Enzymatic synthesis of fructooligosaccharides from sucrose by endo-inulinase-catalyzed transfructosylation reaction in biphasic systems

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A B S T R A C T

The biocatalytic approach based on the endo-inulinase-catalyzed transfructosylation reaction of sucrose in organic solvent/buffer biphasic reaction systems was investigated for the synthesis of fructooligosaccharides (FOSs). Combining kinetics and end-product profiling contributed to the understanding of the reaction selectivity and to the identification of the best biphasic system. Indeed, the highest FOS bioconversion yield (60.2%) was obtained in butyl acetate/buffer system, with nystose being the main end-product. The effects of the reaction parameters were studied using response surface methodology. Enzyme unit exhibited the most significant linear effect in the kestose concentration model, whereas the reaction time was the most significant parameter in the nystose concentration model. However, in the fructosyl-nystose concentration model, all linear terms exerted significant effects. Among the interactive effects, the interaction between reaction time and solvent proportion had the most significant effect on the kestose and nystose concentrations and on the bioconversion yield of FOS. The developed models are expected to provide the capability to FOS with targeted composition. FOS production by endo-inulinase was investigated in maple syrup-based biphasic media. The highest production of kestose was obtained using maple syrup 15’Bx, whereas the 66’Bx maple syrup resulted in the highest productions of nystose and fructosyl-nystose.

1. Introduction

Non-digestible fructooligosaccharides (FOSs) containing monomers with β-configured anomer C4 are a well-recognized class of prebiotics, and the utilization of commercial inulin and oligofructose by probiotic bacteria has been proven [1]. In addition, they show favorable properties as a food ingredient, such as noncariogenic properties and low calorie and low sweetness (30–50% of the sucrose sweetness) [2,3]. Biocatalytic production of FOS can be obtained using β-fructofuranosidases (EC 3.2.1.26) or β-fructosyltransferases (EC 2.4.1.9) as enzymes. Despite their wide availability, only few fructofuranosidases exhibit significant transfructosylation activity. Also, their use in the production of FOS is limited owing to their low yield (<20%) and low regio-selectivity [4–6]. However, the synthesis of FOS through transfructosylation reaction can be favored at high substrate concentration, high temperature, and in nonconventional reaction media [4,7].

The catalytic action of inulinas (EC 3.2.1.7) includes two parallel paths: transfructosylation activity that produces FOS and the hydrolytic activity that produces fructose and inulooligosaccharides [8]. Inulinas have mainly been used for the production of high-fructose syrup and/or inulooligosaccharides by the controlled hydrolysis of inulin. For instance, Han et al. [9] produced inulinase from Aspergillus niger by overexpressing the gene into Yarrowia lipolytica, which was used to hydrolyze inulin into FOS. Agavins (branched neo-fructans) are another example of the substrate that was used to generate branched FOS through the hydrolytic activity of inulinas [10]. Inulinas from selected microbial sources including Kluyveromyces, Aspergillus, Staphylococcus, Xanthomonas, and Pseudomonas have been used for the production of inulin-type carbohydrates [11]. The structural modeling and mechanistic studies of the hydrolytic activity of selected inulinas were reported [12,13]. However, inulinas have been little explored for their transfructosylation activities with sucrose as a substrate [14]. Inulinas from A. niger was reported to produce FOS from sucrose as substrate at yields ranging between 8.5% and 30.6% [2]. Significantly higher yields (14.0–77.2%) of FOS were produced by the same enzyme when used with inulin as a substrate [2]. According to Santos and Maugeri [15], temperature and sucrose concentration are the most crucial parameters in the production of FOS from sucrose by inulina from Kluyveromyces marxianus var. bulgaricus.

The synthesis of FOS in organic solvent/aqueous biphasic system presents several advantages compared with the conventional aqueous systems, including the shift of the reaction equilibrium toward synthesis...
rather than hydrolysis, the reduction of substrate and product inhibition, the specificity variations for some enzymes, the reusability of enzyme, and the simplicity of the separation of products [2,16]. Yet, the biphasic system has some limitations that should not be overlooked when deciding which system to adopt [16]. Only few studies have focused on FOS production in organic solvent/aqueous biphasic system [2,16,17]. Risso et al. [17] reported that FOS was produced from sucrose at higher yield (16.7%) in the biphasic system using inulinase from K. marxianus than in the aqueous solution where the yield was 12.8%.

As a sucrose-rich and renewable product, maple syrup is a potential substrate for FOS production. Indeed, maple syrup consists of sugars such as sucrose (68%), with small amounts of glucose (0.43%, w/w) and fructose (0.34%, w/w) [18]. Therefore, it appears to be an interesting reaction medium to favor transfructosylation reactions. It is worth mentioning that Canada contributes 85% of the annual global production of maple syrup [6,19]. The use of maple syrup for FOS production at the industrial level would thus be technically and economically an interesting option for the country. To our knowledge, only our previous research work has addressed the production of FOS from maple syrups by using levansucrase from Bacillus amyloliquefaciens to produce FOS, oligolevan, and levan [6]. In this study, the enzymatic synthesis was performed in an aqueous system, and the major product (>80%) had a degree of polymerization of 10–30.

In the present study, the biocatalytic approach based on the endo-inulinase-catalyzed transfructosylation reaction of sucrose in biphasic reaction media systems was investigated for the production of FOS. To optimize and better modulate the biocatalytic process, the effects of reaction parameters and their interactions on the yield and the end-product profile were studied by response surface methodology (RSM). RSM is a useful empirical model comparing mathematical and statistical techniques. The objective is to establish the relationship between response and different variables to yield an optimum level for the best system performance [20]. In addition, maple syrups (15°Bx, 30°Bx, and 66°Bx) were evaluated as sucrose-rich reaction media, and the results were compared with those using sucrose as a substrate.

2. Material and methods

2.1. Chemicals

The chemical reagents purchased from Sigma Chemical Co. (St Louis, Missouri) were Inulin, 3,5-dinitrosalicylic acid (DNS), D-fructose, D-glucose, D-maltose, α-lactose, D-raffinose, sucrose, 2-heptanone, dextran standards (50–670 kDa), acetic acid, and sodium acetate. The chemical reagents purchased from Fisher Scientific (Fair Lawn, New Jersey) were cyclohexane, ethyl acetate, heptane, sodium chloride and potassium phosphate dibasic (K2HPO4). Carbohydrate standards including 1-kestose, nystose, and 1-fructosylfructose were purchased from Wako Pure Chemical (Osaka, Japan). The organic solvents n-butyl acetate and 2-Octanone were obtained from Acros Organics (New Jersey, USA). Potassium phosphate monobasic (KH2PO4) was purchased from MP Biomedicals (Fair Lawn, New Jersey) and sodium hydroxide (NaOH) was from Fluka Chemicals (Fair Lawn, New Jersey). Maple syrups 15°Bx, 30°Bx, and 66°Bx were kindly offered by the Centre de recherche, de développement et de transfert technologique acéricole inc. (Centre ACER, St Hyacinthe, Quebec). The commercial endo-inulinase produced from A. niger was purchased from Sigma Chemical Co. (St Louis, Missouri).

2.2. Purification of endo-inulinase

The commercial endo-inulinase from A. niger may contain exo-inulinase enzyme. To purify the endo-inulinase, the enzyme preparation was subjected to an anion exchange chromatography on a MonoQ column at a flow rate of 0.6 mL/min. The column was conditioned using potassium phosphate buffer (20 mM, pH 6.0) as the running buffer. After injection of the samples, the column was first washed with the running buffer (2 column volumes [cv]), and an NaCl elution gradient from 0 to 0.5 M (4 cv) was applied thereafter. The purified enzyme was dialyzed against 5 mM of potassium phosphate buffer (pH 6.0) using a membrane with a 5–6 kDa cut-off for 48 h at 4 ºC. The dialyzed enzyme was freeze dried and stored at −80 ºC. The total protein content of the purified endo-inulinase was measured using the Standard Bio-Rad Protein Assay. The concentration of protein was estimated using a standard curve of bovine serum albumin (0.0625–1 mg/mL).

2.3. Enzymatic assays of inulinase activity

The endo-inulinase activity of the purified enzyme was carried out using sucrose and inulin as substrates (2%, w/v) and incubated with enzyme (1:1, v/v) at 45 ºC for 20 min. The DNS assay was used to quantify the amount of total released reducing sugars [21] using DU800 spectrophotometer (Beckman Coulter). A standard curve was constructed using fructose as a standard (0.5–10 mM). The specific activity was expressed as the enzymatic unit of endo-inulinase per milligram of protein.

The hydrolytic and transfructosylation activities of endo-inulinase were measured by quantifying glucose and fructose, respectively, using a Dionex ICS-3000 high-performance anion exchange chromatography system equipped with pulsed amperometric detector (HPAEC-PAD), a CarboPac PA20 column (3 × 150 mm), and a Chromeleon Software version 7.0. An isoteric elution of the reaction components were carried out using a 10 mM NaOH solution as the mobile phase at a flow rate of 0.5 mL/min for a total elution time of 30 min. The concentration of each product was determined by constructing standard curves of glucose and fructose. All assays were run in duplicates. One unit of total endo-inulinase activity was defined as the amount of biocatalyst to liberate 1 μmol of the reducing sugars (glucose and fructose) from sucrose at the initial stage of the reaction per min. One hydrolytic unit of endo-inulinase is defined as the amount of the biocatalyst to produce 1 μmol of fructose per min at the initial stage of the reaction, whereas one transfructosylation unit of endo-inulinase is defined as the amount of the biocatalyst to release 1 μmol of glucose per min as a result of transferring fructose. The subtraction of the total amount of fructose from that of glucose will provide the amount of glucose resulting from the transferring fructose.

2.4. Transfructosylation reaction catalyzed by endo-inulinase in biphasic systems

The transfructosylation reaction of sucrose was carried out according to a modification of the method described by Li et al. [6]. Before each enzymatic reaction, a stock solution of sucrose (1.2 M) was prepared in a sodium acetate buffer solution (0.1 M, pH 5). An organic solvent was added to the reaction mixture to achieve a solvent:buffer ratio of 1:3 (v/v). The organic solvents studied were butyl acetate, cyclohexane, ethyl acetate, heptane, heptanone, octanone, and hexane with a logarithm of the 1-octanol/water partition coefficient (log P) of 1.25, 2.67, 0.28, 3.58, 2.14, 2.4, and 3.6, respectively. The transfructosylation reaction was initiated by the addition of purified endo-inulinase (4–10 U/mL) to the reaction mixture. The reactions were carried under vacuum at 40 °C, with continuous vortex at 150 rpm for up to 120 h of reaction. However, the temperature was 35 °C when heptanone was used as the organic solvent. Aliquots of 1 mL reaction mixture were collected and boiled for 5 min to stop the enzymatic reaction. Ethanol was then added to the recovered aliquot at a ratio of 1:1 (v/v) to precipitate the enzymes. Control reactions, containing all components except endo-inulinase, were carried out in tandem with the enzymatic reactions. To determine the total sucrose conversion yield and the concentration of transfructosylation products, sucrose, glucose, and