ENZYMATIC PRODUCTION OF FRUCTOSE

Field of the Invention

[0001] The invention relates to processes for the enzymatic production of fructose.

Background

[0002] Fructose is a simple ketonic monosaccharide found in many plants, where it is often bonded to glucose to form the disaccharide, sucrose. Commercially, fructose is derived from sugar cane, sugar beets, and maize. The primary reason that fructose is used commercially in foods and beverages, besides its low cost, is its high relative sweetness. It is the sweetest of all naturally occurring carbohydrates. Fructose is also found in the manufactured sweetener, high-fructose corn syrup (HFCS), which is produced by treating corn syrup with enzymes, converting glucose into fructose with yields limited by equilibrium. (en.wikipedia.org/wiki/Fructose#Physical_and_functional_properties-- accessed 3/7/18). Alternatively, fructose can be used as a precursor for the production of the alternative sweetener allulose (*see*, for example, WO2016160573A1, WO2015032761A1, WO2014049373A1) or as a precursor to hydroxymethylfurfural which can then be turned into various useful chemicals such as 2,5-furandicarboxylic acid or 2,5-dimethylfuran (en.wikipedia.org/wiki/Hydroxymethylfurfural). These applications demand high purity fructose, whereas the sweetener application can be utilized as either high purity or low purity fructose.

[0003] There is a need to develop cost-effective synthetic pathways for high-yield, high purity production of the fructose where at least one step of the processes involves an energetically favorable chemical reaction. Furthermore, there is a need for production processes where the process steps can be conducted in one tank or bioreactor and/or where costly separation steps are avoided or eliminated. There is also a need for high-yield, high purity processes of fructose production that can be conducted at a relatively low concentration of phosphate, where phosphate can be recycled and/or the process does not require using adenosine triphosphate (ATP) as an added source of phosphate.

[0004] Enzymatic processes for preparing fructose are provided in PCT Pat. Appl. No. WO 2018/169957, which is incorporated by reference in its entirety. It is desirable to improve fructose yields and enzyme activity to reduce the cost of the process. In that regard, as described below, the use of certain divalent cations in processes for producing fructose result in significant improvements fructose 6-phosphate phosphatase activity and fructose yield.

Summary ~~of the Invention~~

[0005] The ~~inventions~~ disclosure described herein generally relate to improved processes for the enzymatic production of fructose. In one aspect, the disclosure described herein generally relate to process for preparing fructose from a saccharide comprising: a step of converting fructose 6-phosphate (F6P) to a fructose catalyzed by fructose 6-phosphate phosphatase (F6PP) in the presence of a divalent cation selected from the group consisting of Mg^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+}, and combinations thereof. More particularly, a process of the ~~invention~~ disclosure includes a step of converting

fructose 6-phosphate to fructose by a reaction catalyzed by fructose 6-phosphate phosphatase (F6PP) in the presence of one or more divalent cations, including, for example, Mg^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+}, and combinations thereof. Accordingly, a process according to of the ~~invention~~ disclosure can include a step of converting fructose 6-phosphate to fructose by a reaction catalyzed by F6PP in the presence of Mg^{2+} and a divalent cation selected from the group consisting of Zn^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+}, and combinations thereof.

Brief Description of the Figures

[0006] FIG. 1 is a schematic diagram showing an enzymatic pathway converting starch or its derived products to fructose. The following abbreviations are used: IA, isoamylase; PA, pullulanase; aGP, alphaglucan phosphorylase or starch phosphorylase; MP, maltose phosphorylase; PGM, phosphoglucomutase; PPGK, polyphosphate glucokinase; PGI, phosphoglucoisomerase; F6PP, fructose 6-phosphate phosphatase.

[0007] FIG. 2 is a schematic diagram showing an enzymatic pathway converting sucrose to fructose. The following abbreviations are used: SP, sucrose phosphorylase; PGM, phosphoglucomutase; PGI, phosphoglucoisomerase; F6PP, fructose 6-phosphate phosphatase.

[0008] FIG. 3 shows a chromatogram of a study of the effect of various divalent cations, with magnesium, on the yield of fructose from maltodextrin. Fructose is seen at a retention time of ~19 minutes, starting material between 8 and 11.5 minutes, maltotriose at 11.75 min, maltose at 12.75 minutes, and glucose at 14 minutes. No additional divalent cation = blue; calcium = red; cobalt = green; manganese = pink; copper = gold; and zinc = purple.

[0009] FIG. 4 shows a chromatogram of a study of different concentrations of Co2+ and Mn2+, with magnesium, in the reaction mixture. 1 mM cobalt = blue; 0.5 mM cobalt = red; 0.1 mM cobalt = green; 1 mM manganese = pink; 0.5 mM manganese = gold; and 0.1 mM manganese = purple.

Detailed Description

[0010] The inventions described herein provide improved enzymatic pathways, or processes, for producing fructose with a high product yield, while also decreasing fructose production costs. Also described herein is fructose produced by these process. Improved processes of the invention for the enzymatic production of fructose include a step of converting fructose 6-phosphate to fructose catalyzed by fructose 6-phosphate phosphatase (F6PP), in the presence of a divalent cation. For example, a process of the invention for converting F6P to fructose can be performed in the presence of one or more divalent cations selected from the group consisting of Mg^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+}, and combinations thereof. Some embodiments include a step of converting fructose 6-phosphate to fructose catalyzed by F6PP in the presence of Mg^{2+} and a divalent cation selected from the group