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(54) Title: PRODUCTION AND COMPOSITION OF FRUCTOSE SYRUP

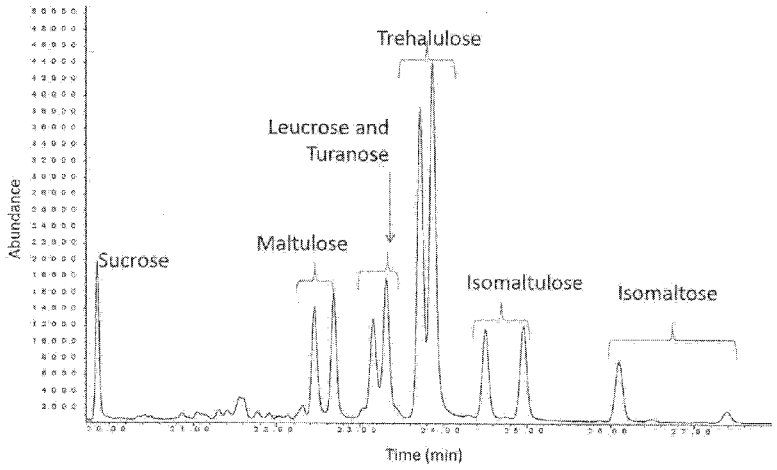

FIG. 1

(57) Abstract: Disclosed herein are aqueous compositions comprising fructose and methods of production thereof. A method for producing an aqueous composition comprising fructose can comprise, for example, conducting an enzymatic reaction by contacting water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3- glucan having at least 30% alpha-1,3-linkages. A soluble fraction produced by such a reaction comprises at least about 55% fructose on a dry weight basis, and can be separated from insoluble poly alpha-1,3-glucan product(s), thereby providing an aqueous composition comprising fructose.

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TITLE

PRODUCTION AND COMPOSITION OF FRUCTOSE SYRUP

This application claims the benefit of U.S. Application No. 14/967,659 (filed December 14, 2015), which is incorporated herein by reference in its entirety.

FIELD

This disclosure is in the field of saccharides. For example, this disclosure pertains to the production of compositions comprising a high fructose content.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named CL6456WOPCT_SequenceListing_ST25_ExtraLinesRemoved created on December 9, 2016, and having a size of 68 kilobytes and is filed concurrently with the specification. The sequence listing contained in this ASCII-formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

Fructose syrup (FS) such as high fructose corn syrup (HFCS) is commonly employed as a sweetener, in part since it is easier to handle than granulated sugar. Starch and table sugar (sucrose) represent the most common feedstocks for producing FS.

Processes for producing FS from starch begin by hydrolyzing this polymer to its monomer, glucose. The glucose is then treated with a glucose isomerase enzyme to convert the glucose to fructose, up to an approximately 50:50 mixture on a dry basis. The isomerization typically yields a syrup ("F42") containing about 42% (dry weight basis [dwb]) fructose, at least about 50% (dwb) glucose, and a small fraction of oligosaccharides. The end point for the isomerization is selected to optimize the process economics. The fructose fraction of the F42 syrup is then enriched by chromatography to yield a syrup ("F90") containing roughly 90% (dwb) fructose. The glucose-enriched fraction from the F42 syrup is recycled and further isomerized into fructose, which is then fractionated by chromatography to increase the overall amount of F90 syrup produced. F90 syrup is typically blended with F42 syrup to produce commercial sweeteners such as HFCS 55, which contains about 55% fructose (dwb), at least about 40% (dwb) glucose, and a small fraction of oligosaccharides.

Processes for producing FS from sucrose involve inverting this disaccharide to glucose and fructose using an immobilized invertase enzyme. The glucose and fructose are then fractionated by chromatography to yield a FS containing at least 90% (dwb) fructose. The glucose fraction is isomerized to F42 using an immobilized glucose isomerase and then further fractionated by chromatography to increase the overall amount of high purity fructose produced.

However, the chromatographic purification steps involved in producing FS from starch and sucrose feedstock are expensive and capital-intensive due to the large amount of evaporation required.

Other processes involving enzymatic approaches have been disclosed attempting to produce FS, but have fallen short in certain respects. U.S. Patent Appl. Publ. No. 2009/0123603, for example, appears to disclose using alternan sucrase to produce a syrup containing about 40% (dwb) fructose from sucrose feedstock. This low fructose level, coupled with the presence of soluble alternan polymer, would necessitate further processing in order to obtain a more useful FS. As additional examples, U.S. Patent Nos. 8828689 and 8962282 disclose enzymatic production of fructose-comprising aqueous compositions. Such compositions, however, are not suitable for use in food applications given the presence of borate therein. Also, though U.S. Patent No. 6242225 discloses enzymatic production of fructose, it is believed this process is limited to producing compositions with fructose and byproduct levels unsuitable for use as FS.

Thus, new processes for enzymatic production of FS are sought that require less processing steps and/or additives. To that end, disclosed herein is FS comprising at least 55% (dwb) fructose and production methods thereof employing a glucosyltransferase reaction.

SUMMARY

In one embodiment, the present disclosure concerns a method for producing an aqueous composition comprising fructose, the method comprising:

(a) contacting water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan having at least 30% alpha-1,3-linkages to produce a soluble fraction and an insoluble fraction, wherein the insoluble fraction comprises the poly alpha-1,3-glucan, and further wherein the soluble fraction comprises at least about 55% fructose on a dry weight basis, and

(b) separating the soluble fraction from the insoluble fraction, thereby providing an aqueous composition comprising fructose.

In another embodiment, the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having at least 95% alpha-1,3-linkages.

In another embodiment, the soluble fraction further comprises soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15.

In another embodiment, the soluble fraction comprises less than about 30% of the soluble oligosaccharides on a dry weight basis.

In another embodiment, the method further comprises contacting the soluble fraction with an alpha-glucosidase enzyme to hydrolyze at least one glycosidic linkage of the oligosaccharides, thereby increasing the monosaccharide content in the soluble fraction.

In another embodiment, the soluble fraction comprises at least about 75% fructose on a dry weight basis.

In another embodiment, the method further comprises a process step that increases the content of monosaccharides relative to the content of other saccharides in the soluble fraction, wherein the process step optionally is nanofiltration or enzymatic hydrolysis.

In another embodiment, the method does not comprise chromatography as a process step to increase the content of fructose relative to the content of other saccharides in the soluble fraction

Another embodiment of the present disclosure concerns an aqueous composition produced by a method herein, such as any of the above embodiments. In another embodiment, such an aqueous composition is comprised within, or is, an ingestible product, and optionally is used as a sweetener of the ingestible product.

Another embodiment of the present disclosure concerns an aqueous composition comprising: (i) at least about 55% fructose on a dry weight basis, (ii) about 3% to about 24% glucose on a dry weight basis, and (iii) soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15, wherein the oligosaccharides comprise glucose and/or fructose.

In another embodiment of the aqueous composition, the soluble oligosaccharides are selected from the group consisting of sucrose, leucrose, trehalulose, isomaltulose, maltulose, isomaltose, and nigerose.

In another embodiment of the aqueous composition, the oligosaccharides comprise (i) at least about 90 wt% glucose, and (ii) about 60-99% alpha-1,3 and about 1-40% alpha-1,6 glucosidic linkages.

In another embodiment, the aqueous composition comprises at least about 75% fructose on a dry weight basis.

In another embodiment, the aqueous composition comprises less than about 30% of the soluble oligosaccharides on a dry weight basis.

In another embodiment, the aqueous composition is comprised within, or is, an ingestible product, and optionally is used as a sweetener of the ingestible product.

In another embodiment, the aqueous composition has a conductivity less than about 50 μS/cm at about 30 wt% dry solids and an ICUMSA value less than about 50.

Another embodiment of the present disclosure concerns an ingestible product comprising an aqueous composition produced by a method herein, wherein the ingestible product is a food, beverage, animal feed, human or animal nutritional product, pharmaceutical product, or oral care product.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCES

FIG. 1 shows a distribution of disaccharides present in the hydrolyzed fructose syrup of Table 6, as detected by gas chromatography-mass spectroscopy.

FIG. 2 shows a distribution of disaccharides present in the hydrolyzed fructose syrup of Table 6, as detected by HPAEC-PAD-MS.

Table 1. Summary of Protein SEQ ID Numbers

Description	Protein SEQ ID NO.
"GTF 7527" (short version of GTFJ), *Streptococcus salivarius*. The first 178 amino acids of the protein are deleted compared to GENBANK Identification No. 47527; a start methionine is included.	1 (1341 aa)
"GTF 2678", *Streptococcus salivarius* K12. The first 188 amino acids of the protein are deleted compared to GENBANK Identification No. 400182678; a start methionine is included.	2 (1341 aa)
"GTF 6855", *Streptococcus salivarius* SK126. The first 178 amino acids of the protein are deleted compared to GENBANK Identification No. 228476855; a start methionine is included.	3 (1341 aa)
"GTF 2919", *Streptococcus salivarius* PS4. The first 92 amino acids of the protein are deleted compared to	4 (1340 aa)

GENBANK Identification No. 383282919; a start methionine is included.	
"GTF 2765", unknown *Streptococcus* sp. C150. The first 193 amino acids of the protein are deleted compared to GENBANK Identification No. 322372765; a start methionine is included.	5 (1340 aa)
GTFJ, *Streptococcus salivarius*. The first 42 amino acids of the protein are deleted compared to GENBANK Identification No. 47527; a start methionine is included.	6 (1477 aa)

DETAILED DESCRIPTION

The disclosures of all cited patent and non-patent literature are incorporated herein by reference in their entirety.

Unless otherwise disclosed, the terms "a" and "an" as used herein are intended to encompass one or more (i.e., at least one) of a referenced feature.

Where present, all ranges are inclusive and combinable, except as otherwise noted. For example, when a range of "1 to 5" is recited, the recited range should be construed as including ranges "1 to 4", "1 to 3", "1-2", "1-2 & 4-5", "1-3 & 5", and the like.

Where present, all ranges are inclusive and combinable, except as otherwise noted. For example, when a range of "1 to 5" is recited, the recited range should be construed as including ranges "1 to 4", "1 to 3", "1-2", "1-2 & 4-5", "1-3 & 5", and the like.

The term "saccharide" as used herein refers to monosaccharides and/or disaccharides/oligosaccharides, unless otherwise noted. A "disaccharide" herein refers to a carbohydrate having two monosaccharides joined by a glycosidic linkage. An "oligosaccharide" herein refers to a carbohydrate that having 2 to 15 monosaccharides, for example, joined by glycosidic linkages. An oligosaccharide can also be referred to as an "oligomer". Monosaccharides (e.g., glucose, fructose) comprised within disaccharides/oligosaccharides can be referred to as "monomer units", "monosaccharide units", or other like terms. The terms "fructose" and "fructose content" herein refer to free fructose, unless otherwise disclosed (this logic similarly applies to other saccharides including glucose).

The terms "alpha-glucan", "alpha-glucan polymer" and the like are used interchangeably herein. An alpha-glucan is a polymer comprising glucose monomeric units linked together by alpha-glucosidic linkages. Poly alpha-1,3-glucan is an example of an alpha-glucan.

The terms "poly alpha-1,3-glucan", "alpha-1,3-glucan polymer", "glucan polymer" and the like are used interchangeably herein. Poly alpha-1,3-glucan is a polymer comprising glucose monomeric units linked together by glucosidic linkages, wherein at least about 30% of the glucosidic linkages are alpha-1,3-glucosidic linkages. Poly alpha-1,3-glucan in certain embodiments comprises at least 95% alpha-1,3-glucosidic linkages.

The terms "glycosidic linkage", "glycosidic bond" and the like are used interchangeably herein and refer to the covalent bond that joins a carbohydrate molecule to another carbohydrate molecule. The terms "glucosidic linkage", "glucosidic bond" and the like are used interchangeably herein and refer to a glycosidic linkage between two glucose molecules. The term "alpha-1,3-glucosidic linkage" as used herein refers to the type of covalent bond that joins alpha-D-glucose molecules to each other through carbons 1 and 3 on adjacent alpha-D-glucose rings. The term "alpha-1,6-glucosidic linkage" as used herein refers to the covalent bond that joins alpha-D-glucose molecules to each other through carbons 1 and 6 on adjacent alpha-D-glucose rings. Herein, "alpha-D-glucose" is referred to as "glucose." All glucosidic linkages disclosed herein are alpha-glucosidic linkages, except as otherwise noted.

The glycosidic linkage profile of an alpha-glucan herein can be determined using any method known in the art. For example, a linkage profile can be determined using methods that use nuclear magnetic resonance (NMR) spectroscopy (e.g., ^{13}C NMR or ^{1}H NMR). These and other methods that can be used are disclosed in Food Carbohydrates: Chemistry, Physical Properties, and Applications (S. W. Cui, Ed., Chapter 3, S. W. Cui, Structural Analysis of Polysaccharides, Taylor & Francis Group LLC, Boca Raton, FL, 2005), which is incorporated herein by reference.

The "molecular weight" of poly alpha-1,3-glucan herein can be represented as weight-average molecular weight (Mw) or number-average molecular weight (Mn), the units of which are in Daltons or grams/mole. Alternatively, molecular weight can be represented as DPw (weight average degree of polymerization) or DPn (number average degree of polymerization). Various means are known in the art for calculating these molecular weight measurements such as with high-pressure liquid chromatography (HPLC), size exclusion chromatography (SEC), or gel permeation chromatography (GPC).

The degree of polymerization (DP) number of an oligosaccharide herein refers to the number of monomeric units in the oligosaccharide. For example, a DP3 oligosaccharide has 3 monomeric units.

The term "sucrose" herein refers to a non-reducing disaccharide composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1,2-glycosidic bond. Sucrose is known commonly as table sugar.

The terms "leucrose" and "D-glucopyranosyl-alpha(1-5)-D-fructopyranose" are used interchangeably herein and refer to a disaccharide containing an alpha-1,5 glucosyl-fructose linkage.

The term "trehalulose" as used herein refers to the disaccharide 1-*O*-alpha-D-glucopyranosyl-beta-D-fructose.

The term "isomaltulose" as used herein refers to the disaccharide 6-*O*-alpha-D-glucopyranosyl-D-fructose.

The term "maltulose" as used herein refers to the disaccharide 4-*O*-alpha-D-glucopyranosyl-D-fructose.

The term "isomaltose" as used herein refers to the disaccharide 6-*O*-alpha-D-glucopyranosyl-D-glucose.

The term "nigerose" as used herein refers to the disaccharide 3-*O*-alpha-D-glucopyranosyl-D-glucose.

The term "turanose" as used herein refers to the disaccharide 3-*O*-alpha-D-glucopyranosyl-D-fructose.

The terms "glucosyltransferase", "glucosyltransferase enzyme", "GTF", "glucansucrase" and the like are used interchangeably herein. The activity of a glucosyltransferase herein catalyzes the reaction of the substrate sucrose to make the products alpha-glucan and fructose. Other products (byproducts) of a glucosyltransferase reaction can include glucose and various soluble oligosaccharides (e.g., DP2-DP7) including leucrose. Wild type forms of glucosyltransferase enzymes generally contain (in the N-terminal to C-terminal direction) a signal peptide, a variable domain, a catalytic domain, and a glucan-binding domain. A glucosyltransferase herein is classified under the glycoside hydrolase family 70 (GH70) according to the CAZy (Carbohydrate-Active EnZymes) database (Cantarel et al., *Nucleic Acids Res.* 37:D233-238, 2009).

The term "glucosyltransferase catalytic domain" herein refers to the domain of a glucosyltransferase enzyme that provides alpha-glucan-synthesizing activity to a glucosyltransferase enzyme. A glucosyltransferase catalytic domain preferably does not require the presence of any other domains to have this activity.

The terms "enzymatic reaction", "glucosyltransferase reaction", "glucan synthesis reaction", "reaction composition" and the like are used interchangeably herein and generally refer to a reaction that initially comprises water, sucrose, at least one active glucosyltransferase enzyme, and optionally other components. Components that can be further present in a glucosyltransferase reaction typically after it has commenced include fructose, glucose, soluble oligosaccharides (e.g., DP2-DP7) such as leucrose, and soluble and/or insoluble alpha-glucan product(s) of DP8 or higher (e.g., DP100 and higher). It would be understood that certain glucan products, such as poly alpha-1,3-glucan with a degree of polymerization (DP) of at least 8 or 9, are water-insoluble and thus not dissolved in a glucan synthesis reaction, but rather may be present out of solution. It is in a glucan synthesis reaction where the step of contacting water, sucrose and a glucosyltransferase enzyme is performed. The term "under suitable reaction conditions" as used herein refers to reaction conditions that support conversion of sucrose to poly alpha-1,3-glucan via glucosyltransferase enzyme activity. An enzymatic reaction herein is not believed to occur in nature.

The term "aqueous composition" herein refers to an aqueous solution comprising at least about 55% fructose on a dry weight basis. A solid material that is not dissolved in the aqueous solution may optionally be present therein (i.e., an aqueous solution may itself be part of a mixture, for example). A soluble fraction herein is an example of an aqueous composition. The solvent of an aqueous composition comprises about, or at least about 70, 75, 80, 85, 90, 95, or 100 wt% water (or any integer value between 70 and 100 wt%), for example.

The term "soluble fraction" herein refers to a solution portion of a glucan synthesis reaction that has produced insoluble poly alpha-1,3-glucan. A soluble fraction can be a portion of, or all of, the solution of a glucan synthesis reaction, and typically has been (or will be) separated from an insoluble poly alpha-1,3-glucan product synthesized in the reaction. A soluble fraction herein comprises at least 55% fructose on a dry weight basis. An example of a soluble fraction is a filtrate of a glucan synthesis reaction. A soluble fraction can contain dissolved sugars such as sucrose, fructose, glucose, and

soluble oligosaccharides (e.g., DP2-DP7) such as leucrose. Thus, a soluble fraction can optionally be referred to as a "fructose stream", "fructose syrup", or "sugar solution". The term "hydrolyzed fraction" and the like herein refer to a soluble fraction that has been treated with an alpha-glucosidase herein to hydrolyze leucrose and/or oligosaccharides present in the soluble fraction.

The term "insoluble fraction" herein refers to insoluble poly alpha-1,3-glucan formed in a glucan synthesis reaction. Other components, such as impurities, can optionally be comprised within an insoluble fraction herein (e.g., colorants).

The terms "dry weight basis" (dwb), "dry solids basis" (dsb) and the like are used interchangeably herein. The amount of a material (e.g., fructose) on a dry weight basis in a soluble fraction, for example, refers to the weight percentage of the material as it exists in all the dissolved material (e.g., fructose, sucrose, glucose, soluble DP2-7 oligosaccharides, optionally salts and impurities) in the soluble fraction. For example, if a soluble fraction comprises 75% (dwb) fructose, there would be 75 wt% fructose in the dry matter resulting from removing all the water from the soluble fraction.

The "percent dry solids" (percent DS) of a solution herein (e.g., soluble fraction, aqueous composition) refers to the wt% of all the materials (i.e., the solids) dissolved in the solution. For example, a 100 g solution with 10 wt% DS comprises 10 g of dissolved material.

The "yield" of poly alpha-1,3-glucan by a glucan synthesis reaction herein represents the weight of poly alpha-1,3-glucan product expressed as a percentage of the weight of sucrose substrate that is converted in the reaction. For example, if 100 g of sucrose in a reaction solution is converted to products, and 10 g of the products is poly alpha-1,3-glucan, the yield of the poly alpha-1,3-glucan would be 10%. This yield calculation can be considered as a measure of selectivity of the reaction toward poly alpha-1,3-glucan.

An "ICUMSA" (International Commission for Uniform Methods of Sugar Analysis) value, or "standard ICUMSA" value, is an international unit for expressing the purity of a sugar sample in solution, and is directly related to the color of the dissolved sugar. The greater the ICUMSA value of a sugar sample, the darker the sugar sample is. Methods of determining ICUMSA values for sugar samples are well known in the art and are disclosed by the International Commission for Uniform Methods of Sugar Analysis in ICUMSA Methods of Sugar Analysis: Official and Tentative Methods Recommended by

the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) (Ed. H.C.S. de Whalley, Elsevier Pub. Co., 1964), for example, which is incorporated herein by reference. ICUMSA can be measured, for example, by ICUMSA Method GS1/3-7 as described by R.J. McCowage, R.M. Urquhart and M.L. Burge (Determination of the Solution Colour of Raw Sugars, Brown Sugars and Coloured Syrups at pH 7.0 – Official, Verlag Dr Albert Bartens, 2011 revision), which is incorporated herein by reference. ICUMSA values can be expressed in "reference base units" (RBU), for example. ICUMSA values herein can be measured, for example, following ICUMSA Method GS1/3-7 or the protocol provided in Table 2. For example, an ICUMSA value of a fructose syrup (soluble fraction) herein can be measured by at least (i) diluting about 3 g of the syrup in about 7 g of water, (ii) adjusting the pH of the diluted syrup to about 7.0 (e.g., using 0.1 M NaOH or 0.1 M HCl), (iii) filtering the diluted syrup (e.g., with a ~0.45-micron filter), (iv) measuring the Brix (g solid/100 g) of the diluted syrup, (v) measuring the density (g/mL) of the diluted syrup, (vi) calculating the concentration, *c*, (g solid/mL) of the diluted syrup (i.e., Brix × density/100), (vii) measuring the absorbance, *As*, at about 420 nm using pathlength, *b*, in cm, (viii), and (ix) calculating ICUMSA as $1000 \times As/(b \times c)$.

The terms "conductivity", "electrical conductivity" and the like are used interchangeably herein. Conductivity generally refers to the ability of an aqueous solution to conduct an electric current between two electrodes at a particular temperature and/or pH. Since a current flows by ion transport in solution, the higher the level of salts dissolved in an aqueous solution, the greater the conductivity of the solution. Thus, conductivity can be used herein to gauge the level ash (i.e., inorganic components) in a sugar solution herein. A suitable unit of measurement for conductivity herein is microSiemens per centimeter (μS/cm), and can be measured using a standard conductivity meter. In general, in order for a sugar solution to be suitable as a sweetener, it should have a conductivity below about 50 μS/cm. The conductivity of a sugar solution herein can be measured following any method known in the art, such as methodology disclosed in U.S. Patent No. 8097086, which is incorporated herein by reference.

The terms "percent by volume", "volume percent", "vol %", "v/v %" and the like are used interchangeably herein. The percent by volume of a solute in a solution can be determined using the formula: [(volume of solute)/(volume of solution)] x 100%.

The terms "percent by weight", "weight percentage (wt%)", "weight-weight percentage (% w/w)" and the like are used interchangeably herein. Percent by weight refers to the percentage of a material on a mass basis as it is comprised in a composition, mixture, or solution.

The terms "alpha-glucosidase", "alpha-1,4-glucosidase", and "alpha-D-glucoside glucohydrolase" are used interchangeably herein. Alpha-glucosidases (EC 3.2.1.20) ("EC" refers to Enzyme Commission number) have previously been recognized as enzymes that catalyze hydrolytic release of terminal, non-reducing (1,4)-linked alpha-D-glucose residues from oligosaccharide (e.g., disaccharide) and polysaccharide substrates. Alpha-glucosidases also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages, and toward alpha-1,3 and alpha-1,6 glucosyl-glucose linkages (refer to U.S. Patent Appl. Publ. Nos. 2015/0240278 and 2015/0240279). Transglucosidase and glucoamylase enzymes are examples of alpha-glucosidases with such activity.

The terms "transglucosidase" (TG), "transglucosidase enzyme", and "1,4-alpha-glucan 6-alpha-glucosyltransferase" are used interchangeably herein. Transglucosidases (EC 2.4.1.24) are D-glucosyltransferase enzymes that catalyze both hydrolytic and transfer reactions on incubation with certain alpha-D-gluco-oligosaccharides. Transglucosidases also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages, and toward alpha-1,3 and alpha-1,6 glucosyl-glucose linkages.

The terms "glucoamylase" (GA), "glucoamylase enzyme", and "alpha-1,4-glucan glucohydrolase" are used interchangeably herein. Glucoamylases (EC 3.2.1.3) are exo-acting enzymes that catalyze hydrolysis of both alpha-1,4 and alpha-1,6 glycosidic linkages from non-reducing ends of glucose-containing di-, oligo- and poly-saccharides. Glucoamylases also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages.

The term "hydrolysis" herein refers to a process in which the glycosidic linkages of an disaccharide/oligosaccharide are broken in a reaction involving water, thereby producing the constituent monosaccharides of the disaccharide/oligosaccharide. The term "enzymatic hydrolysis" herein can refer to, for example, a process in which an alpha-glucosidase is contacted with a soluble fraction to catalyze hydrolysis of leucrose

and/or other oligosaccharides dissolved therein. The term “saccharification” herein refers to a process of breaking a saccharide (disaccharide/oligosaccharide) into its monosaccharide components. A saccharide can be saccharified in a hydrolysis reaction herein.

The term “nanofiltration” as used herein refers to the filtration process in which a low to moderately high pressure (typically 5-30 bar) transports solvent and some solutes through a semi-permeable membrane with some solutes being retained. A semi-permeable membrane for nanofiltration herein typically has pore sizes between 0.1 nm to 10 nm and/or molecular weight cut-off (MWCO) between 100-5000 Daltons and/or magnesium sulfate rejection between 50-99% (e.g., at a pressure of 9 bar, 2000 ppm feed concentration, 25 °C). Generally, the nanofiltration range is between “loose” reverse osmosis (RO) and "tight” ultrafiltration (UF). The material that passes through the membrane of a nanofiltration unit can be referred to as “permeate”, whereas the material that does not pass through the membrane can be referred to as either “concentrate” or “retentate”.

The terms “ingestible product” and “ingestible composition” are used interchangeably herein, and refer to any substance that, either alone or together with another substance, may be taken orally (i.e., by mouth) whether intended for consumption or not. Thus, an ingestible product includes food/beverage products, as well as otherwise non-edible products that can be used orally. “Food/beverage products" refer to any edible product intended for consumption (e.g., for nutritional purposes) by humans or animals, including solids, semi-solids, or liquids. “Non-edible products” (“non-edible compositions”) refer to any composition that can be taken by the mouth for purposes other than food or beverage consumption. Examples of non-edible products herein include supplements, nutraceuticals, functional food products, pharmaceutical products, oral care products (e.g., dentifrices, mouthwashes), and cosmetic products such as sweetened lip balms.

The terms “sequence identity” or “identity” as used herein with respect to polynucleotide or polypeptide sequences refer to the nucleic acid bases or amino acid residues in two sequences that are the same when aligned for maximum correspondence over a specified comparison window. Thus, “percentage of sequence identity” or “percent identity” refers to the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide

or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the results by 100 to yield the percentage of sequence identity. It would be understood that, when calculating sequence identity between a DNA sequence and an RNA sequence, T residues of the DNA sequence align with, and can be considered "identical" with, U residues of the RNA sequence. For purposes of determining percent complementarity of first and second polynucleotides, one can obtain this by determining (i) the percent identity between the first polynucleotide and the complement sequence of the second polynucleotide (or vice versa), for example, and/or (ii) the percentage of bases between the first and second polynucleotides that would create canonical Watson and Crick base pairs.

The Basic Local Alignment Search Tool (BLAST) algorithm, which is available online at the National Center for Biotechnology Information (NCBI) website, may be used, for example, to measure percent identity between or among two or more of the polynucleotide sequences (BLASTN algorithm) or polypeptide sequences (BLASTP algorithm) disclosed herein. Alternatively, percent identity between sequences may be performed using a Clustal algorithm (e.g., ClustalW, ClustalV, or Clustal-Omega). For multiple alignments using a Clustal method of alignment, the default values may correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using a Clustal method may be KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids, these parameters may be KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. Alternatively still, percent identity between sequences may be performed using an EMBOSS algorithm (e.g., needle) with parameters such as GAP OPEN=10, GAP EXTEND=0.5, END GAP PENALTY=false, END GAP OPEN=10, END GAP EXTEND=0.5 using a BLOSUM matrix (e.g., BLOSUM62).

Various polypeptide amino acid sequences and polynucleotide sequences are disclosed herein as features of certain embodiments. Variants of these sequences that

are at least about 70-85%, 85-90%, or 90%-95% identical to the sequences disclosed herein can be used. Alternatively, a variant amino acid sequence or polynucleotide sequence can have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with a sequence disclosed herein. The variant amino acid sequence or polynucleotide sequence may have the same function/activity of the disclosed sequence, or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the function/activity of the disclosed sequence. Any polypeptide amino acid sequence disclosed herein not beginning with a methionine can typically further comprise at least a start-methionine at the N-terminus of the amino acid sequence. Any polypeptide amino acid sequence disclosed herein beginning with a methionine can optionally be considered without this methionine residue (i.e., a polypeptide sequence can be referred to in reference to the position-2 residue to the C-terminal residue of the sequence).

All the amino acid residues at each amino acid position of the proteins disclosed herein are examples. Given that certain amino acids share similar structural and/or charge features with each other (i.e., conserved), the amino acid at each position of a protein herein can be as provided in the disclosed sequences or substituted with a conserved amino acid residue ("conservative amino acid substitution") as follows:

1. The following small aliphatic, nonpolar or slightly polar residues can substitute for each other: Ala (A), Ser (S), Thr (T), Pro (P), Gly (G);
2. The following polar, negatively charged residues and their amides can substitute for each other: Asp (D), Asn (N), Glu (E), Gln (Q);
3. The following polar, positively charged residues can substitute for each other: His (H), Arg (R), Lys (K);
4. The following aliphatic, nonpolar residues can substitute for each other: Ala (A), Leu (L), Ile (I), Val (V), Cys (C), Met (M); and
5. The following large aromatic residues can substitute for each other: Phe (F), Tyr (Y), Trp (W).

The term "isolated" as used herein refers to any cellular component that has been completely or partially purified from its native source (e.g., an isolated polynucleotide or polypeptide molecule). In some instances, an isolated polynucleotide or polypeptide molecule is part of a greater composition, buffer system or reagent mix. For example,

an isolated polynucleotide or polypeptide molecule can be comprised within a cell or organism in a heterologous manner. Another example is an isolated glucosyltransferase enzyme or reaction. "Isolated" herein can also characterize an aqueous composition herein. As such, an aqueous composition of the present disclosure is synthetic/man-made, and/or has properties that are not naturally occurring.

The term "increased" as used herein can refer to a quantity or activity that is at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 50%, 100%, or 200% more than the quantity or activity for which the increased quantity or activity is being compared. The terms "increased", "elevated", "enhanced", "greater than", "improved" and the like are used interchangeably herein.

New processes for enzymatic production of fructose syrup (FS) are sought after that require less processing steps and/or additives. To that end, disclosed herein is FS comprising at least 55% fructose on a dry weight basis (dwb) and production methods thereof employing a glucosyltransferase reaction.

Certain embodiments of the present disclosure concern a method for producing an aqueous composition comprising fructose. This method comprises at least:

(a) contacting water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan having at least 30% alpha-1,3-linkages to produce a soluble fraction and an insoluble fraction, wherein the insoluble fraction comprises the poly alpha-1,3-glucan, and wherein the soluble fraction comprises at least about 55% fructose on a dry weight basis, and

(b) separating the soluble fraction from the insoluble fraction, thereby providing an aqueous composition comprising fructose.

Significantly, although one or more chromatographical process can be applied if desired to enhance results, such additional processing is not necessary to reach the 55% fructose (dwb) level. It would be understood that a soluble fraction produced in the glucosyltransferase reaction of this method represents the aqueous composition as directly produced by the method (i.e., the aqueous composition likewise comprises 55% fructose [dwb]). Thus, disclosures herein regarding a soluble fraction can likewise apply to the disclosure of an aqueous composition, unless otherwise noted. Step (a) can

optionally be characterized as providing a reaction by virtue of contacting each of the water, sucrose and enzyme components with each other.

A soluble fraction (and therefore an aqueous composition produced by the above method) herein comprises at least about 55% fructose (dwb). For example, there can be at least about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98% fructose on a dry weight basis in a soluble fraction herein. In other examples, there can be about 55-95%, 55-90%, 55-85%, 55-80%, 55-75%, 55-70%, 60-95%, 60-90%, 60-60%, 60-80%, 60-75%, 60-70%, 65-95%, 65-90%, 65-85%, 65-80%, 65-75%, 65-70%, 70-95%, 70-90%, 70-85%, 70-80%, 70-75%, 75-95%, 75-90%, 75-85%, 75-80%, 80-95%, 80-90%, 80-85%, 85-95%, 85-90%, or 90-95% fructose on a dry weight basis in a soluble fraction. In some embodiments in which a soluble fraction has been subjected at least to an oligosaccharide (e.g., leucrose) hydrolytic procedure (e.g., enzymatic hydrolysis) and/or nanofiltration, there can be at least about 65% fructose on a dry weight basis in the soluble fraction.

A soluble fraction in some aspects can comprise about 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 3-24%, 4-24%, 6-24%, 8-24%, 10-24%, 12-24%, 14-24%, 16-24% glucose on a dry weight basis. In some embodiments in which a soluble fraction has been subjected at least to an oligosaccharide (e.g., leucrose) hydrolytic procedure (e.g., enzymatic hydrolysis) and/or nanofiltration, there can be about 14-24%, 14-20%, 14-18%, 14-16%, 15-24%, 15-20%, 15-18%, or 15-16% (dwb) glucose.

A soluble fraction in some aspects can comprise soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15. The DP of such oligosaccharides can range from 2-7, 2-8, 2-9, 2-10, 2-11, 2-12, 2-13, 2-14, or 2-15, for example. Examples of the oligosaccharides include one or more of sucrose (i.e., residual sucrose that was not converted in the glucosyltransferase reaction), leucrose, trehalulose, isomaltulose, maltulose, isomaltose, nigerose and turanose. All of these oligosaccharides, at least, can be present in a soluble fraction in certain embodiments. The oligosaccharides present can be maltulose, leucrose, trehalulose, isomaltulose, and isomaltose in some

aspects. In these and/or certain other embodiments, oligosaccharides of a soluble fraction can comprise glucose and/or fructose. There can be at least about 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95 wt% glucose content in the soluble oligosaccharides, for example. In some embodiments, oligosaccharides can include fructan disaccharides (i.e., DP2 oligomers comprising only fructose). The relative amount of trehalulose in a soluble fraction can be about, or at least about, 2, 2.5, 3, 3.5, 4, or 5 times more, for example, than the relative amount of sucrose, maltulose, leucrose, turanose, isomaltulose, and/or isomaltose also present in the soluble fraction. In some aspects, the relative amount of leucrose in a soluble fraction can be nearly the same (e.g., ± 5%, 10%, 15%) as the relative amount of isomaltose and/or maltulose. Still in some aspects, the aforementioned relative amounts of disaccharides can characterize a soluble fraction that has been subject to an enzymatic hydrolysis procedure.

A soluble fraction in some aspects can comprise less than about 30% of soluble oligosaccharides on a dry weight basis. For example, there may be less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, for example, of soluble oligosaccharides on a dry weight basis in the soluble fraction. In some embodiments in which a soluble fraction has been subjected at least to an oligosaccharide (e.g., leucrose) hydrolytic procedure (e.g., enzymatic hydrolysis) and/or nanofiltration, there may be less than about 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, for example, of soluble oligosaccharides on a dry weight basis in the soluble fraction. In some aspects of the present disclosure, there is some amount of soluble oligosaccharides present in a soluble fraction (i.e., the level of oligosaccharides is not 0% dwb).

A soluble fraction in some aspects can comprise less than about 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% leucrose on a dry weight basis. In some embodiments in which a soluble fraction has been subjected at least to an oligosaccharide (e.g., leucrose) hydrolytic procedure (e.g., enzymatic hydrolysis) and/or nanofiltration, there may be less than about 3%, 2%, or 1% (dwb) leucrose.

A sub-population of the oligosaccharides in a soluble fraction in some aspects (e.g., DP2-7 or DP2-8) can comprise alpha-1,3 glucosidic linkages and/or alpha-1,6 glucosidic linkages. Such a sub-population of oligosaccharides can optionally be said to comprise only glucose (no fructose), whereas in other aspects some oligosaccharides of this sub-population can comprise fructose. Oligosaccharides in certain embodiments of this sub-population can comprise about 60-99% alpha-1,3 glucosidic linkages and about 1-40% alpha-1,6 glucosidic linkages. Such oligosaccharides alternatively can comprise about 60-95% or 70-90% alpha-1,3 glucosidic linkages, and about 5-40% or 10-30% alpha-1,6 glucosidic linkages. Alternatively still, such oligosaccharides herein can comprise about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% alpha-1,3 glucosidic linkages, and about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40% alpha-1,6 glucosidic linkages. The aforementioned oligosaccharides can collectively comprise any of the foregoing linkage profiles. By “collectively comprise”, it is meant that the total or average linkage profile of a mixture of the oligosaccharides is any of the foregoing linkage profiles. The aforementioned oligosaccharides can contain mostly alpha-1,3 and alpha-1,6 glucosidic linkages. For example, at least 95%, 96%, 97%, 98%, 99%, or 100% of the linkages of the oligosaccharide sub-population are alpha-1,3 and alpha-1,6 glucosidic linkages. Other linkages, if present in the oligosaccharides, may be alpha-1,4 or alpha-1,2 glucosidic linkages, and/or fructose linkages, for example.

In some aspects, a sub-population of oligosaccharides as described above can represent about 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 32-45, 32-42, 32-39, 35-45, 35-42, 35-39, 38-45, or 38-42 wt% of all the oligosaccharides of a soluble fraction. The percent dry solids basis (dsb) of these oligosaccharides in a soluble fraction can be about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 2-10%, 4-10%, 6-10%, 2-8%, 4-8%, 6-8%, 2-6%, or 4-6%, for example. These percentages/amounts are typically observed in a soluble fraction herein in which there has been no oligosaccharide (e.g., leucrose) hydrolytic procedure (e.g., enzymatic hydrolysis) and/or nanofiltration. Alternatively, a sub-population of oligosaccharides as described above can represent about 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 85-95, 87.5-95, 90-95, 85-

92.5, 87.5-92.5, 90-92.5, 85-90, or 87.5-90 wt%, for example, of all the oligosaccharides of a soluble fraction that has been treated with an oligosaccharide (e.g., leucrose) hydrolytic procedure (e.g., enzymatic hydrolysis) (but optionally no nanofiltration). The percent dry solids basis (dsb) of these oligosaccharides in a soluble fraction that has been hydrolytically treated can be about 15%, 16%, 17%, 18%, 19%, 20%, 15-20%, 16-19%, or 17-18%, for example.

A soluble fraction can be a portion of (e.g., at least about 70-80%), or all of, the liquid solution from a glucosyltransferase reaction. Typically, a soluble fraction is separated from an insoluble glucan product(s) synthesized in the reaction. For example, a soluble fraction can be separated from one or more glucan products that are insoluble in water (e.g., poly alpha-1,3-glucan) which fall out of solution during their synthesis. A soluble fraction in certain embodiments of the present disclosure is from a poly alpha-1,3-glucan synthesis reaction.

The volume of a soluble fraction (before optionally diluting or concentrating the fraction, see below) in certain embodiments can be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (or any integer value between 10% and 90%) of the volume of the glucosyltransferase reaction from which it is obtained, for example. A soluble fraction can be obtained at any stage of a glucosyltransferase reaction, but is preferably obtained near (e.g., greater than 80 or 90% complete) or after completion of the reaction.

Examples of a soluble fraction of a glucosyltransferase reaction in certain embodiments include filtrates and supernatants; a filtrate or supernatant can also be considered as an aqueous composition of the present disclosure. Thus, a soluble fraction herein can be obtained (separated) from a glucosyltransferase reaction using a funnel, filter (e.g., a surface filter such as a rotary vacuum-drum filter, cross-flow filter, screen filter, belt filter, screw press, or filter press with or with membrane squeeze capability; or a depth filter such as a sand filter), centrifuge, and/or any other method or equipment known in the art that allows removal of some or all liquids from solids. Any configuration useful for separating soluble and insoluble fraction can be used. Filtration can be by gravity, vacuum, or press filtration, for example. Filtration preferably removes all or most of an insoluble glucan; any filter material (e.g., cloth, metal screen or filter paper) with an average pore size (e.g., ~10-50 micron) sufficient to remove solids from

liquids can be used. A soluble fraction typically retains all or most of its dissolved components, such as certain byproducts (e.g., glucose, oligosaccharides such as leucrose) of the glucosyltransferase reaction. Optionally, all or most of any residual amount of soluble fraction remaining with the insoluble fraction following a separation step herein can be obtained by washing the insoluble fraction one or more times with water or an aqueous solution. Such washing can be done one, two, or more times with a wash volume that is at least about 10-100% of the volume of the gtf reaction used to produce the soluble and insoluble fractions, for example. Washing can be done by various modes, such as displacement or reslurry washing. Wash liquid that is separated from the insoluble fraction can be combined with the originally isolated soluble fraction.

A soluble fraction herein can optionally be diluted or concentrated, if desired. Concentration of a soluble fraction can be performed using any method and/or equipment known in the art suitable for concentrating a solution. Concentration of a soluble fraction is preferably performed in such a way as to minimize color production. For example, a fraction can be concentrated by evaporation, such as with a rotary evaporator. Other suitable types of evaporation equipment include forced circulation or falling film evaporators. A preferred evaporator is a falling film evaporator, which in some aspects uses multiple effects or vapor recompression to minimize energy consumption. Concentration is typically done at pressures below atmospheric pressure to minimize color formation. Concentration may be done in multiple stages if desired. For example, a soluble fraction herein can be evaporated to about 30-60 wt% dry solids (DS) at one temperature and pressure, and then evaporated to about 30-80 wt% DS (~30-60 wt% DS, ~70-77 wt% DS) at a lower temperature and pressure (compared to the first stage) to minimize formation of color in the final syrup. A concentrated soluble fraction (e.g., concentrated filtrate) can optionally be referred to as a syrup. Fructose syrup herein can be concentrated to about 65-77 wt% solids to increase shelf life and prevent microbial growth, for example.

A soluble fraction in certain embodiments is from a poly alpha-1,3-glucan synthesis reaction; such a fraction is preferably a filtrate. A fraction of a poly alpha-1,3-glucan synthesis reaction herein can comprise at least water, fructose and one or more other saccharides (e.g., glucose and oligosaccharides such as leucrose), for instance. Other components that may be in this type of soluble fraction include sucrose (i.e., residual sucrose not consumed in the gtf reaction), proteins, ions, and/or organic acids,

for example. Minimally, the components of a soluble fraction from a poly alpha-1,3-glucan synthesis reaction include water, fructose, glucose, one or more oligosaccharides (DP2-7 or DP2-8, including leucrose, for example), and optionally sucrose, for example. It would be understood that the composition of a soluble fraction depends, in part, on the conditions of the glucosyltransferase reaction from which the fraction is obtained.

In certain embodiments, a soluble fraction herein does not contain any borates (e.g., boric acid, tetraborate), or contains less than 50, 100, 150, 200, 250, or 300 mM of a borate. It would be understood that, in cases in which a soluble fraction of a reaction herein is intended for use as an ingestible product such as a food or pharmaceutical product, borate is not present or is otherwise undetectable in the soluble fraction.

It should be understood that the exact distribution of sugar byproducts produced in a glucosyltransferase reaction can vary based on the reaction conditions and gtf enzyme used, especially on temperature and sucrose concentration. Generally, as the amount of sucrose is increased, the selectivity of the reaction towards both leucrose and oligosaccharides will increase. Conversely, as the temperature increases, the selectivity of the reaction towards leucrose tends to decrease, while the selectivity towards oligosaccharides is largely unaffected. It should also be understood that the ratio of sugars to water, i.e., wt% DS, which is calculated by dividing the mass of sugar to total solution weight, can be adjusted either by evaporation or addition of water without a significant impact to the relative distribution of sugars in the soluble fraction. It is also possible to increase the percentage of sucrose in a fraction by stopping the gtf reaction before complete conversion (to glucan) is achieved, either by reducing the pH below the active range for the gtf enzyme or by thermal deactivation of the gtf enzyme.

An insoluble fraction is produced in the glucosyltransferase reaction of the disclosed method that comprises poly alpha-1,3-glucan. It would therefore be understood that such glucan polymer is aqueous-insoluble under the conditions of a glucosyltransferase reaction herein (e.g., pH 4-9), which are non-caustic. Poly alpha-1,3-glucan herein comprises at least 30% alpha-1,3-glucosidic linkages.

Poly alpha-1,3-glucan in certain embodiments has at least about 95%, 96%, 97%, 98%, 99%, or 100% alpha-1,3 glucosidic linkages. In some embodiments, accordingly, poly alpha-1,3-glucan has less than about 5%, 4%, 3%, 2%, 1%, or 0% of glucosidic linkages that are not alpha-1,3. It should be understood that the higher the percentage

of alpha-1,3-glucosidic linkages present in poly alpha-1,3-glucan, the greater the probability that the poly alpha-1,3-glucan is linear, since there are lower occurrences of certain linkages forming branch points in the polymer. Thus, poly alpha-1,3-glucan with 100% alpha-1,3 glucosidic linkages is believed to be completely linear. In certain embodiments, poly alpha-1,3-glucan has no branch points or less than about 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer. Examples of branch points include alpha-1,6 branch points.

Poly alpha-1,3-glucan (≥95% 1,3 linkages) herein can have a molecular weight in DP_w or DP_n of at least about 100 in some aspects. For example, the molecular weight can be at least about 400 DP_w or DP_n. DP_w or DP_n in still another embodiment can be at least about 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, or 1500 (or any integer between 100 and 1500).

Poly alpha-1,3-glucan (≥95% 1,3 linkages) is insoluble in most aqueous systems. In general, the solubility of a glucan polymer in an aqueous systems is related to its linkage type, molecular weight and/or degree of branching. Poly alpha-1,3-glucan (≥95% 1,3 linkages) is generally insoluble at a DP_w of 8 and above in aqueous (or mostly aqueous) liquids at 20 °C.

In some other embodiments, an insoluble poly alpha-1,3-glucan can comprise at least 30% alpha-1,3-glucosidic linkages and a percentage of alpha-1,6-glucosidic linkages that brings the total of both the alpha-1,3 and -1,6 linkages in the poly alpha-1,3-glucan to 100%. For example, the percentage of alpha-1,3 linkages can be at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, while the percentage of alpha-1,6 linkages can be that which brings the total of both the alpha-1,3 and -1,6 linkages in the in the poly alpha-1,3-glucan to 100%. Poly alpha-1,3-glucan in these embodiments does not comprise alternan (alternating 1,3 and 1,6 linkages).

Step (a) of a method herein of producing an aqueous composition comprising fructose includes forming a glucosyltransferase reaction. Such an enzymatic reaction

employs a glucosyltransferase that synthesizes poly alpha-1,3-glucan comprising at least about 30% alpha-1,3-glucosidic linkages. A glucosyltransferase enzyme herein can produce any aqueous-insoluble poly alpha-1,3-glucan molecule as disclosed above, such as one comprising at least 95% alpha-1,3-glucosidic linkages.

A glucosyltransferase enzyme in certain embodiments for producing poly alpha-1,3-glucan (≥95% 1,3 linkages) can comprise, or consist of, an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 99%, or 99.5% identical to SEQ ID NOs:1, 2, 3, 4, or 5. Further, this glucosyltransferase can produce (along with poly alpha-1,3-glucan as an insoluble product) soluble products comprising at least 55% fructose on a dry weight basis of the soluble products. A glucosyltransferase in some aspects produces soluble products comprising about, or at least about, any of the fructose amounts disclosed above (e.g., at least 65% or 80% dwb).

A glucosyltransferase enzyme in certain embodiments can comprise, or consist of, a glucosyltransferase catalytic domain having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 99%, or 99.5% identical to amino acid positions 54-957 of SEQ ID NO:1, and can produce soluble products comprising at least 55% fructose on a dry weight basis (or any higher amount as disclosed above) of the soluble products. A glucosyltransferase enzyme with amino acid positions 54-957 of SEQ ID NO:1 can produce poly alpha-1,3-glucan with 100% alpha-1,3 linkages and a DPw of at least 400 (data not shown, refer to Table 6 of U.S. Pat. Appl. No. 62/180,779), for example.

SEQ ID NOs:1 (GTF 7527), 2 (GTF 2678), 3 (GTF 6855), 4 (GTF 2919), and 5 (GTF 2765) each represent a glucosyltransferase that, compared to its respective wild type counterpart, lacks the signal peptide domain and all or a substantial portion of the variable domain. Thus, each of these glucosyltransferase enzymes has a catalytic domain followed by a glucan-binding domain. The approximate location of catalytic domain sequences in these enzymes is as follows: 7527 (residues 54-957 of SEQ ID NO:1), 2678 (residues 55-960 of SEQ ID NO:2), 6855 (residues 55-960 of SEQ ID NO:3), 2919 (residues 55-960 of SEQ ID NO:4), 2765 (residues 55-960 of SEQ ID NO:5). The amino acid sequences of catalytic domains of GTFs 2678, 6855, 2919 and 2765 have about 94.9%, 99.0%, 95.5% and 96.4% identity, respectively, with a catalytic domain sequence of GTF 7527 (i.e., amino acids 54-957 of SEQ ID NO:1). These

particular glucosyltransferase enzymes can produce poly alpha-1,3-glucan with 100% alpha-1,3 linkages and a DPw of at least 400 (data not shown, refer to Table 4 of U.S. Pat. Appl. No. 62/180,779). Thus, a glucosyltransferase enzyme in certain embodiments can (i) comprise, or consist of, a glucosyltransferase catalytic domain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 99%, or 99.5% identical to the amino acid sequence of a catalytic domain of GTF 2678, 6855, 2919, or 2765, and (ii) produce soluble products comprising at least 55% fructose on a dry weight basis (or any higher amount as disclosed above) of the soluble products. In some alternative embodiments, a glucosyltransferase catalytic domain sequence does not comprise residues 54-957 of SEQ ID NO:1, residues 55-960 of SEQ ID NO:2, residues 55-960 of SEQ ID NO:3, residues 55-960 of SEQ ID NO:4, or residues 55-960 of SEQ ID NO:5.

Although it is believed that a glucosyltransferase enzyme herein need only have a catalytic domain sequence, such as one comprising an amino acid sequence that is at least 90% identical to amino acid positions 54-957 of SEQ ID NO:1 (or positions 55-960 of SEQ ID NO:2, positions 55-960 of SEQ ID NO:3, positions 55-960 of SEQ ID NO:4, or positions 55-960 of SEQ ID NO:5), the glucosyltransferase enzyme can be comprised within a larger amino acid sequence. For example, the catalytic domain may be linked at its C-terminus to a glucan-binding domain, and/or linked at its N-terminus to a variable domain and/or signal peptide.

Still further examples of glucosyltransferase enzymes can be any as disclosed herein and that include 1-300 (or any integer there between [e.g., 10, 15, 20, 25, 30, 35, 40, 45, or 50]) residues on the N-terminus and/or C-terminus. Such additional residues may be from a corresponding wild type sequence from which the glucosyltransferase enzyme is derived, or may be a heterologous sequence such as an epitope tag (at either N- or C-terminus) or a heterologous signal peptide (at N-terminus), for example. A glucosyltransferase enzyme herein typically lacks an N-terminal signal peptide.

The amino acid sequence of a glucosyltransferase enzyme in certain aspects has been modified such that the enzyme produces more products (poly alpha-1,3-glucan and fructose), and less byproducts (e.g., glucose, oligosaccharides such as leucrose), from a given amount of sucrose substrate. For example, one, two, three, or more amino acid residues of the catalytic domain of a glucosyltransferase herein can be modified to

obtain an enzyme that produces at least about 55% fructose (or any higher amount as disclosed above) on a dry weight basis of the soluble products of the enzyme.

A glucosyltransferase enzyme herein can be derived from any microbial source, such as a bacteria or fungus. Examples of bacterial glucosyltransferase enzymes are those derived from a *Streptococcus* species, *Leuconostoc* species or *Lactobacillus* species. Examples of *Streptococcus* species include *S. salivarius*, *S. sobrinus*, *S. dentirousetti*, *S. downei*, *S. mutans*, *S. oralis*, *S. gallolyticus* and *S. sanguinis*. Examples of *Leuconostoc* species include *L. mesenteroides*, *L. amelibiosum*, *L. argentinum*, *L. carnosum*, *L. citreum*, *L. cremoris*, *L. dextranicum* and *L. fructosum*. Examples of *Lactobacillus* species include *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*, *L. casei*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. brevis*, *L. buchneri*, *L. fermentum* and *L. reuteri*.

A glucosyltransferase enzyme herein can be prepared by fermentation of an appropriately engineered microbial strain, for example. Recombinant enzyme production by fermentation is well known in the art using microbial strains such as *E. coli*, *Bacillus* strains (e.g., *B. subtilis*), *Ralstonia eutropha*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha*, and species of *Aspergillus* (e.g., *A. awamori*) and *Trichoderma* (e.g., *T. reesei*) (e.g., see Adrio and Demain, *Biomolecules* 4:117-139, which is incorporated herein by reference). A nucleotide sequence encoding a glucosyltransferase enzyme amino acid sequence is typically linked to a heterologous promoter sequence to create an expression cassette for the enzyme. Such an expression cassette may be incorporated on a suitable plasmid or integrated into the microbial host chromosome, using methods well known in the art. The expression cassette may include a transcriptional terminator nucleotide sequence following the amino acid coding sequence. The expression cassette may also include, between the promoter sequence and amino acid coding sequence, a nucleotide sequence encoding a signal peptide that is designed to direct secretion of the glucosyltransferase enzyme. At the end of fermentation, cells may be ruptured accordingly and the glucosyltransferase enzyme can be isolated using methods such as precipitation, filtration, and/or concentration. Alternatively, a lysate comprising a glucosyltransferase can be used without further isolation. The activity of a glucosyltransferase enzyme can be confirmed by biochemical assay, such as measuring its conversion of sucrose to glucan polymer.

A glucosyltransferase enzyme in certain embodiments does not occur in nature. For example, an enzyme herein is not believed to be one that is naturally secreted (i.e., mature form) from a microbe (from which the glucosyltransferase enzyme herein could possibly have been derived).

The temperature of a glucosyltransferase reaction herein can be controlled, if desired. In certain embodiments, the temperature of the reaction can be between about 5 °C to about 50 °C. The temperature in certain other embodiments can be between about 20 °C to about 40 °C, or about 20 °C to about 30 °C (e.g., about 22-25 °C).

The initial concentration of sucrose in a reaction solution herein can be about 20 g/L to about 400 g/L, for example. Alternatively, the initial concentration of sucrose can be about 75 g/L to about 175 g/L, or from about 50 g/L to about 150 g/L. Alternatively still, the initial concentration of sucrose can be about 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, or 160 g/L (or any integer value between 40 and 160 g/L), for example. "Initial concentration of sucrose" refers to the sucrose concentration in a glucosyltransferase reaction just after all the reaction components have been added (e.g., at least water, sucrose, glucosyltransferase enzyme).

Sucrose used in a glucosyltransferase reaction herein can be highly pure (≥ 99.5%) or be of any other purity or grade. For example, sucrose can have a purity of at least 99.0%, or can be reagent grade sucrose.

The pH of a glucosyltransferase reaction in certain embodiments can be between about 4.0 to about 8.0, or between about 5.0 to about 6.0. Alternatively, the pH can be about 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0, for example. The pH can be adjusted or controlled by the addition or incorporation of a suitable buffer, including but not limited to: phosphate, tris, citrate, or a combination thereof. Buffer concentration in a glucan synthesis reaction can be from 0 mM to about 100 mM, or about 10, 20, or 50 mM, for example. A glucan synthesis reaction in some preferred aspects uses a minimal amount of buffer, such as about 10 mM or less.

One or more different glucosyltransferase enzymes may be used in certain aspects. A glucosyltransferase reaction herein may contain one, two, or more glucosyltransferase enzymes, for example.

A method as presently disclosed for producing an aqueous composition with fructose comprises providing a reaction by contacting at least water, sucrose, and a glucosyltransferase enzyme as described herein. These and optionally other reagents can be added altogether or added in any order. It will be understood that as the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan, the reaction, which initially is in the form of a solution, becomes a mixture since insoluble poly alpha-1,3-glucan falls out of solution. The contacting step of the disclosed method can be performed in any number of ways. For example, the desired amount of sucrose can first be dissolved in water (optionally, other components may also be added at this stage of preparation, such as buffer components), followed by the addition of at least one glucosyltransferase enzyme. The solution may be kept still, or agitated via stirring or orbital shaking, for example. Typically, a glucan synthesis reaction is cell-free.

Additional components of a glucosyltransferase reaction can be any of those as disclosed above and/or in the instant Examples. In some instances, no borate (e.g., boric acid, tetraborate) or less than 50, 100, 150, 200, 250, or 300 mM of a borate is used in a reaction. A borate may optionally be present and/or be above any of the foregoing concentrations, in a glucosyltransferase reaction that (i) otherwise has the capability to yield, without borate addition, a soluble fraction with at least about 65%, 70%, or 75% fructose on a dry weight basis, and (ii) is not used for preparing an ingestible product. It would be understood that, in cases in which a soluble fraction of a reaction herein is intended for use as an ingestible product such as a food or pharmaceutical product, borate is not present or is otherwise undetectable in the soluble fraction. In some instances, a glucosyltransferase reaction comprises, or does not comprise, a semi-permeable membrane (e.g., molecular weight cut-off from 12,000 to 100,000 Daltons).

Completion of a reaction in certain embodiments can be determined visually (no more accumulation of insoluble poly alpha-1,3-glucan) and/or by measuring the amount of sucrose left in the solution (residual sucrose), where a percent sucrose consumption of over about 90% can indicate reaction completion, for example. Typically, a reaction of the disclosed process will take about 12, 24, 36, 48, 60, 72, 84, or 96 hours to complete, depending on certain parameters such as the amount of sucrose and glucosyltransferase enzyme used in the reaction, and/or the temperature of the reaction.

The percent sucrose consumption of a glucosyltransferase reaction in certain embodiments is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the sucrose initially contacted with water and a glucosyltransferase enzyme. Alternatively, the percent sucrose consumption may be >95% or >99%.

The yield of poly alpha-1,3-glucan produced in some aspects of a glucosyltransferase reaction herein can be at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% based on the weight of sucrose converted in the reaction.

The yield of fructose produced in some aspects of a glucosyltransferase reaction herein is at least about 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50% based on the weight of sucrose converted in the reaction.

Poly alpha-1,3-glucan produced in a glucosyltransferase reaction of the instantly disclosed method can be isolated. Such isolation can be characterized to represent the method step herein of separating the soluble fraction from the insoluble fraction, thereby providing an aqueous composition comprising fructose.

A method of producing an aqueous composition comprising fructose in certain aspects of the instant disclosure can further comprise contacting a soluble fraction and/or a glucosyltransferase reaction with an alpha-glucosidase enzyme to hydrolyze at least one glycosidic linkage of one or more oligosaccharides present in the soluble fraction and/or glucosyltransferase reaction, thereby increasing the monosaccharide content in the soluble fraction. Thus, a soluble fraction herein can be contacted with an alpha-glucosidase after its separation from an insoluble fraction comprising poly alpha-1,3-glucan, or before its separation (e.g., while it is being formed in the reaction, and/or after completion of the reaction) (i.e., in contacting step [a] and/or after separation step [b]). The activity of an alpha-glucosidase hydrolyzes one or more different oligosaccharides herein (e.g., leucrose and/or oligosaccharides comprising only glucose) to its constituent monosaccharides (fructose and/or glucose). Embodiments herein comprising alpha-glucosidase treatment can optionally be characterized as further comprising an enzymatic hydrolysis step. Where treatment is with an alpha-glucosidase that hydrolyzes a fructose-comprising oligosaccharide such as leucrose, such treatment will increase the fructose content (dwb) of the soluble fraction.

An alpha-glucosidase herein, and methods of its use in a soluble fraction and/or glucosyltransferase reaction, can be as disclosed in U.S. Patent Appl. Publ. Nos. 2015/0240278 and 2015/0240279, which are incorporated herein by reference. An alpha-glucosidase (EC 3.2.1.20) can be used in certain embodiments herein to hydrolyze an alpha-1,5 glucosyl-fructose linkage in a saccharide comprising at least one alpha-1,5 glucosyl-fructose linkage (e.g., leucrose). A transglucosidase (EC 2.4.1.24; 1,4-alpha-glucan 6-alpha-glucosyltransferase) or glucoamylase (EC 3.2.1.3; alpha-1,4-glucan glucohydrolase) can be used, for example, as an alpha-glucosidase to hydrolyze an alpha-1,5 glucosyl-fructose linkage in a saccharide comprising at least one alpha-1,5 glucosyl-fructose linkage (e.g., leucrose). Examples of suitable alpha-glucosidases (disclosed in U.S. Appl. Publ. Nos. 2015/0240278 and 2015/0240279) include the mature forms of "TG L-2000" (*Aspergillus niger* transglucosidase), "GC 321 Glucoamylase" (*Trichoderma reesei* glucoamylase, TrGA), "Aclglu1" (*Aspergillus clavatus* alpha-glucosidase), "Aclglu1" (*Aspergillus clavatus* alpha-glucosidase), "Nfiglu1" (*Neosartorya fischeri* alpha-glucosidase), "Nfiglu1" (*Neosartorya fischeri* alpha-glucosidase), "Ncrglu1" (Neurospora crassa alpha-glucosidase), "Ncrglu1" (*Neurospora crassa* alpha-glucosidase), "TauSec098" (*Rasamsonia composticola* alpha-glucosidase), "TauSec098" (*Rasamsonia composticola* alpha-glucosidase), "TauSec099" (*Rasamsonia composticola* alpha-glucosidase), "TauSec099" (*Rasamsonia composticola* alpha-glucosidase), "BloGlu1" (*Bifidobacterium longum* (subsp. longum JDM301) alpha-glucosidase), "BloGlu2" (*Bifidobacterium longum* alpha-glucosidase), "BloGlu3" (*Bifidobacterium longum* (subsp. F8) alpha-glucosidase), "BpsGlu1" (*Bifidobacterium pseudolongum* alpha-glucosidase), "BthGlu1" (*Bifidobacterium thermophilum* RBL67 alpha-glucosidase), "BbrGlu2" (*Bifidobacterium breve* alpha-glucosidase), and "BbrGlu5" (*Bifidobacterium breve* ACS-071-V-Sch8b alpha-glucosidase), or any amino acid sequence that (i) is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of these enzymes and (ii) has hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages in saccharides. One or more of alpha-glucosidase enzymes herein may be used in a hydrolysis reaction in certain embodiments. Both a transglucosidase and glucoamylase can be used in a reaction, for example.

Typically, a soluble fraction and/or a glucosyltransferase reaction herein can be contacted directly, without modification, with one or more alpha-glucosidases. The following conditions, however, may be useful for performing enzymatic hydrolysis. The

pH can be about 3.0 to 9.0 (e.g., 4.0-5.0), for example. The temperature can be about 20 °C to about 80 °C (e.g., 55-65 °C, or 60 °C), for example. Enzymatic hydrolysis can be performed for a period of at least about 10 minutes to about 90 hours, for example. In certain embodiments, such as for hydrolyzing leucrose, a hydrolysis reaction can be performed in less than 4 hours (e.g., 0.5-4 hours). The duration of the hydrolysis reaction is typically impacted by the amount of enzyme added and the amount of oligosaccharides to be hydrolyzed. A soluble fraction that has been concentrated down to a syrup can be used in a hydrolysis reaction in some aspects. One or more gtf enzymes present in a soluble fraction of glucosyltransferase reaction can optionally be deactivated (e.g., heat-deactivated) before use thereof in an enzymatic hydrolysis. The hydrolysis enzymes present in the hydrolyzed fraction are typically destroyed using thermal denaturation after the completion of hydrolysis.

An alpha-glucosidase in certain embodiments may be immobilized. The enzyme may be immobilized using any method and/or means known in the art, such as those disclosed in U.S. Pat. Nos. 5541097 and 4713333, which are incorporated herein by reference. For example, one or more enzymes can be immobilized by contacting the enzyme(s) with a solution of an amine-reactive material (e.g., glutaraldehyde) to form an adduct (e.g., enzyme-glutaraldehyde adduct), after which the adduct is bonded to a solid carrier that has been treated with a polyamine (e.g., a polyethylenimine such as EPOMIN P-1050). A solid carrier (solid support) to which an alpha-glucosidase enzyme can be immobilized in certain embodiments can be an inorganic or organic material. Such materials include, for example, gamma-alumina, titania, activated granular carbon, granular diatomaceous earth, glass beads, porous glass, pumice-stone, silica gel, metal oxide and aluminum oxide.

An increase of the monosaccharide content (dwb) (e.g., fructose plus glucose) in a soluble fraction resulting from alpha-glucosidase treatment herein can be about, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 50-80%, 50-90%, 60-80%, or 60-90% as compared to the monosaccharide content in the soluble fraction as it existed before alpha-glucosidase treatment (separated or not separated from ongoing or completed glucosyltransferase reaction). An increase of the fructose content (dwb) in a soluble fraction resulting from alpha-glucosidase treatment can be by about, at least about, or no more than about, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 30%, 40%, 50%, 10-40%, 10-50%, 20-40%, or 20-50%, for

example, as compared to the fructose content in the soluble fraction as it existed before alpha-glucosidase treatment (separated or not separated from ongoing or completed glucosyltransferase reaction).

A method of producing an aqueous composition comprising fructose in certain aspects can further comprise a process step that increases the content of monosaccharides (e.g., dwb) relative to the content of other saccharides (e.g., oligosaccharides) in a soluble fraction. Such a process step can be in addition to, or instead of, performing an enzymatic hydrolysis step as disclosed above, for example. Also, such a process step is typically performed after separating insoluble poly alpha-1,3-glucan from a soluble fraction of a glucosyltransferase reaction.

A process comprising nanofiltration can be used in some aspects of the present disclosure, and is generally suitable for increasing the monosaccharide content by removing oligosaccharides from a soluble fraction. Nanofiltration typically realizes separations on the basis of size: smaller molecules such as fructose and glucose can pass through a membrane, and larger molecules such as oligosaccharides cannot pass through the membrane. Several structural factors, including surface chemistry, porosity, and geometric configuration, can impact the performance of a nanofiltration membrane. Nanofiltration membranes in some aspects can be flat sheets or spiral-wound; the latter membrane type is preferred as it achieves a higher surface area to volume ratio in a skid. The operating conditions of the membrane can also impact nanofiltration performance. Temperature, pH, trans-membrane pressure, and the viscosity and concentration of retentate and permeate can impact membrane performance. Higher temperatures, pH, and transmembrane pressures are preferred. The exact configuration of a nanofiltration unit herein, including the membrane and operating conditions, is not critical to the present disclosure. A commercial nanofiltration setup can optionally be used, comprising either a single stage or multiple stages. A multi-stage nanofiltration system herein is typically operated by recirculating material in a given stage around the membrane and sending a slip stream of the retentate to subsequent stages. A preferred option minimizes the amount of membrane area necessary to achieve a given purification and may use multiple stages. To achieve high yields, diafiltration water can be used to dilute the retentate to maintain a lower viscosity, and may be added to some or all stages of the process. A preferred nanofiltration operation uses less diafiltration

water. Nanofiltration membranes herein are typically characterized on the basis of their flux of permeate through the membrane. Preferred membranes have a high flux of desired compounds, such as fructose and glucose. Another consideration for a nanofiltration membrane is that it sufficiently rejects unwanted materials, such as oligosaccharides, protein, and/or salts. Preferred membranes have a high rejection of unwanted materials. Several commercially available nanofiltration membranes, including OSMONICS DESAL 5 DL and OSMONICS DK produced by General Electric (New York, United State); TS40, XN45, P4 and UA60 produced by Trisep Corporation (Galeta, CA); NF 245 produced by Dow (Midland, MI); or NFX and NFW produced by Synder (Vacaville, CA), and other similar membranes are suitable for use herein.

Nanofiltration in certain embodiments can be performed on a soluble fraction that comprises about, or at least about, 20, 25, 30, 35, 40, 45, 50, 55, 60, 30-50, 30-45, 30-40, 35-50, 35-45, 35-40 wt% dry solids. The pH of a soluble fraction to be entered into a nanofiltration process herein can be about 4.0, 4.25, 4.5, 4.75, 5.0, 5.25., 5.5, 5.75, 6.0, 4.0-6.0, 4.0-5.5, 4.0-5.0, 4.0-4.75, 4.0-4.5, 4.25-5.0, 4.25-4.75, or 4.25-4.5. Nanofiltration can be performed, if desired, by continuously recirculating the sample being filtered. About 0.1, 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 0.1-2.0, 0.1-1.5, 1.25-2.0, 1.25-1.75, 1.25-1.5, 1.5-2.0, or 1.5-1.75 parts diafiltration water can be added per 1 part dry solids of the sample being filtered, for example.

An increase of the monosaccharide content (dwb) (e.g., fructose plus glucose) in a soluble fraction resulting from a process herein such as nanofiltration can be by about, or at least about, 5%, 10%, 15%, 20%, 25%, 30%, 10-30%, 10-25%, 10-20%, 10-15%, 15-30%, 15-25%, or 15-20% as compared to the monosaccharide content in the soluble fraction as it existed before conducting such process. An increase of the fructose content (dwb) in any of these embodiments can be by about, or at least about, 5%, 10%, 15%, 20%, 25%, 30%, 10-30%, 10-25%, 10-20%, 10-15%, 15-30%, 15-25%, or 15-20% as compared to the fructose content in the soluble fraction as it existed before conducting such process. A decrease in the oligosaccharide content (dwb) of a soluble fraction in any of these embodiments can be by about, or at least about, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 70-95%, 70-90%, 80-95%, 80-90%, 85-95%, 85-90%, or 90-95%.

A soluble fraction that has been treated by nanofiltration (and also enzymatic hydrolysis in some aspects) can have, for example, (i) an ICUMSA value (measured as

disclosed herein) of about 700-900, 800-900, or below about 900, and/or (ii) a conductivity of about 3200, 3000-3400, 3000-3300, 3100-3400, 3100-3300, or 3200 μS/cm or below about 3300 μS/cm. Such a soluble fraction can have about 10-30, 15-30, 20-30, 10-25, or 15-25 wt% dry solids, for example.

Other processes besides, or in addition to, nanofiltration that can optionally be used herein to increase the content of monosaccharides relative to the content of other saccharides (e.g., oligosaccharides) in a soluble fraction include, for example, crystallization (e.g., see U.S. Patent No. 4634472) and chromatography such as size-exclusion chromatography, ligand chromatography (ligand conversion or ligand-exchange chromatography such as that based on differential binding to calcium ions), partition chromatography, or anion exchange chromatography.

In some embodiments of the present disclosure, a method of producing an aqueous composition comprising fructose does not comprise a process step that increases the content of fructose relative to the content of other saccharides (e.g., oligosaccharides and/or glucose) in a soluble fraction. For instance, a method in some cases does not comprise crystallization (e.g., see U.S. Patent No. 4634472) and/or chromatography such as size-exclusion chromatography, ligand chromatography (ligand conversion or ligand-exchange chromatography), partition chromatography, or anion-exchange chromatography. In some embodiments, there is no chromatographic process for particularly removing glucose, such as ligand chromatography (e.g., ligand-exchange based on differential binding to calcium ions). Any of these embodiments may, however, optionally comprise a nanofiltration and/or hydrolysis process as disclosed.

A method of producing an aqueous composition comprising fructose in some embodiments can further comprise a polishing step in which ions and/or color are removed from a soluble fraction herein. Such polishing can be performed immediately following preparation of an insoluble fraction from a glucosyltransferase reaction, and/or after its treatment to one or more of the above-disclosed processes (e.g., hydrolysis alone, nanofiltration alone, hydrolysis and nanofiltration). The dry solids content of an aqueous composition herein can optionally be adjusted before polishing; preferred dry solids contents may be 30-70 wt% DS.

Polishing can be achieved, for example, using ion exchange chromatography (typically for ion removal) and/or carbon treatment (typically for color removal) in some certain aspects. For example, a polishing process can employ a series of columns containing either strong acid cation (SAC) ion exchange resin (e.g., IMAC C16 P), weak base anion (WBA) ion exchange resin (e.g., AMBERLITE IRA 92, Dow, Midland, MI), strong base anion (SBA) ion exchange resin (e.g. DOWEX 22), and/or activated carbon (AC) (CHEMVIRON CPG, Calgon Carbon Corporation). The columns can contain one or more of these resins, including combinations of strong acid cation exchange resin and one of either weak base anion or strong base anion resins in the same configuration. Such a mixture is referred to in the art as a mixed bed resin. An aqueous composition herein may contact the ion exchange units one or multiple times in one or multiple passes. A preferred configuration removes both cations and anions, including organic acids, from the aqueous composition. Such columns can be configured in series such as SAC→WBA→SAC→WBA→AC. The exact configuration of columns, choice of cation and/or anion exchange resins, and choice of AC are not critical to the present disclosure, and other configurations of columns are feasible herein, including configurations that may use strong base anion ion exchange resins or mixed bed resins. Numerous commercially available ion exchange resins, such as those sold by Dow, Purolite, or others are also suitable. Use of monodisperse particles may be advantageous for reduced fouling in some aspects. Other parameters, such as contact time and temperature, may be adjusted to achieve target specifications. Typical operating conditions for ion exchange resins herein are 2-6 bed volumes (BV) per hour at concentrations from 10-80 wt% (e.g., ~50 wt%) dry solids (DS), where a bed volume is defined as the total volume of settled resin in a column, which includes both the resin and the interstitial space. The temperature can be about 20 °C to about 80 °C (e.g., 55-65 °C, or 60 °C), for example. Faster flow rates, lower temperatures, and lower concentrations may be advantageous to reduce epimerization of fructose to glucose, or production of disaccharides. Once an ion exchange resin is partially or completely saturated, the ability of the resin to remove further ions is diminished. The column may then be regenerated through the use of either acid or base, and consequently reused. Activated carbon columns may be regenerated but are more typically disposed or sent back to the vendor for purification. Regeneration procedures for these adsorbents are well known by those of ordinary skill in the art.

The amount of ions and/or color removed from a soluble fraction by applying a polishing treatment herein can be gauged, for example, by monitoring conductivity and/or ICUMSA, respectively, of the soluble fraction resulting from polishing. In some aspects, a soluble fraction that has been polished can have (i) an ICUMSA value (measured as disclosed herein) of about 1-25 or 1-20, or less than 25 or 20, and/or (ii) a conductivity of about 2.5-25, 2.5-20, 2.5-15, 5-25, 5-20, 5-15, 7.5-25, 7.5-20, or 7.5-15 μS/cm, or less than about 25 or 20 μS/cm. Such a soluble fraction can have about 5-15 wt% (e.g., ~10 wt%) dry solids, for example. In some aspects, a soluble fraction herein comprising about 20-40 wt% dry solids (e.g., ~30 wt% DS) can have (i) an ICUMSA value (measured as disclosed herein) of about 30-60, or less than about 50 or 40, and/or (ii) a conductivity less than about 50, 40, or 30 μS/cm. Conductivity herein can be measured at any temperature between about 20-80 °C or 50-70 °C (e.g., ~60 °C), and/or a pH of about 3, 4, 5, 6, 7, 8, or 9, for example. Such a soluble fraction herein can constitute an ingestible product in some embodiments.

An aqueous composition produced by any process disclosed herein, or a related process, is yet another aspect of the present disclosure. Any soluble fraction as presently disclosed can constitute such an aqueous composition, for example. Alternatively, an aqueous composition optionally can replicate a soluble fraction as now presently disclosed, but be produced in a different manner (e.g., components of a soluble fraction as presently disclosed can be brought together, such as in providing a formulation). The following embodiments represent examples of an aqueous composition.

An aqueous composition in some aspects can comprise: (i) at least about 55% fructose on a dry weight basis, (ii) about 3% to about 24% glucose on a dry weight basis, and (iii) soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15, wherein said oligosaccharides comprise glucose and/or fructose. Certain embodiments of such an aqueous composition can comprise any other fructose content disclosed herein for a soluble fraction, such as at least about 75% fructose on a dry weight basis. Still additional embodiments can comprise any fructose, glucose and/or soluble oligosaccharide (DP2-15) content disclosed herein for a soluble fraction.

Soluble oligosaccharides of an aqueous composition can be, for example, selected from the group consisting of sucrose, leucrose, trehalulose, isomaltulose, maltulose, isomaltose, and nigerose. Soluble oligosaccharides in some embodiments can comprise (i) at least about 90 wt% glucose, and (ii) about 60-99% alpha-1,3 and about 1-40% alpha-1,6 glucosidic linkages. An aqueous composition can comprise less than about 30% of soluble oligosaccharides on a dry weight basis in some aspects.

An aqueous composition in some embodiments can (i) be comprised within, or be, an ingestible product, and/or (ii) have a conductivity less than about 50 μS/cm at 30% dry solids and an ICUMSA value less than about 50. In other aspects, an aqueous composition can have any conductivity level and/or ICUMSA level as disclosed elsewhere herein. An aqueous composition in still further aspects can be comprised within, or be, an ingestible product, regardless of its conductivity and/or ICUMSA levels.

An aqueous composition herein can be comprised within, or be, a consumer product such as an ingestible product or non-ingestible product. Examples of an ingestible product are foods/beverages and pharmaceutical products. Examples of non-ingestible products include cosmetics. In certain aspects, an aqueous composition herein can be used as a partial or complete substitute for high fructose corn syrup (HFCS) in a consumer product that typically incorporate HFCS. An aqueous composition herein can be used as a sweetener and/or preservative in an ingestible product or consumer product, for example.

An aqueous composition herein can be utilized in a food, beverage, animal feed, an animal health and nutrition product, pharmaceutical product, and/or cosmetic product, for example. The intended use of an aqueous composition herein in foods and feeds can be to soften texture, add volume, thicken, prevent crystallization of sugar, and/or enhance flavor or sweetness, for example.

Further examples of using an aqueous composition of the present disclosure include its use as: a bulking, binding and/or coating ingredient; a carrier for coloring agents, flavors/fragrances, and/or high intensity sweeteners; a spray drying adjunct; a bulking, bodying and/or dispersing agent; and an ingredient for promoting moisture retention (humectant). Illustrative examples of products that can be prepared using an aqueous composition disclosed herein include food products, beverage products, pharmaceutical products, nutritional products, sports products and cosmetic products.

Particular examples of beverage products that can comprise an aqueous composition herein include beverage products such as concentrated beverage mixes, carbonated beverages, non-carbonated beverages, fruit-flavored beverages, fruit juices, teas, coffee, milk nectars, powdered soft drinks, liquid concentrates, milk drinks, smoothies, alcoholic beverages, flavored waters and combinations thereof. Particular examples of food products that can comprise an aqueous composition herein include baked goods (e.g., breads), confectioneries, frozen dairy products, meats, cereal products (e.g., breakfast cereals), dairy products (e.g., yogurt), condiments, snack bars, soups, dressings, mixes, prepared foods, baby foods, diet preparations, peanut butter, syrups, sweeteners, food coatings, pet food, animal feed, animal health and nutrition products, dried fruit, sauces, gravies, jams/jellies, dessert products, spreads, batters, breadings, spice mixes, frostings and the like.

An aqueous composition as presently disclosed can be used directly in a consumer product without modification. Alternatively, it can be diluted or concentrated accordingly, depending on how it is to be used in preparing a particular consumer product.

Additional examples of consumer products, and formulations thereof, in which an aqueous composition herein can be useful for preparing are disclosed in U.S. Patent. Appl. Publ. Nos. 2015/0257422, 2015/0282513, 2015/0313265 and 2015/0216219, which are all incorporated herein by reference.

Non-limiting examples of compositions and methods disclosed herein include:

1. A method for producing an aqueous composition comprising fructose, the method comprising:

(a) contacting water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan having at least 30% alpha-1,3-linkages, to produce a soluble fraction and an insoluble fraction, wherein the insoluble fraction comprises the poly alpha-1,3-glucan, and wherein the soluble fraction comprises at least about 55% fructose on a dry weight basis, and

(b) separating the soluble fraction from the insoluble fraction, thereby providing an aqueous composition comprising fructose.

2. The method of embodiment 1, wherein the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having at least 95% alpha-1,3-linkages.

3. The method of embodiment 1 or 2, wherein the soluble fraction further comprises soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15.

4. The method of embodiment 3, wherein the soluble fraction comprises less than about 30% of the soluble oligosaccharides on a dry weight basis.

5. The method of embodiment 3 or 4, further comprising contacting the soluble fraction with an alpha-glucosidase enzyme to hydrolyze at least one glycosidic linkage of the oligosaccharides, thereby increasing the monosaccharide content in the soluble fraction (such contacting may be with the soluble fraction of step [a] and/or step [b]).

6. The method of embodiment 1, 2, 3, 4, or 5, wherein the soluble fraction comprises at least about 75% fructose on a dry weight basis.

7. The method of embodiment 1, 2, 3, 4, 5, or 6, wherein the method further comprises a process step that increases the content of monosaccharides relative to the content of other saccharides in the soluble fraction, wherein the process step optionally is nanofiltration or enzymatic hydrolysis.

8. The method of embodiment 1, 2, 3, 4, 5, or 6, wherein the method does not comprise chromatography as a process step to increase the content of fructose relative to the content of other saccharides in the soluble fraction

9. An aqueous composition produced by a method according to embodiment 1, 2, 3, 4, 5, 6, 7, or 8.

10. The aqueous composition of embodiment 9, wherein the aqueous composition is comprised within, or is, an ingestible product, and optionally is used as a sweetener of the ingestible product.

11. An aqueous composition comprising:

(i) at least about 55% fructose on a dry weight basis,

(ii) about 3% to about 24% glucose on a dry weight basis, and

(iii) soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15, wherein the oligosaccharides comprise glucose and/or fructose.

12. The aqueous composition of embodiment 11, wherein the soluble oligosaccharides are selected from the group consisting of sucrose, leucrose, trehalulose, isomaltulose, maltulose, isomaltose, and nigerose.

13. The aqueous composition of embodiment 11 or 12, wherein the oligosaccharides comprise (i) at least about 90 wt% glucose, and (ii) about 60-99% alpha-1,3 and about 1-40% alpha-1,6 glucosidic linkages.

14. The aqueous composition of embodiment 11, 12, or 13, wherein the aqueous composition comprises at least about 75% fructose on a dry weight basis.

15. The aqueous composition of embodiment 11, 12, 13, or 14, wherein the aqueous composition comprises less than about 30% of the soluble oligosaccharides on a dry weight basis.

16. The aqueous composition of embodiment 11, 12, 13, 14, or 15, wherein the aqueous composition is comprised within, or is, an ingestible product, and optionally is used as a sweetener of the ingestible product.

17. The aqueous composition of embodiment 16, wherein the aqueous composition has a conductivity less than about 50 μS/cm at about 30 wt% dry solids and an ICUMSA value less than about 50.

18. An ingestible product comprising the aqueous composition of any of claims 11, 12, 13, 14, 15, 16, or 17, wherein the ingestible product is a food, beverage, animal feed, human or animal nutritional product, pharmaceutical product, or oral care product.

EXAMPLES

The present disclosure is further exemplified in Examples 2-6 below. It should be understood that these Examples, while indicating certain preferred aspects herein, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of the disclosed embodiments, and without departing from the spirit and scope thereof, can make various changes and modifications to adapt the disclosed embodiments to various uses and conditions.

Abbreviations

The meaning of some of the abbreviations used herein is as follows: “g” means gram(s), “h” means hour(s), “mL” means milliliter(s), “psi” means pound(s) per square inch, “wt%” means weight percentage, “μm” means micrometer(s), “°C” means degrees Celsius, “mg” means milligram(s), “mm” means millimeter(s), “m” means meter(s), “μL” means microliter(s), “mmol” means millimole(s), “min” means minute(s), “mol%” means mole percent, “M” means molar, “mg/g” means milligram per gram, “rpm” means revolutions per minute, “MPa” means megaPascals, “BV” means bed volume, “lbm” means pound-mass, “psia” means pounds per square inch absolute.

GENERAL METHODS

Analysis of Carbohydrate Composition

An Aminex® HPX-42A column (BioRad, Hercules, CA) having deionized water at a flow rate of 0.6 mL/min and a temperature of 85 °C was used to quantitate soluble oligosaccharide byproducts (DP2-DP7). An Aminex® HPX-87C column (BioRad) having deionized water at a flow rate of 0.6 mL/min and a temperature of 85 °C was used to quantitate the level of sucrose, glucose, leucrose and fructose in the soluble reaction products. Use of 2x Aminex® HPX-87C columns in series at the same flow rate and temperature was performed to quantitate the level of all carbohydrates on de-ashed, purified fructose products.

ICUMSA Color Analysis

ICUMSA is a measure of the color of sugar solutions/syrups. This parameter can be measured according to the following protocol using a spectrophotometer. The method used herein for measuring ICUMSA (Table 2) is somewhat iterative, but for very low color materials, it is ideal to measure color using long cells.

Table 2

Protocol to Measure ICUMSA of a Syrup

Step	Description
1	While agitating with PTFE stir blade, dilute 3.0 g fructose syrup (soluble fraction) with 7.0 g DI water.
2	Adjust pH to 7.0 using 0.1 M NaOH or 0.1 M HCl.
3	Filter solution through 0.45-micron filter.
4	Measure Brix (g solid/100 g) using a SPER SCIENTIFIC digital refractometer.
5	Measure density (g/mL) using densitometer.
6	Calculate concentration *c* (g solid/mL) as Brix*density/100.
7	Measure absorbance *As* at 420 nm using indicated pathlength *b* in cm.
8	Calculate ICUMSA as 1000**As*/(*b***c*).

Conductivity

Conductivity measurements were made using a commercially available conductivity meter (VWR, Radnor, PA). Fructose syrups were diluted to 10 wt% dry solids (DS) with deionized water before conductivity measurements were made.

EXAMPLE 1 (Comparative)

Production of Fructose Syrup Using GTFJ Enzyme

This example discloses the composition of a fructose syrup produced in a poly alpha-1,3-glucan synthesis reaction catalyzed by a GTFJ enzyme (SEQ ID NO:6), and its subsequent composition following leucrose hydrolysis.

GTFJ synthesizes fructose and poly alpha-1,3-glucan coproducts, as well as glucose and oligosaccharide (e.g., DP2-7) byproducts. The poly alpha-1,3-glucan product comprises about 100% alpha-1,3 linkages.

White crystalline sucrose (3000 g) was added to a clean 5-gallon polyethylene bucket. Water (18.1 L) and Fermasure™ (10 mL) were added to the bucket, and the pH was adjusted to 7.0 by addition of 5 vol% NaOH and/or 5 vol% H_2SO_4. The final volume was ~20 L and the initial concentration of sucrose as measured by HPLC was 155.3 g/L. A poly alpha-1,3-glucan synthesis reaction was initiated by adding crude gtfJ enzyme (SEQ ID NO:6) extract (prepared as described in the General Methods section) to a concentration of 0.3 vol%. Agitation of the reaction was provided using an overhead mechanical motor equipped with a glass shaft and PTFE blade.

HPLC analysis of the glucan synthesis reaction that had commenced for 65 hours revealed that the sucrose concentration in the soluble fraction was 6.3 g/L; the reaction was deemed to be complete. The soluble fraction of the finished reaction was then filtered away from the insoluble poly alpha-1,3-glucan product, thereby providing a filtrate. The GTF enzyme was deactivated by heating the filtrate to 60 °C for 30 minutes. The filtrate was then treated with TG L-2000 transglucosidase enzyme (1 vol%, DuPont, Wilmington, DE) at 60 °C for 48 hours under gentle agitation to hydrolyze leucrose. The hydrolyzed filtrate was then concentrated to a total concentration of 870 g/L dry solids using a rotary evaporator, thereby providing a syrup. Table 3 provides the sugar content (dwb) of the filtrate before and after hydrolysis. While hydrolysis of leucrose in the filtrate was successful, the fructose content of the resulting syrup was lower than predicted apparently due to competitive trans-glucosylation and trans-fructosylation reactions occurring during the transglucosidase treatment, whereby the concentration of the oligosaccharides increased (Table 3). The oligosaccharide content comprised primarily DP2-7, though some oligosaccharides up to DP8 or DP9 may have been present.

Table 3

Carbohydrate Composition of Filtrates (wt% – Dry Weight Basis)

Material	Fructose	Glucose	Leucrose	Other DP2	DP3+	Total
Filtrate (not hydrolyzed)	35.1	7.5	38.6	10.0	8.8	100
Hydrolyzed Filtrate (Actual)	47.0	33.7	1.1	8.7	9.3	100
Predicted composition of Hydrolyzed Filtrate	54.4	27.8	2.0	8.8	7.0	100

The relatively low content of fructose and high content of oligosaccharides in the hydrolyzed filtrate generally renders this syrup unsuitable for use in large-scale applications, such as in the sweetener industry. Furthermore, the final concentration of fructose that was obtained in the glucan synthesis reaction using GTFJ followed by leucrose hydrolysis using TG L-2000 is not higher than the fructose content that can be obtained by inverting sucrose, which can yield a fructose composition of up to 50 wt% (dwb) without further processing.

Thus, alternative methods of using a glucan synthesis reaction to produce fructose syrup were pursued, and are disclosed below.

EXAMPLE 2

Production of Fructose Syrup Using an Improved GTF Enzyme

This example discloses the composition of a fructose syrup produced in a poly alpha-1,3-glucan synthesis reaction catalyzed by an improved GTF, and its subsequent composition following leucrose hydrolysis.

The amino acid sequence of an *S. salivarius* GTF enzyme that produces poly alpha-1,3-glucan with about 100% alpha-1,3 linkages was modified in its catalytic domain such that the enzyme could produce more products (poly alpha-1,3-glucan and fructose), and less byproducts (e.g., glucose, oligosaccharides such as leucrose), from sucrose substrate, as compared to the enzyme's unmodified counterpart.

Poly alpha-1,3-glucan synthesis reactions using the improved GTF were run in a 5000-gal stainless steel vessel comprising 94 g/L white crystalline sucrose dissolved in water. The pH of the reaction was maintained using 10 mM potassium phosphate as a buffer and adjusted to 5.5 using 2 N H_2SO_4. An antimicrobial, FermaSure® XL, was added at 100 ppmv to prevent contamination during the reaction. The reactor contained

three pitched blade impellers set to 33 rpm and was controlled at 23 °C using cooling water flowing into the jacket of the reactor. The reaction was initiated by adding 30 pounds of the improved GTF enzyme, and deemed complete after 14 hours at which time the sucrose concentration reached less than 2 g/L. At the end of the reaction, the GTF enzyme was deactivated by heating the reaction contents to 65 °C for 30 minutes using an external heat exchanger. This process was repeated in a second reaction ("2.2") comprising a portion of the soluble fraction (490 gallons) produced in the first reaction ("2.1"). The initial contents of reactions 2.1 and 2.2 are summarized in Table 4.

Table 4

Polymerization conditions for production of fructose syrup

Rxn	Water Volume (gal)	Buffer Mass (lbm)	FermaSure® Mass (lbm)	Sucrose Mass (lbm)	Enzyme Mass (lbm)	Soluble Fraction from Rxn 2.1 (gal)
2.1	4751	57.4	4.2	3962	30	N/A
2.2	4261	57.4	4.2	3962	30	490

The insoluble poly alpha-1,3-glucan polymer (i.e., insoluble fraction) produced in each reaction was separated from each respective soluble fraction using a filter press, thereby providing a filtrate. Though a filter press was employed to provide a soluble fraction separated from an insoluble fraction, any number of separation processes known in the art could have been used for this purpose. Table 5 provides the sugar content (dwb) of each filtrate.

Table 5

Carbohydrate Composition of Reaction 2.1 and 2.2 Filtrates (wt% – Dry Weight Basis)

Rxn	Fructose	Glucose	Sucrose	Leucrose	Other Oligomers	Total
2.1	70.3	7.2	2.0	13.6	6.9	100
2.2	64.6	7.3	2.5	17.7	7.8	100

It is apparent from Table 5 that the improved GTF could produce at least about 65-70% fructose on a dry weight basis of the soluble products of the enzyme.

For purposes of leucrose hydrolysis, the remaining filtrate of reaction 2.1 was combined with the filtrate of reaction 2.2 to provide a total of 4740 gal of dilute fructose syrup. The combined dilute syrup (comprising about 67.5 wt% fructose dwb) was treated with sodium thiosulfate for 30 min and adjusted to pH 4.4 and 55 °C. TG L-2000

transglucosidase enzyme was added to initiate a hydrolysis reaction, which was agitated for 8.5 hours. Table 6 provides the sugar content (dwb) of the hydrolyzed syrup. Its oligosaccharide content comprised primarily DP2-7, though some oligosaccharides up to DP8 or DP9 may have also been present.

Table 6

Carbohydrate Composition of Hydrolyzed Syrup (wt% – Dry Weight Basis)

Fructose	Glucose	Leucrose	Other DP2[a]	DP3+	Total
70.7	15.7	<1.0	10.2	3.3	100

[a] Includes at least sucrose.

Table 6 indicates that, while hydrolysis of leucrose in the filtrate was successful, the fructose content of the resulting syrup was not substantially higher than the pre-hydrolysis fructose level (Table 5). This result may have been due to competitive trans-glucosylation and trans-fructosylation reactions occurring during the transglucosidase treatment, whereby the concentration of non-leucrose oligosaccharides increased.

The hydrolyzed fructose syrup was then concentrated to 70 wt% dry solids (DS) using a plate-and-frame forced circulation evaporator operated with an outlet temperature of 143 °F and a pressure of 1.5-1.6 psia. The concentrated fructose syrup had an ICUMSA color of 2240 and a conductivity of 2200 μS/cm at 10 wt% DS; it was apparent that the evaporation process resulted in color formation in the syrup.

Thus, a syrup comprising at least about 70 wt% fructose on a dry weight basis was prepared. This fructose content (dwb) is sufficiently high for the syrup to be used as a sweetener; however, the amount of oligosaccharides is still quite high. Additionally, the conductivity and color of this material are too high for this syrup to be generally useful in large-scale sweetener applications. Nevertheless, such fructose syrup will lend itself useful to various other downstream applications. Such a feedstock would be suitable for use in fermentation feeds (e.g., to produce ethanol or other fermentation products), animal feeds, or in specialty sweetener applications.

EXAMPLE 3

Removal of Oligosaccharides from Fructose Syrup by Nanofiltration

This example discloses removing oligosaccharides from the fructose syrup produced in Example 2. This step increased the content of monosaccharides (fructose

and glucose) in the syrup relative to other saccharides, and in so doing raised the percent fructose (dwb) of the syrup.

The fructose syrup prepared in Example 2 (sugar content provided in Table 6) was diluted to 41 wt% DS and adjusted to pH 4.45, thereby providing a feed for nanofiltration. The feed was introduced to a nanofiltration system at 65 °C and constantly recirculated until a total fructose yield of 91% was achieved. The unit was equipped with two 4-inch spiral wound modules in parallel. One module contained an OSMONICS DK (General Electric, New York, United States) spiral wound membrane unit containing 6.2 m^2 surface area, and the other module contained a TS40 (Trisep Corporation, Galeta, CA) spiral wound membrane unit containing 7.2 m^2 surface area. Permeate from the two membranes was combined; the mass balance for the experiment is provided in Table 7. The feed concentration was maintained to the initial concentration by the addition of diafiltration (DF) water. A total of 1.66 kg DF water was added per kg DS in the feed. Two permeate fractions, F1 and F2, were collected. The first fraction, F1, was collected from 0-75% yield and the second fraction, F2, was collected from 75-91% yield, where the yield is defined as kg fructose in the permeate divided by kg fructose in the feed. Both permeates F1 and F2 constituted enriched fructose streams comprising, respectively, 79 and 76 wt% of the dry matter as fructose (Table 7).

Table 7

Nanofiltration Mass Balance

Fraction	wt% DS	kg DS	Fructose Purity (%)
Permeate F1	23	66	79
Permeate F2	19	11	77
Concentrate	36	25	26
Feed	41	102	68

The detailed sugar composition of permeate F1 is provided in Table 8. The concentration (dwb) of fructose in F2 was lower than in F1 due to breakthrough of disaccharides. A preferred membrane herein has low breakthrough of disaccharides at even very high yields of fructose, such as 95% or even 98%. Overall, however, it is apparent from comparing Table 8 with Table 6 that removal of oligosaccharides via nanofiltration is useful for further increasing the content (dwb) of fructose in a syrup. (It

should also be apparent that this process would be able to increase the fructose content of a syrup that was not hydrolyzed per Example 2.)

Table 8

Carbohydrate Composition of Nanofiltration Permeate F1 (wt% – Dry Weight Basis)

Fructose	Glucose	DP2[a]	DP3+	Total
79.0	19.4	1.6	<0.1	100

[a] Includes at least sucrose and leucrose.

The ICUMSA color of permeate F1 was 870, and a corresponding increase in color of the retentate was observed. The conductivity of permeate F1 was 3400 μS/cm, indicating that many of the ions present in the syrup were not rejected during the nanofiltration process.

Thus, a syrup comprising at least about 79 wt% fructose on a dry weight basis was prepared. The use of nanofiltration removed nearly all of the DP3+ oligosaccharides as well as the majority of the DP2 oligosaccharides while simultaneously increasing the fructose content (dwb) in the syrup. While a substantial amount of color was removed from the syrup (compare to ICUMSA >2000 observed in Example 2), its high conductivity and color are generally unsuitable for use in large scale sweetener applications. Again, however, such fructose syrup will lend itself useful to various other downstream applications.

EXAMPLE 4

Polishing of Fructose Syrup

This example discloses the removal of ions and color by treatment of a fructose syrup with adsorption technology. In particular, the fructose syrups prepared in Examples 2 and 3 were subjected to ion exchange chromatography and activated carbon treatment to decrease the conductivity and ICUMSA of each syrup, while preserving their high fructose content.

Columns containing either strong acid cation (SAC) ion exchange resin (IMAC C16 P), weak base anion (WBA) ion exchange resin (AMBERLITE IRA 92, Dow, Midland, MI), or activated carbon (AC) (CHEMVIRON CPG, Calgon Carbon Corporation) were provided accordingly. The columns were configured in series as 1) SAC, 2) WBA, 3) SAC, 4) WBA, and 5) AC. The fructose syrup described in Table 8 was adjusted to a concentration of 52 wt% DS and heated to 60 °C. The syrup was then fed to the column

series at 2 BV/hr. The conductivity of the syrup over 7 hours of running it through the columns was reduced to between 7.4 and 19 μS/cm, and its ICUMSA color was measured between 1 and 20. The fructose syrup described in Table 6 (adjusted to 10 wt% DS) was polished in a similar manner, yielding a syrup with an ICUMSA color less than 10 and conductivity of 5.5 μS/cm.

Thus, fructose syrup disclosed herein can be polished to remove ample amounts of ions and color therefrom. The low color and low conductivity, coupled with high fructose content, of this syrup render it as being particularly suitable for use in large scale sweetener applications.

EXAMPLE 5

Characterization of Oligosaccharides in Fructose Syrup

This example describes the DP3+ oligosaccharides present in fructose syrup prepared in a glucan synthesis reaction.

Fructose syrup was produced and concentrated using the methodology described in Example 2, with the exception that treatment with TG L-2000 transglucosidase was omitted. The composition of this syrup is provided in Table 9. The levels of each sugar component are as expected, being in line with the results shown in Table 5 (Example 2).

Table 9

Carbohydrate Composition of Fructose Syrup (wt% – Dry Weight Basis)

Fructose	Glucose	Leucrose	Other DP2[a]	DP3+	Total
68.8	7.6	13.1	3.5	7.0	100

[a] Includes at least sucrose.

Chromatographic separation employing a strong acid cation-exchange resin was used to isolate the oligosaccharide fraction of the syrup. The physical parameters of the column used for this separation appear in Table 10.

Table 10

Physical Parameters of the Column Used for Chromatographic Separation

Resin Type	FINEX CS11GC, #296
Ion form	Na^+
Crosslinking, % divinyl benzene	5.5%
Particle size (mm)	0.35
Bed length (m)	5.2
Column diameter (m)	0.225

The concentrated syrup was filtered and diluted to 30 g dry solids/100 g solution using ion exchanged tap water. Prior to addition of the diluted syrup to the column resin, the resin was washed with six bed volumes (BV) of sodium chloride solution (three BV at 10 wt% sodium chloride followed by three BV at 5 wt% sodium chloride) to convert the resin to the sodium form. The sugar solution (15 L) was then fed to the column, after which the column was eluted using ion exchanged water at a flow rate of 30 L/h. The run conditions of the chromatographic separation are summarized in Table 11.

Table 11

Chromatographic Separation Run Conditions

Feed size (L)	15
Feed dry solids (g/100 g)	30
Column temp (°C)	70
Flow rate (L/hr)	30

An oligosaccharide solution eluted between 140 and 185 minutes and was recovered. The oligosaccharide fraction thus prepared was analyzed by HPLC to determine its product distribution. Briefly, the composition of the oligosaccharide fraction was measured using an Agilent 1260 HPLC equipped with a refractive index detector. Separation was realized using a BioRad AMINEX HPX-42A column using water as an eluent at 85 °C and a flow rate of 0.6 mL/min. The composition of the oligosaccharides appears in Table 12.

Table 12

Composition of Oligosaccharides Recovered by Fractionation (wt% – Dry Weight Basis)

DP2	DP3	DP4	DP5	DP6	DP7+
11	23	28	21	12	5

The oligosaccharide fraction described in Table 12 was subjected to partially methylated alditol acetate (PMAA) analysis (following methodology in Pettolino et al., *Nature Protocols* 7:1590-1607) and analyzed by GC-MS. Briefly, the sample was treated with DMSO anion and iodomethane to methylate hydroxyl groups, and then hydrolyzed with trifluoroacetic acid. The hydroxyl groups resulting from the broken glycosidic linkages were then acetylated with acetic anhydride, and the resulting glucitols were analyzed by GC/MS. The oligosaccharides were found to have the distribution described in Table 13 (all linkages therein believed to be alpha). The dominant linkage was alpha-1,3. No terminal fructose was detected in this oligosaccharide fraction.

Table 13

Linkage Distribution of Oligosaccharides

Linkage	Linkage %
1→3	87.5
1→6	7.3
1→3,6	2.8
1→4	1.0
1→2,3	0.7
1→2	0.6
1→3,4	0.3

Thus, DP3+ oligosaccharides present in fructose syrup prepared in a glucan synthesis reaction herein were characterized.

EXAMPLE 6

Characterization of Disaccharides Present in Fructose Syrup

This example discloses the nature of the disaccharides present in fructose syrups prepared herein. It was found that fructose syrup can comprise at least the disaccharides sucrose, maltulose, leucrose, trehalulose, isomaltulose, and isomaltose. While not directly detected in the present composition, the high level of alpha 1→3 linkages in the oligosaccharides suggests that nigerose could be present in aqueous compositions produced using the techniques described in the present disclosure.

The purified fructose syrups described in Tables 6 (hydrolyzed syrup) and 8 (hydrolyzed and nanofiltrated syrup) were characterized extensively to identify the oligosaccharides present therein. Specifically, disaccharides were identified in syrup by two different methods: GC-MS and HPAEC-PAD-MS (High Performance Anion

Exchange Chromatography-Pulsed Amperometric Detection and Mass Spectrometric detection).

The materials were derivatized by oximation and silylation before being analyzed by GC-MS (*J. Agric. Food Chem.*, 19:551-554). Briefly, each syrup was dried down and the material of each was treated with hydroxylamine hydrochloride in pyridine, and then with hexamethyldisilazane and trifluoroacetic acid to complete the reaction. Separation was done on a 14% cyanopropyl-phenyl 86% dimethyl polysiloxane column with a mass-selective detector operating in full scan mode. FIG. 1 shows a distribution of certain disaccharides (sucrose, maltulose, leucrose, turanose, trehalulose, isomaltulose, isomaltose) present in the fructose syrup of Table 6, as detected by GC-MS.

Disaccharides were also identified by HPAEC-PAD-MS using a PA20 column with sodium hydroxide gradient, or LC-MS with separation on two in-series HPX-87C columns. Disaccharides such as sucrose, maltulose, leucrose, trehalulose, isomaltulose, and isomaltose were identified in the fructose syrup of Table 6 by matching the retention time and mass spectra (product ion spectra for HPAEC-PAD-MS and electron ionization [EI] spectra for GC-MS) of a disaccharide authentic standard with those of the disaccharide peaks detected in the material separated on the PA20 column (FIG. 2). Some disaccharide peaks could not be matched to any of the commercially available disaccharide standards composed of glucose or glucose and fructose. These unmatched peaks were postulated to be fructan disaccharides that are present, for example, in honey. Although the exact structure and linkage of these DP2 fructans were not established, the disaccharide fructans present in the fructose syrup were identical to those observed in alfalfa honey (data not shown) and a commercial sample of ISOCLEAR 42 HFCS obtained from Cargill (Wayzata, MN) (data not shown). The identity of the fructans present in the fructose syrup was established by comparing the product ion spectra and retention times of fructan disaccharides versus those of the alfalfa honey or ISOCLEAR 42 HFCS samples.

Thus, the disaccharide content of fructose syrup prepared in a glucan synthesis reaction herein was characterized.

CLAIMS

What is claimed is:

1. A method for producing an aqueous composition comprising fructose, said method comprising:

(a) contacting water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan having at least 30% alpha-1,3-linkages to produce a soluble fraction and an insoluble fraction,
wherein the insoluble fraction comprises said poly alpha-1,3-glucan, and
wherein the soluble fraction comprises at least about 55% fructose on a dry weight basis, and

(b) separating the soluble fraction from the insoluble fraction, thereby providing an aqueous composition comprising fructose.

2. The method of claim 1, wherein the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having at least 95% alpha-1,3-linkages.

3. The method of claim 1, wherein the soluble fraction further comprises soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15.

4. The method of claim 3, wherein the soluble fraction comprises less than about 30% of said soluble oligosaccharides on a dry weight basis.

5. The method of claim 3, further comprising contacting the soluble fraction with an alpha-glucosidase enzyme to hydrolyze at least one glycosidic linkage of said oligosaccharides, thereby increasing the monosaccharide content in the soluble fraction.

6. The method of claim 1, wherein the soluble fraction comprises at least about 75% fructose on a dry weight basis.

7. The method of claim 1, wherein the method further comprises a process step that increases the content of monosaccharides relative to the content of other

saccharides in the soluble fraction, wherein the process step optionally is nanofiltration or enzymatic hydrolysis.

8. The method of claim 1, wherein the method does not comprise chromatography as a process step to increase the content of fructose relative to the content of other saccharides in the soluble fraction

9. An aqueous composition produced by a method according to claim 1.

10. The aqueous composition of claim 9, wherein the aqueous composition is comprised within, or is, an ingestible product, and optionally is used as a sweetener of the ingestible product.

11. An aqueous composition comprising:
(i) at least about 55% fructose on a dry weight basis,
(ii) about 3% to about 24% glucose on a dry weight basis, and
(iii) soluble oligosaccharides with a degree of polymerization (DP) of 2 to about 15, wherein said oligosaccharides comprise glucose and/or fructose.

12. The aqueous composition of claim 11, wherein the soluble oligosaccharides are selected from the group consisting of sucrose, leucrose, trehalulose, isomaltulose, maltulose, isomaltose, and nigerose.

13. The aqueous composition of claim 11, wherein said oligosaccharides comprise (i) at least about 90 wt% glucose, and (ii) about 60-99% alpha-1,3 and about 1-40% alpha-1,6 glucosidic linkages.

14. The aqueous composition of claim 11, wherein the aqueous composition comprises at least about 75% fructose on a dry weight basis.

15. The aqueous composition of claim 11, wherein the aqueous composition comprises less than about 30% of said soluble oligosaccharides on a dry weight basis.

16. The aqueous composition of claim 11, wherein the aqueous composition is comprised within, or is, an ingestible product, and optionally is used as a sweetener of the ingestible product.

17. The aqueous composition of claim 16, wherein the aqueous composition has a conductivity less than about 50 μS/cm at about 30 wt% dry solids and an ICUMSA value less than about 50.

18. An ingestible product comprising the aqueous composition of claim 11, wherein the ingestible product is a food, beverage, animal feed, human or animal nutritional product, pharmaceutical product, or oral care product.

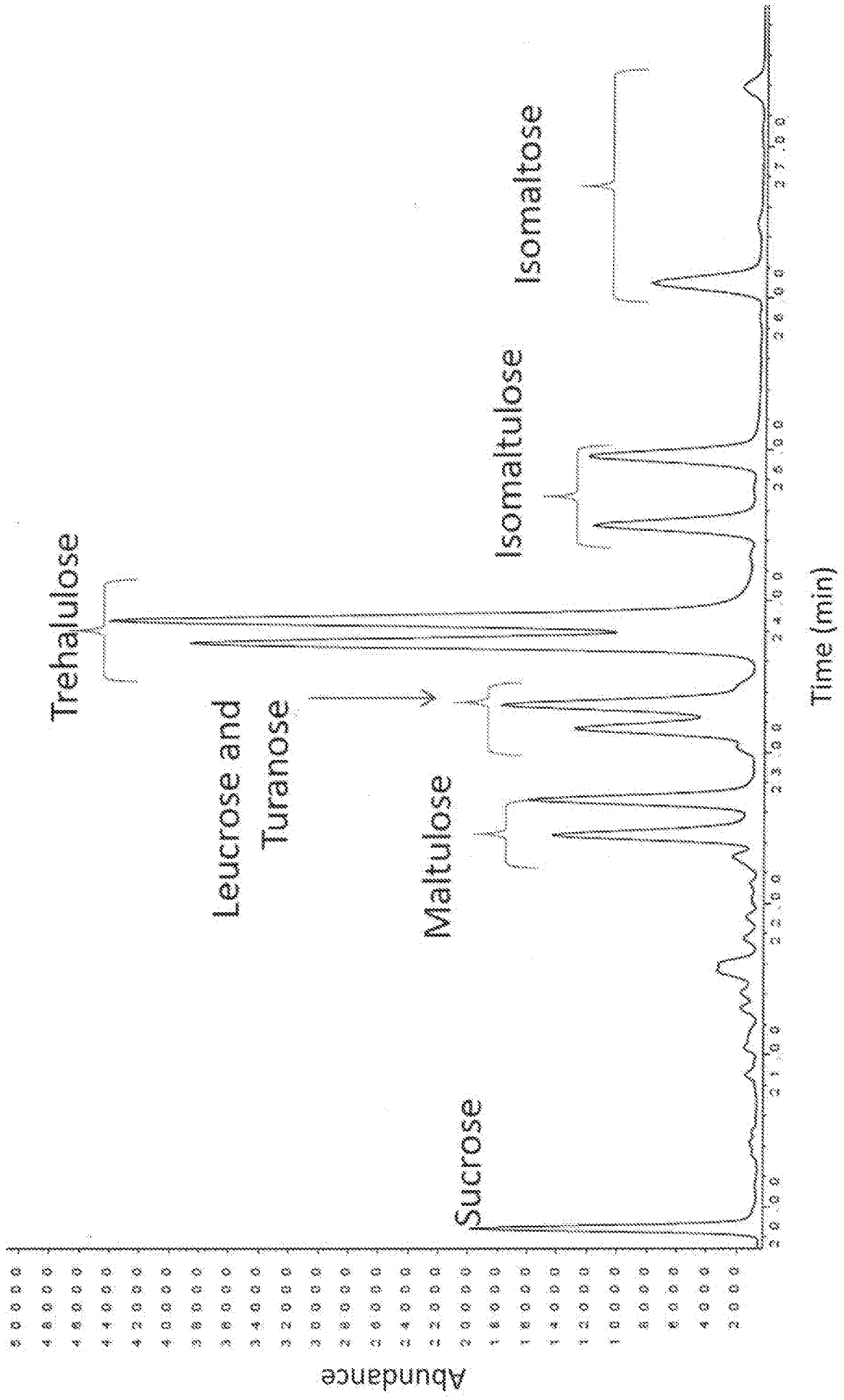
Trehalulose
Leucrose and
Turanose
Maltulose
Sucrose
Isomaltulose
Isomaltose
Abundance
Time (min)

FIG. 1

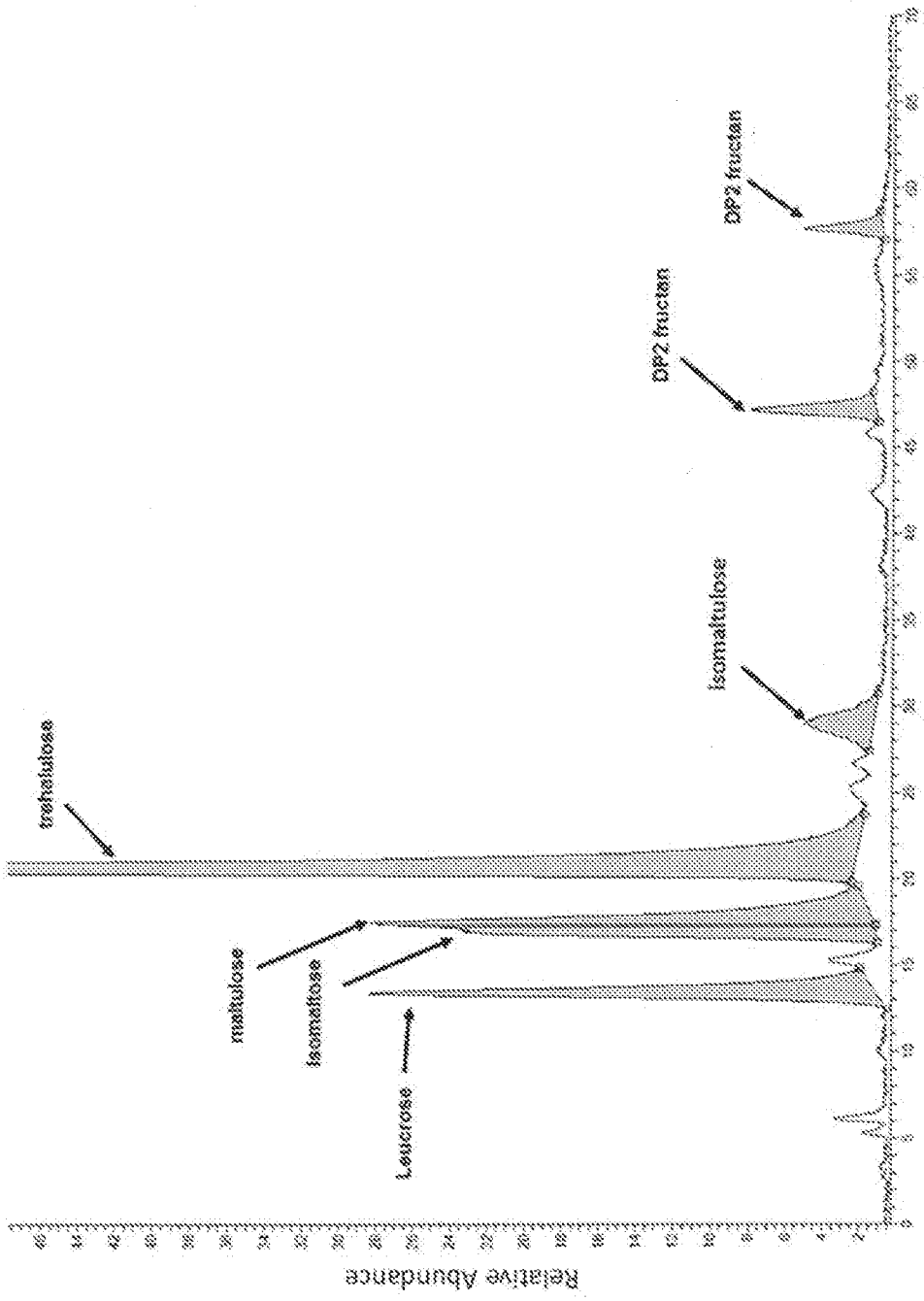
trehalulose
maltulose
isomaltose
Leucrose
isomaltulose
DP2 fructan
DP2 fructan
Relative Abundance
Time (min)

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/066517

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P19/02 C12P19/18 C13K11/00 A23L29/30
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P C13K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, COMPENDEX, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 962 282 B2 (CAIMI PERRY G [US] ET AL) 24 February 2015 (2015-02-24) cited in the application example 4; table 3	1-4,7-10
X	US 8 828 689 B2 (CAIMI PERRY G [US] ET AL) 9 September 2014 (2014-09-09) cited in the application examples 4, 5; tables 4, 5 column 1, lines 56-65	1-4,7-10
Y	US 2015/240278 A1 (NAGY KEVIN D [US] ET AL) 27 August 2015 (2015-08-27) cited in the application	5
A	abstract; claim 14; examples 1-16	1-4,6-9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2017	22/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schröder, Gunnar

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/066517

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/240279 A1 (NAGY KEVIN D [US] ET AL) 27 August 2015 (2015-08-27) cited in the application	5
A	abstract; claim 14; examples 1-16	1-4,6-9

A	WO 2015/123063 A1 (DANISCO US INC [US]) 20 August 2015 (2015-08-20) claim 2	1-10

A	S. A. F. T. VAN HIJUM ET AL: "Structure-Function Relationships of Glucansucrase and Fructansucrase Enzymes from Lactic Acid Bacteria", MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, vol. 70, no. 1, 1 March 2006 (2006-03-01), pages 157-176, XP055179067, ISSN: 1092-2172, DOI: 10.1128/MMBR.70.1.157-176.2006 section "Glucansucrases"; page 158 - page 166	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/066517

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-10

   Method for producing an aqueous composition comprising fructose, and aqueous composition obtained thereby

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2. claims: 11-18

   Aqueous composition comprising fructose, glucose and soluble oligosaccharides of DP 2-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/066517

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 8962282 B2	24-02-2015	US 2013196384 A1	01-08-2013
		WO 2013096511 A1	27-06-2013
US 8828689 B2	09-09-2014	US 2013157316 A1	20-06-2013
		WO 2013096502 A1	27-06-2013
US 2015240278 A1	27-08-2015	US 2015240278 A1	27-08-2015
		US 2015240279 A1	27-08-2015
US 2015240279 A1	27-08-2015	US 2015240278 A1	27-08-2015
		US 2015240279 A1	27-08-2015
WO 2015123063 A1	20-08-2015	EP 3104717 A1	21-12-2016
		US 2017006902 A1	12-01-2017
		WO 2015123063 A1	20-08-2015