U/g. (3) Transglycosylation: An-TGase was added to the reaction mixture (500 U/g dried starch), and the reaction was performed at 55°C for up to 30 h. For single parameter optimization, the above parameters for IMO preparation were fixed except for changes in one parameter. For the scaling-up preparation of IMOs in a 10-L reactor, 25% (*w*/*v*) liquefied corn starch with a DE value of approximately 25 was used as substrate and the last two steps, i.e., saccharification and transglycosylation, were combined and conducted at 55°C. The samples were collected, and the enzymes were inhibited by adjusting the pH to 9.0 with 1 mol/L sodium hydroxide and incubating at 100°C for 10 min.

2.4. Analytical methods

Total dry matter was determined as described previously [28]. The DE value of liquefied starch was determined by the titrimetric method with glucose as the reference, as described previously [29]. The content and components of IMOs and the sugar profiles during IMO preparation were analyzed according to a previously described method with some modification [30]. Briefly, the analysis was performed on an HPLC system (Agilent 1200 Series HPLC System) with an evaporative light-scattering detector (Alltech ELSD detector 2000s, Grace Co. Ltd.) using a TSKgel Amide-80 column (4.6 mm IDx250 mm, 5 μm, Tosoh, Japan). Acetonitrile:water (67:33, *v*/*v*) was used as solvent at a flow rate of 1 mL min^{-1} at 30°C. Glucose (G1) and maltose (G2) used as HPLC standards were purchased from Shanghai BioTech Co. Ltd., China. Isomaltose (IG_2), maltotriose (G3), isomaltotriose (IG_3), panose (P), maltotetraose (G4), maltopentaose

(G5), and maltohexaose (G6) used as HPLC standards were purchased from Sigma (St. Louis, MO). The MOS and IMO references were obtained from Jiangsu Ruiyang Biotech Co. Ltd., China.

iMTP value, a measure of the IMO content, was calculated as the percentage of the combined amounts of IG_2, IG_3, and P in the dried matter. The specific value of iMTP was calculated as iMTP (%, *w*/w) divided by the reaction time.

3. Result and discussion

3.1. Effects of the degree of starch liquefaction on IMO yield

It is well established that the liquefaction degree of starch affects the quality and quantity of its final sugar products. For example, a certain degree of starch liquefaction (~DE 12) is required for higher glucose content formation during high-glucose syrup manufacturing, and a much lower degree of starch liquefaction (~DE 6 ~ 8) is preferred for higher maltose content during maltose syrup preparation [31]. However, in IMO preparation, not only maltose but also maltotriose and other MOSs formed during the starch liquefaction and saccharification are the main precursors of IMOs and favorite substrates for transglycosylation with An-TGase [32,33,34,35,36]. We therefore determined the effect of starch liquefaction degree on IMO formation. Starch slurry of 25% (*w*/*v*) was liquefied at different times using BLA at 100°C, and liquefied starch with DE values of 12, 20, or 30 was obtained. PulA, BBA, and An-TGase were then added, and the reaction mixture was incubated at 55°C for up to 24 h. The sugar profiles were analyzed and are summarized in Fig. 1, which shows that increasing starch liquefaction degree shortened the transglycosylation time required for maximum yields of iMTP, indicating that well-liquefied starch was more beneficial to IMO formation with increased yields per reaction time.

3.2. pH and temperature optima for simultaneous saccharification and transglycosylation in the preparation of IMOs

During the saccharification and transglycosylation steps of IMO production, BBA or PulA can be used to generate more maltose and MOSs, which serve as substrates for An-TGase. However, each enzyme has its own pH and temperature optimum. Studies have shown that BBA shows maximum activity at pH 5.5 and 55°C [35], PulA at pH 4.5 and 60°C [36], and An-TGase at pH 4.5 and 60°C [32]. We evaluated the properties of these commercially available enzymes in this study (Fig. 2) and found that the values either agreed with or deviated only slightly from the published values. An-TGase and PulA showed the pH optimum at pH 5.0 and BBA at pH 5.5. All three enzymes retained more than 50% of their maximum activity at pH 5.0–6.0 (Fig. 2a). As for their temperature optima, PulA, BBA, and An-TGase displayed the maximum activity at 60°C, 50°C, and 55°C, respectively. All three enzymes retained more than 50% of the maximum activity at temperatures from 45°C to 65°C (Fig. 2b).

To further examine the possibility of requiring pH and temperature control during IMO preparation, yields of iMTP were evaluated at different initial pH values and temperatures. As shown in Fig. 2c and Fig. 2d, saccharification and transglycosylation were only slightly affected by pH between 4.5 and 6.0 and temperatures between 45°C and 60°C. An optimal pH of 5.8 and optimal temperature of 55°C were selected for subsequent experiments.

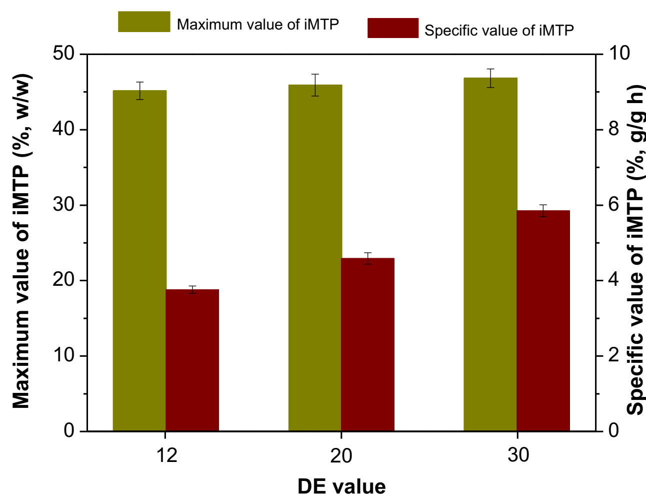

Fig. 1. Effect of starch liquefaction degree (DE) on the yield and specific production of isomaltose, isomaltotriose, and panose (iMTP).

3.3. Dosage of An-TGase affects IMO formation efficiency

The effects of the amount of An-TGase on iMTP preparation were examined, and the results are summarized in Fig. 3. The use of An-TGase at dosages of 500, 1000, and 2000 U/g resulted in maximum IMO yields of 46.47% at 13 h, 45.80% at 8 h, and 45.46% at 4 h, respectively. These results show that high dosage of An-TGase can significantly reduce the transglycosylation time. However, from the economic point of view (An-TGase is one of the most expensive enzymes used in IMO production), 500–1000 U/g dosage is more suitable for industrial-scale preparation of IMOs.

3.4. Roles of PulA, BBA, and BAA in IMO preparation

Pullulanase is an enzyme that specifically hydrolyzes α-1,6-linkages in starch molecules and is known as a debranching enzyme [32]. To determine its effect on IMO preparation, different amounts of PulA were added to the reaction mixture. Although the control showed a maximum of 35% iMTP formation, addition of PulA significantly increased iMTP synthesis, achieving a maximum value of 46% when 0.9 U/g of PulA was added (Table 1). However, treatment of IMO products with the same amount of PulA under similar conditions showed no significant effects on IMO quality, as analyzed by HPLC (data not shown). From this, it can be said that PulA is beneficial for IMO formation as it debranches liquefied starch, thus facilitating the consequent saccharification, but hardly hydrolyzes α-1,6-linkages in oligosaccharides.

β-Amylase specifically hydrolyzes α-1,4-linkages in starch molecules to form maltose [35]. To clarify the effect of BBA on IMO preparation, different amounts of BBA were added to the reaction mixture. In the absence of BBA, only about 33% IMOs were formed. The addition of 0.5°DP/g of BBA increased both the percentage of iMTP and the specific value of iMTP (Table 1). These results indicate that BBA enhances IMO formation by enhancing the yields of maltose and maltotriose and thus promotes transglycosylation by An-TGase. Furthermore, the activity of BBA, which catalyzes the hydrolysis of maltodextrin to form MOSs, is necessary for IMO production as re-confirmed in this study (Table 1). With the addition of a certain amount of BBA (0.3–0.7°DP/g), iMTP values could be significantly increased, and the maximum yield was obtained when ≥0.5°DP/g of BBA was added.

Therefore, increasing the dosages of PulA and BBA and the degree of starch liquefaction resulted in elevated levels of substrates for transglycosylation, especially higher contents of maltose and maltotriose. This leads to an improved IMO production efficiency through transglycosylation by An-TGase, which rapidly transfers glucosyl residues to MOSs, followed by gradual hydrolysis of both α-(1 → 4) linkages and α-(1 → 6) linkages at the nonreducing end to generate smaller molecules with mainly α-(1 → 6) linkages [33,34]. Given that BAA catalyzes the hydrolysis of maltodextrin to form MOSs, it is expected that the use of BAA will generate more preferred

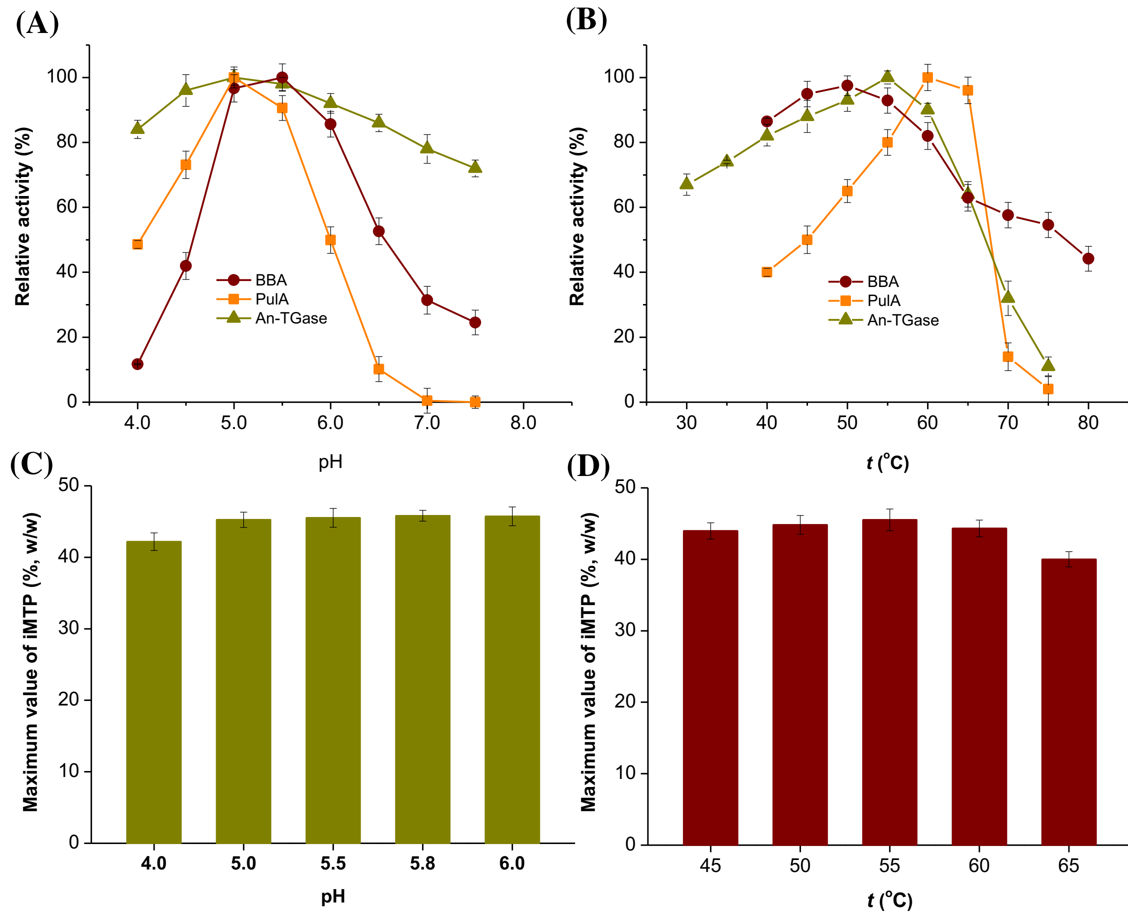

Fig. 2. Optimal pH and temperature used for IMO preparation. (a) optimum pH profile of enzymes, (b) optimum temperature profile of enzymes. (c) optimum pH for saccharification and transglycosylation. (d) optimum temperature for saccharification and transglycosylation.

substrates for BBA, which in turn produces higher levels of suitable substrates for An-TGase, thus accelerating the transglycosylation process during IMO preparation. Therefore, the effects of BAA (10–18 U/g) on IMO production were investigated with the use of 0.5°DP/g of BBA. As expected, increasing the dosage of BAA greatly enhanced the iMTP yield and especially the specific value of iMTP and shortened the transglycosylation process (Table 1). The use of 16 U/g of BAA resulted in improved iMTP yield, with a 22% increase in the specific value of iMTP (from 3.01 to 3.68) (Table 1). These results also suggest a novel application of BAA in the preparation of long-chain or middle-chain isomalto/malto-polysaccharides, a novel soluble dietary fiber prepared through the enzymatic conversion of starch [37,38].

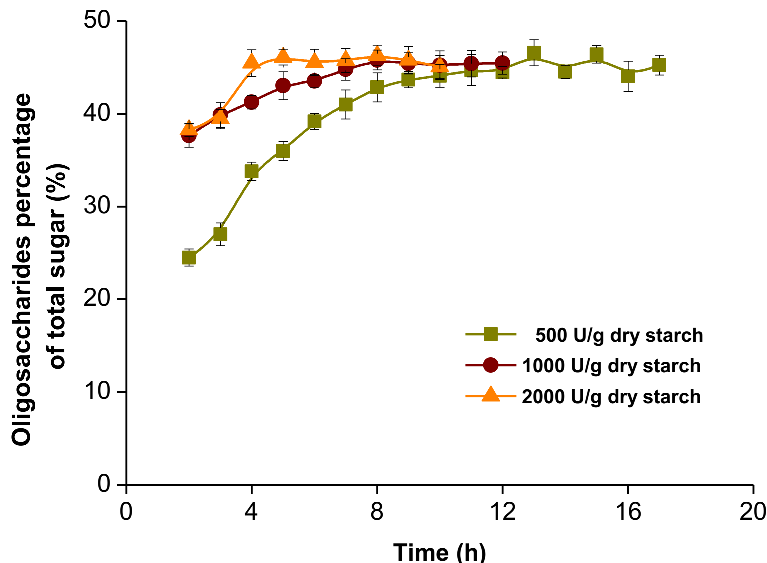

Fig. 3. Effects of α-glucosyltransferase dosage on IMO formation.

Table 1
Effects of pullulanase, BBA, and BAA on IMO formation during saccharification and transglycosylation stages.

Dosage of enzyme	Maximum value of iMTP (%, *w*/w)	Specific value of iMTP (%, g/g·h^{-1})
PulA (U/g)		
0	35.01 ± 1.33	2.33 ± 0.09
0.3	40.42 ± 1.23	2.70 ± 0.08
0.6	43.85 ± 1.42	2.92 ± 0.09
0.9	45.38 ± 1.33	3.03 ± 0.09
1.8	45.51 ± 1.23	3.03 ± 0.08
BBA (°DP/g)		
0	33.18 ± 1.33	2.21 ± 0.05
0.3	43.45 ± 1.23	2.90 ± 0.07
0.4	45.04 ± 1.42	2.92 ± 0.08
0.5	46.29 ± 1.33	3.01 ± 0.06
0.6	46.02 ± 1.23	3.07 ± 0.09
0.7	45.74 ± 1.13	3.05 ± 0.06
BAA (U/g)		
10	46.55 ± 1.25	3.58 ± 0.09
12	46.80 ± 1.36	3.60 ± 0.08
14	47.07 ± 1.29	3.62 ± 0.07
16	47.79 ± 1.20	3.68 ± 0.08
18	47.32 ± 1.09	3.64 ± 0.09

3.5. Improved processes for IMO production

The above results show that several microbial amylases are capable of catalyzing maltodextrin hydrolysis to form MOSs during enhanced IMO formation, and this prompted us to develop an enzyme cocktail-based process for IMO preparation. The reaction mixture in a 10-L reactor contained 25% (*w*/*v*) liquefied starch with a DE value of 25 and an enzyme cocktail. In the proposed process (Table 2) for IMO production using an enzyme cocktail of BAA, BBA, PulA, and An-TGase, 49.09% iMTP was obtained in 13 h, a significant improvement over the currently used process for commercial production with 39–41% iMTP yields (personal communication). Interestingly, the process proposed here can be easily modified to accommodate a Do-It-Yourself (DIY) style by increasing the dosage of An-TGase (Table 2), which would enable people to prepare their own IMOs using an enzyme cocktail of BAA, BBA, PulA, and An-TGase (2000 U/g). This rapid preparation of IMOs was completed in 4 h, making it suitable for the production of IMOs at home.

In the traditional method for the production of IMOs, α-amylase is added to starch slurry (30%), followed by maltogenase such as β-amylase for saccharification and then transglucosidase for transglycosylation. The resulting IMOs are finally purified with yeast fermentation and chromatography [39]. The entire process requires approximately 120 h to yield a mixture with 58% IMOs [39]. However, in the currently used process for the commercial-scale production of IMOs, a thermostable α-amylase is added to starch slurry, followed by a jet liquefaction process at 107–109°C. After cooling to 55°C, fungal α-amylase is used for saccharification for 4 h and subsequently transglucosidase is added to perform transglycosylation for another 40–60 h. The resulting IMOs are finally physically or biotechnologically purified to prepare IMOs of different grades. A lengthy process and low yields are the main disadvantages of this process, which takes 45–65 h to produce 39–41% IMOs (unpublished data from a Chinese IMO manufacturer, personal communication). The new process developed in this study uses PulA, BAA, BBA, and An-TGase to perform simultaneous saccharification and transglycosylation to process the liquefied starch. After 13 h of reaction time, 49.09% IMOs (calculated from the total amount of isomaltose, isomaltotriose, and panose) were produced. A rapid process for IMO preparation was also tested for a DIY purpose, with a yield of 45.5% IMOs in 4 h (Table 2).

An alternative method has been developed in which *Geobacillus stearothermophilus* maltogenic amylase and *T. maritima* α-glucanotransferase were simultaneously used to process the liquefied starch solution. After 14 h of reaction, a yield of 68% IMOs was obtained [20]. This relatively lower yield is possibly due to the formation of DP_4- or larger IMOs, which are not calculated as the contents of IMOs.

4. Conclusion

In the present study, a novel process for high-efficiency production of IMOs was developed based on the combined functions of commercially available *B. naganoensis* pullulanase, *B. amyloliquefaciens* α-amylase, barley bran β- amylase, and *A. niger* α-transglucosidase. Pullulanase is a crucial factor to accelerate saccharification and MOSs formation and significantly shortened the duration of transglycosylation. Saccharification and transglycosylation can be done simultaneously with acceptable IMO productivity, thus providing a new possibility to simplify and improve the IMO production from starch with an enzyme cocktail.

Financial support

This study was funded in part by the Tianjin Research Program of Application Foundation and Advanced Technology (grant number 14JCQNJC09200), the Science and Technology Development Foundation of Fujian Higher Education (grant number JA15049), the Priming Scientific Research Foundation of Fuzhou University (grant number XRC-1564), the Science and Technology Development Foundation of Tianjin Higher Education (grant number 20130628), and the National Natural Science Foundation of China (grant number 31461143026).

Conflict of Interest Statement

The authors declare that they have no competing interests.

References

[1] Yun J, Suh JH, Song S. Kinetic study and mathematical model for the production of isomalto-oligosaccharides from maltose by transglucosylation of *Aureobasidium pullulans*. J Korean Inst Chem Eng 1994;32:875–80.
[2] Goffin D, Delzenne N, Blecker C, Hanon E, Deroanne C, Paquot M. Will isomalto-oligosaccharides, a well-established functional food in Asia, break through the European and American market? The status of knowledge on these prebiotics. Crit Rev Food Sci Nutr 2011;51:394–409. http://dx.doi.org/10.1080/10408391003628955.
[3] Goffin D, Wathelet B, Blecker C, Deroanne C, Malmendier Y, Paquot M. Comparison of the glucooligosaccharide profiles produced from maltose by two different transglucosidases from *Aspergillus niger*. Biotechnol Agron Soc Environ 2010;14: 607–16.
[4] Lina BAR, Jonker D, Kozianowski G. Isomaltulose (Palatinose®): A review of biological and toxicological studies. Food Chem Toxicol 2002;40:1375–81. http://dx.doi.org/10.1016/S0278-6915(02)00105-9.
[5] Konishi Y, Shindo K. Production of nigerose, nigerosyl glucose, and nigerosyl maltose by *Acremonium* sp. S4G13. Biosci Biotechnol Biochem 1997;61:439–42. http://dx.doi.org/10.1271/bbb.61.439.
[6] Chaen H, Nishimoto T, Nakada T, Fukuda S, Kurimoto M, Tsujisaka Y. Enzymatic synthesis of kojioligosaccharides using kojibiose phosphorylase. J Biosci Bioeng 2001;92:177–82. http://dx.doi.org/10.1016/S1389-1723(01)80221-8.

Table 2
IMO production using different processes.

Enzyme cocktails	Maximum value of iMTP (%, *w*/w)	Specific value of iMTP (%, g/g·h^{-1})	Process parameters
BAA/BBA/PulA/An-TGase	49.09 ± 1.56	3.78 ± 0.12	16.0 U/g BAA, 0.9 U/g PulA, 0.5°DP/g BBA, 500 U/g An-TGase at pH 5.8 and 55°C for 13 h
BAA/BBA/PulA/An-TGase (DIY)	45.52 ± 0.91	11.38 ± 0.23	16.0 U/g BAA, 0.9 U/g PulA, 0.5°DP/g BBA, 2000 U/g An-TGase at pH 5.8 and 55°C for 4 h

[7] Fabre E, Bozonnet S, Arcache A, Willemot RM, Vignon M, Monsan P, et al. Role of the two catalytic domains of DSR-E dextransucrase and their involvement in the formation of highly α-1,2 branched dextran. J Bacteriol 2005;187:296–303. http://dx.doi.org/10.1128/JB.187.1.296-303.2005.

[8] Leemhuis H, Pijning T, Dobruchowska JM, van Leeuwen SS, Kralj S, Dijkstra BW, et al. Glucansucrases: Three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. J Biotechnol 2013;163:250–72. http://dx.doi.org/10.1016/j.jbiotec.2012.06.037.

[9] Mountzouris KC, Gilmour SG, Rastall RA. Continuous production of oligodextrans via controlled hydrolysis of dextran in an enzyme membrane reactor. J Food Sci 2002; 67:1767–71. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08720.x.

[10] Demuth K, Jordening HJ, Buchholz K. Oligosaccharide synthesis by dextransucrase: New unconventional acceptors. Carbohydr Res 2002;337:1811–20. http://dx.doi.org/10.1016/S0008-6215(02)00272-0.

[11] Moynihan PJ. Update on the nomenclature of carbohydrates and their dental effects. J Dent 1998;26:209–18. http://dx.doi.org/10.1016/S0300-5712(97)00010-9.

[12] Mukai K, Watanabe H, Kubota M, Chaen H, Fukuda S, Kurimoto M. Purification, characterization, and gene cloning of a novel maltosyltransferase from an *Arthrobacter globiformis* strain that produces an alternating α-1,4- and α-1,6-cyclic tetrasaccharide from starch. Appl Environ Microbiol 2006;72:1065–71. http://dx.doi.org/10.1128/AEM.72.2.1065-1071.2006.

[13] Florowska A, Krygier K, Florowski T, Dłużewska E. Prebiotics as functional food ingredients preventing diet-related diseases. Food Funct 2016;7:2147–55. http://dx.doi.org/10.1039/C5FO01459J.

[14] Bharti SK, Krishnan S, Kumar A, Gupta AK, Ghosha AK, Kumard A. Mechanism-based antidiabetic activity of fructo- and isomalto-oligosaccharides: Validation by in vivo, in silico and in vitro interaction potential. Process Biochem 2015;50:317–27. http://dx.doi.org/10.1016/j.procbio.2014.10.014.

[15] Tungland BC, Meyer D. Nondigestible oligo-and polysaccharides (dietary fiber): their physiology and role in human health and food. Compr Rev Food Sci Food Saf 2002;3:73–92. http://dx.doi.org/10.1111/j.1541-4337.2002.tb00009.x.

[16] White JW, Hoban N. Composition of honey. IV. Identification of the disaccharides. Arch Biochem Biophys 1959;80:386–92. http://dx.doi.org/10.1016/0003-9861(59)90267-X.

[17] Kim IC, Cha JH, Kim JR, Jang SY, Seo BC, Cheong TK, et al. Catalytic properties of the cloned amylase from *Bacillus licheniformis*. J Biol Chem 1992;267:22108–14.

[18] Cha HJ, Yoon HG, Kim YW, Lee HS, Kim JW, Kweon KS, et al. Molecular and enzymatic characterization of a novel maltogenic amylase that hydrolyzes and transglycosylates acarbose. Eur J Biochem 1998;253:251–62. http://dx.doi.org/10.1046/j.1432-1327.1998.2530251.x.

[19] Abdul Manas NH, Pachelles S, Mahadi NM, Illias RM. The characterisation of an alkali-stable maltogenic amylase from *Bacillus lehensis* G1 and improved malto-oligosaccharide production by hydrolysis suppression. PLoS One 2014;9:e106481. http://dx.doi.org/10.1371/journal.pone.0106481.

[20] Lee SJ, Yoo SH, Kim MJ, Kim JW, Seok HM, Park KH. Production and characterization of branched oligosaccharides from liquefied starch by the action of *Bacillus licheniformis* amylase. Starch/Starke 1995;47:127–34. http://dx.doi.org/10.1002/star.19950470403.

[21] Lee HS, Auh JH, Yoon HG, Kim MJ, Park JH, Hong SS, et al. Cooperative action of α-glucanotransferase and maltogenic amylase for an improved process of isomaltooligosaccharide (IMO) production. J Agric Food Chem 2002;50:2812–7. http://dx.doi.org/10.1021/jf011529y.

[22] Rudeekulthamrong P, Sawasdee K, Kaulpiboon J. Production of long-chain isomaltooligosaccharides from maltotriose using the thermostable amylomaltase and transglucosidase enzymes. Biotechnol Bioprocess Eng 2013;18:778–86. http://dx.doi.org/10.1007/s12257-012-0777-8.

[23] Naumthong W, Ito K, Pongsawasdi P. Acceptor specificity of amylomaltase from *Corynebacterium glutamicum* and transglucosylation reaction to synthesize palatinose glucosides. Process Biochem 2015;50:1825–34. http://dx.doi.org/10.1016/j.procbio.2015.07.003.

[24] Liu Y, Shen W, Shi GY, Wang ZX. Role of the calcium-binding residues Asp231, Asp233, and Asp438 in alpha-amylase of *Bacillus amyloliquefaciens* as revealed by mutational analysis. Curr Microbiol 2010;60:162–6. http://dx.doi.org/10.1007/s00284-009-9517-5.

[25] Niu D, Zuo Z, Shi GY, Wang ZX. High yield recombinant thermostable α-amylase production using an improved *Bacillus licheniformis* system. Microb Cell Fact 2009; 8:58. http://dx.doi.org/10.1186/1475-2859-8-58.

[26] Wang Y, Liu Y, Wang Z, Lu F. Influence of promoter and signal peptide on the expression of pullulanase in *Bacillus subtilis*. Biotechnol Lett 2014;36:1783–9. http://dx.doi.org/10.1007/s10529-014-1538-x.

[27] Li M, Zhou L, Liu M, Huang Y, Sun X, Lu F. Construction of an engineering strain producing high yields of α-transglucosidase via *Agrobacterium tumefaciens*-mediated transformation of *Aspergillus niger*. Biosci Biotechnol Biochem 2013;77:1860–6. http://dx.doi.org/10.1271/bbb.130281.

[28] Committee on food chemicals codex. In: Institute of Medicine, editor. Food chemicals codex. 5th ed. Washington DC: The National Academies Press; 2003.

[29] Dziedzic SZ, Kearsley MW. Handbook of starch hydrolysis products and their derivatives. London: Blackie Academic & Professional; 1995. http://dx.doi.org/10.1007/978-1-4615-2159-4.

[30] Ko J, Lee MS, Kwak BM, Ahn JH, Park JS, Kwon JH. Determination of isomaltooligosaccharides in yoghurts by using HPLC-ELSD. Korean J Food Sci Anim Resour 2013;33:417–24. http://dx.doi.org/10.5851/kosfa.2013.33.3.417.

[31] Guzmán-Maldonado H, Paredes-López O, Biliaderis CG. Amylolytic enzymes and products derived from starch: A review. Crit Rev Food Sci Nutr 1995;35:373–403. http://dx.doi.org/10.1080/10408399509527706.

[32] Yan TR, Chiou RD. Catalytic properties of a transglucosidase from *Aspergillus niger* CCRC 31494. Biotechnol Lett 1996;18:553–8. http://dx.doi.org/10.1007/BF00140202.

[33] Ota M, Okamoto T, Wakabayashi H. Action of transglucosidase from *Aspergillus niger* on maltoheptaose and [U-^{13}C]maltose. Carbohydr Res 2009;344:460–5. http://dx.doi.org/10.1016/j.carres.2008.12.004.

[34] Masafumi O, Takeshi O, Wataru H, Hidehiko W. Action of α-$_{D}$-glucosidase from *Aspergillus niger* towards dextrin and starch. Carbohydr Polym 2009;78:287–91. http://dx.doi.org/10.1016/j.carbpol.2009.03.047.

[35] Thacker SP, Ramamurthy V, Kothari RM. Characterization of barley β-amylase for application in maltose production. Starch/Starke 1992;44:339–41. http://dx.doi.org/10.1002/star.19920440906.

[36] Schülein M, Højer-Pedersen B. Characterization of a new class of thermophilic pullulanases from *Bacillus acidopullulyticus*. Ann N Y Acad Sci 1984;434:271–4. http://dx.doi.org/10.1111/j.1749-6632.1984.tb29842.x.

[37] Leemhuis H, Dobruchowska JM, Ebbelaar M, Faber F, Buwalda PL, van der Maarel MJ, et al. Isomalto/malto-polysaccharide, a novel soluble dietary fiber made via enzymatic conversion of starch. J Agric Food Chem 2014;62:12034–44. http://dx.doi.org/10.1021/jf503970a.

[38] Bai Y, van der Kaaij RC, Leemhuis H, Pijning T, van Leeuwen SS, Jin Z, et al. Biochemical characterization of the *Lactobacillus reuteri* glycoside hydrolase family 70 GTFB type of 4,6-α-glucanotransferase enzymes that synthesize soluble dietary starch fibers. Appl Environ Microbiol 2015;81:7223–32. http://dx.doi.org/10.1128/AEM.01860-15.

[39] Yoo SH, Kweon MR, Kim MJ, Auh JH, Jung DS, Kim JR, et al. Branched oligosaccharides concentrated by yeast fermentation and effectiveness as a low sweetness humectant. J Food Sci 1995;60:516–21. http://dx.doi.org/10.1111/j.1365-2621.1995.tb09816.x.