Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Preparation of maltotriitol-rich malto-oligosaccharide alcohol from starch

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

[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] Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa
[e] The Key Lab for Fermentation Microbiology, Ministry of Education, Tianjin University of Science and Technology, Tianjin 300457, China

ARTICLE INFO

Article history:
Received 28 August 2016
Received in revised form 2 November 2016
Accepted 9 November 2016
Available online xxx

Keywords:
Maltotriitol-rich malto-oligosaccharides alcohol
Maltotriose
Enzymatic process

ABSTRACT

Malto-oligosaccharide alcohols (MOSA) are one of the most important sugar alcohols widely used as sweetener in food, cosmetic, and pharmaceutical industries in recent years, of which maltotriitol-rich MOSA is much more recognized. With the aim of preparing maltotriitol-rich MOSA from starch, a novel process was developed and optimized. Starch was first liquefied with thermostable *Bacillus licheniformis* α-amylase. The liquefied starch was then saccharified to yield maltotriose-rich malto-oligosaccharides under the cooperative actions of *Bacillus naganoensis* pullulanase, *Bacillus amyloliquefaciens* α-amylase, and barley bran β-amylase. The maltotriitol-rich MOSA was finally prepared by chemical hydrogenation. Under the optimized conditions, maltotriitol-rich MOSA containing 42.18% maltotriitol was obtained with a conversion rate of 104.57% from starch. The process can be employed for large-scale preparation of maltotriitol-rich MOSA, and a further modification of the process can lead to the formulation of various types of MOSA with different percentages of components of sugar alcohols.

1. Introduction

Sugar alcohols (polyols or polyhydric alcohols), derived from substituting an aldehyde group in a sugar molecule with a hydroxyl one, are low-digestible carbohydrates that are used as sweeteners in food, cosmetic, and pharmaceutical industries [1–3]. Sugar alcohols can be classified as hydrogenated monosaccharides (sorbitol, mannitol), hydrogenated disaccharides (isomaltitol, maltitol, lactitol), and mixtures of hydrogenated mono-, di-and/or oligosaccharides (hydrogenated starch hydrolysates) [1,3,4]. Recently, because of recognition of the beneficial effects of maltitol and maltotriitol by the food industry (especially in baking and beverage) and the pharmaceutical industry (such as their anti-dental plaque effect or drug activity related to their glass-forming structures in freeze state), the hydrogenated starch hydrolysates having high content of maltitol and maltotriitol are becoming increasingly attractive [3–10].

Maltotriitol is a trisaccharide polyol that consists of two glucoses and a sorbitol, while maltitol is a disaccharide polyol of glucose and sorbitol in equal parts. Both sugar alcohols can be obtained from starch hydrolysates with a chemical hydrogenation process in which maltitol is derived from hydrogenation of maltose and maltotriitol from maltotriose. The purified form of maltitol has been commercially produced with purification and crystallization from the hydrogenation mixture of maltose syrup. However, the purified maltotriitol is not routinely prepared because of the high cost and/or low conversion efficiency for the production of maltotriose from either pullulan hydrolyzed with pullulanase or starch with a specific α-amylase [11,12].

At present, the mixture of sugar alcohols consisting of ~70% maltitol and up to ~20% maltotriitol can be prepared from starch, in which maltotriose, the precursor of maltotriitol, is formed as a by-product during high maltose syrup preparation [1,3,4]. Given that a higher maltotriitol content (for example, >40%) is more desirable for certain food formula or manufacturing [4,12], it is of value to

* Corresponding author at: Fujian Provincial Key Laboratory of Marine Enzyme Engineering, College of Biological Science and Engineering, Fuzhou University, Fuzhou 350116, China.
** Corresponding author.
E-mail addresses: ddniu0529@fzu.edu.cn (D. Niu), kangmingtian@tust.edu.cn (K. Tian).

http://dx.doi.org/10.1016/j.procbio.2016.11.007
1359-5113/

develop a new process based on starch hydrolysates to increase the maltotriose yield. In the present work, an enzymatic process was developed to produce a syrup with higher maltotriose content. Thermostable α-amylase was used to convert the starch into soluble maltodextrin, which was further debranched with a pullulanase to form malto-oligosaccharides (MOS) and simultaneously saccharified with bacterial α-amylase and β-amylase to produce maltotriose-rich MOS syrup. With chemical hydrogenation, the hydrogenated malto-oligosaccharide alcohol (MOSA) with a higher percentage of maltotriitol can be conveniently prepared from starch, thus allowing the production of a wide range of hydrogenated starch hydrolysates with different percentages of maltotriitol and other sugar alcohols. In lieu of the above the aim of this study focused on the development of a new process based on starch hydrolysates to increase the maltotriose content in malto-oligosaccharides (MOS).

2. Materials and methods

Cornstarch was purchased from Shandong Baisheng Biotech Co. Ltd. (Shandong, China); glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5), and maltohexaose (G6) were purchased from Jiangsu Ruiyang Biotech Co. Ltd (Jiangsu, China); thermostable α-amylase (BLA) from *Bacillus licheniformis* [13], bacterial α-amylase (BAA) from *Bacillus amyloliquefaciens* [14], and *Bacillus naganoensis* pullulanase (PulA) [15] from the recombinant *B. licheniformis* were obtained from Fuda Biotech Co. Ltd. (Fuzhou, China); and barley β-amylase (BBA) extracted from barley bran was obtained from Shandong Longda Biotech Co Ltd. (Shandong, China).

For standard reference in HPLC analysis, sorbitol and maltitol (purities ≥ 99.9%) were obtained from Shandong Futian Pharmaceutical Co. Ltd. (Shandong, China), maltotriitol (purities ≥ 95.0%) was purchased from Santa Cruz Biotechnology (Shanghai) Co. Ltd (China), and glucose (G1) and maltose (G2) were purchased from Shanghai BioTech Co. Ltd (China). Maltotriose (G3), maltotetraose (G4), maltopentaose (G5), and maltohexaose (G6) were purchased from Sigma (St. Louis, MO, USA).

2.1. Development and optimization of MOS preparation process

The action of enzyme (BBA, BAA, or PulA) on maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, or maltodextrin was carried out at 55 °C and pH 5.8 for up to 3 h with a substrate concentration of 25% (w/v) in a 5-mL volume. The samples were collected, followed by inactivation of enzymes by incubating at 100 °C for 10 min. The sugar profiles were analyzed by HPLCs as described below.

The experiments for MOS preparation were carried out in a 500-mL reactor with the following steps: (1) Starch liquefaction: this was carried out with 25% (w/v, dried starch weight) cornstarch slurry in tap water and 200 U/g (dried starch) of BLA at pH 5.8 and ~100 °C for different time periods, and samples were collected and the dextrose equivalent (DE) values were determined as described below. (2) Debranching: different amounts of PulA were added after the reaction mixture was cooled to 55 °C, and the reaction was carried out at 55 °C for up to 4 h. (3) Saccharification: BAA and BAA plus BBA were added, and the reaction was maintained at 55 °C for up to 12 h.

For scaling-up preparation of MOS in a 10-L reactor, 25% (w/v) liquefied cornstarch with DE value of about 29 was used as substrate, and the debranching and saccharification were carried out at 55 °C. The samples were collected, followed by inactivation of enzymes by adjusting pH to 9.0 with 1 mol/L sodium hydroxide, and incubated at 100 °C for 10 min. The sugar profiles were analyzed by HPLCs as described below.

2.2. Hydrogenation of MOS for MOSA production

The hydrogenation of MOS was carried out in a 20-l reactor catalyzed with Raney Ni RTH-311 under 11 MPa, 120–130 °C, and 500 rpm for 2 h according to the method described in [16]. The sugar profiles were determined by HPLC as described below.

2.3. Analysis methods

The DE value of liquefied starch was determined with the titrimetric method using glucose as reference according to the method described [17]. The total dry matter was determined as described previously [18]. The content and components of low molecular weight monosaccharides of MOS and MOSA were detected with an Agilent 1200 Series HPLC system coupled with a ELSD detector (Alltech, Grace Co. Ltd.). The HPLC system included a carbomyl-silica HILIC column (TSK gel, Amide 80, 5 μm, 80 Å, 250 mm × 4.6 mm I.D., Tosoh, Japan). The conditions for the column, injection of samples and standard curves for the mixture of G1–G6 were as described by Karlsson et al., 2005 [19]. Large molecular weight components were also analysed from MOS using an Agilent 1200 Series HPLC system coupled with a refractive index (RI) detector using a TSK gel G3000PW column (7.8 mm ID × 300 mm, 7 μm, Tosoh, Japan) with water as a solvent at a flow rate of 0.6 mL min^{-1} at 30 °C as per manufacturers' instructions.

3. Results and discussion

3.1. The procedure for maltotriitol-rich MOSA prepared from starch

The general procedure for preparing maltotriitol-rich MOSA from starch is shown in Fig. 1. Three main steps are involved: (1) starch liquefaction to form maltodextrin syrup, (2) maltodextrin debranching and saccharification to form maltotriose-rich malto-oligosaccharides syrup, and (3) chemical hydrogenation to produce maltotriitol-rich MOSA. The protocols outlined below were optimized by multiple laboratory experiments in our quest for the development of a novel process for preparing maltotriitol-rich MOSA from starch (data not shown).

A 25% cornstarch slurry at the laboratory scale or 30–33% cornstarch slurry at the industrial scale in tap water (pH 5.8) was thoroughly mixed with thermostable *B. licheniformis* α-amylase. The starch liquefaction degree was controlled, and the optimized DE value of the resultant maltodextrin syrup was about 29. The maltodextrin syrup was treated at 55 °C to debranch to yield MOS (with G2–G6 as its main components) with pullulanase (the optimized dosage was 8 U/g dried substrate); the debranched MOS was simultaneously digested with various combinations of BAA and BBA to produce the maltotriose-rich MOS (the optimized combination was 16 U BAA per gram of dried substrate and 0.1 DP BBA per gram of dried substrate). The resultant maltotriose-rich MOS syrup was chemically hydrogenated, and the maltotriitol-rich MOSA was produced with the conversion efficiency of up to 98.76%.

3.2. The maltotriose and maltopentaose levels of the syrup were greatly affected by the liquefaction degree of starch

It is expected that maltotriose and maltopentaose levels in the maltodextrin syrup will determine the level of maltotriitol in the final product as maltopentaose may be further converted to maltotriose during the following saccharification stage. Therefore, the

Please cite this article in press as: D. Niu, et al., Preparation of maltotriitol-rich malto-oligosaccharide alcohol from starch, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.11.007