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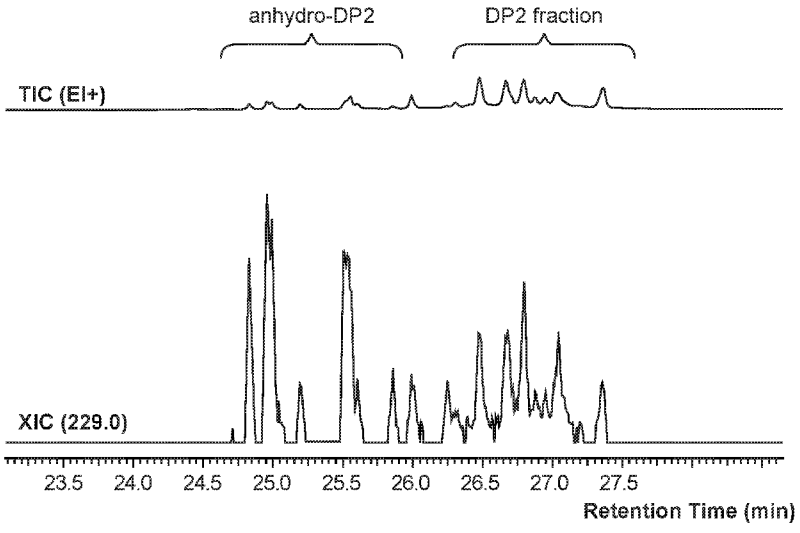


FIG. 6

(57) Abstract: The present disclosure relates to selective analytical methods for the detection and/or quantification of an oligosaccharide preparation in a nutritional composition such as an animal feed. Also disclosed are methods of manufacturing a nutritional composition comprising an oligosaccharide preparation, the presence or concentration of which can be selectively detected or determined.

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METHODS OF QUANTIFYING OLIGOSACCHARIDE PREPARATIONS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/757,231 filed on November 8, 2018, the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Oligosaccharide preparations, which can generally include monosaccharides, oligosaccharides, polysaccharides, functionalized oligosaccharides, or their combinations, are used as additives in nutritional compositions such as animal feed. The addition of oligosaccharide preparations can improve the health and performance of the animal. However, it is challenging to detect or quantify an oligosaccharide preparation additive in a nutritional composition, because nutritional compositions usually contain other carbohydrate sources that can have structural similarities with the oligosaccharide preparations. As a result, a need exists for methods of selectively detecting or quantifying the oligosaccharide preparations in a nutritional composition.

SUMMARY

[0003] Disclosed herein are simple, selective, and sensitive analytical methods for the detection and/or quantification of an oligosaccharide preparation or a composition comprising the oligosaccharide preparation in a nutritional composition. Also disclosed are methods of manufacturing a nutritional composition comprising an oligosaccharide preparation, the presence or concentration of which can be selectively and sensitively detected or determined.

[0004] In one aspect, described herein is a method of correlating a synthetic oligosaccharide preparation in a nutritional composition, wherein the nutritional composition comprises the synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition, the method comprising: (a) providing a sample of the nutritional composition, (b) detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition, and (c) correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition, wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β

glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In one aspect, described herein is a method of performing quality control of a nutritional composition comprising: (a) providing a batch of a nutritional composition, wherein the nutritional composition comprises a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition, (b) obtaining a sample of the nutritional composition from the batch, (c) detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, and (d) accepting or rejectig the batch of the nutritional composition, wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the signal is indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the one or more anhydro-subunit containing oligosaccharides have a degree of polymerization of 2 (DP2). In one aspect, described herein is a method of performing quality control of a nutritional composition comprising: (a) providing a sample of a nutritional composition, wherein the nutritional composition comprises a naturally occurring oligosaccharide composition, and (b) detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, wherein the signal is indicative of one or more anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2). In some embodiments, the nutritional composition comprises a synthetic oligosaccharide preparation. In some embodiments, the method comprises correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition. In some embodiments, the signal is detected by high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, size exclusion chromatography (SEC), field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), weight determination of fractions by preparative chromatography, or any combination thereof. In some embodiments, the nutritional composition comprises a base nutritional composition. In some embodiments, the base nutritional composition comprises the naturally occurring oligosaccharide composition. In some embodiments, the base nutritional composition lacks a detectable level of anhydro-subunit containing oligosaccharides. In some embodiments, the base nutritional composition is essentially free of anhydro-subunit containing oligosaccharides. In some embodiments, the one or more anhydro-subunit containing oligosaccharides originate from the synthetic oligosaccharide

preparation. In some embodiments, the signal is attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 1 (DP1). In some embodiments, the signal is attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof. In some embodiments, the signal is attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 3 (DP3). In some embodiments, the signal is attributed to DP1, DP2, or DP3 anhydro-subunit containing oligosaccharides, or a combination thereof. In some embodiments, the signal is attributed to one or more DP2 anhydro-subunit containing oligosaccharides. In some embodiments, the signal is attributed to anhydro-cellobiose. In some embodiments, the detecting comprises a weight determination of one or more degree of polymerization (DP) fractions of oligosaccharides. In some embodiments, the detecting comprises a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the sample. In some embodiments, the at least a portion of anhydro-subunit containing oligosaccharides have a degree of polymerization of 1, 2, or 3. In some embodiments, the one or more DP fractions of oligosaccharides or the at least a portion of anhydro-subunit containing oligosaccharides are isolated by preparative chromatography. In some embodiments, the signal is detected, at least in part, by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS). In some embodiments, the signal is detected, at least in part, by liquid chromatography–mass spectrometry (LC-MS)/MS. In some embodiments, the signal is detected, at least in part, by GC-flame ionization detector (GC-FID) or GC-MS. In some embodiments, the signal is detected, at least in part, by NMR spectroscopy. In some embodiments, the signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages of oligosaccharides. In some embodiments, the detecting comprises obtaining an NMR spectroscopy. In some embodiments, the signal is associated with α-(1,2) glycosidic linkages. In some embodiments, the signal is associated with α-(1,3) glycosidic linkages. In some embodiments, the signal is associated with α-(1,6) glycosidic linkages. In some embodiments, the signal is associated with β-(1,2) glycosidic linkages. In some embodiments, the signal is associated with β-(1,3) glycosidic linkages. In some embodiments, the signal is associated with β-(1,4) glycosidic linkages. In some embodiments, the signal is associated with β-(1,6) glycosidic linkages. In some embodiments, the detecting comprises determining the presence or absence of the signal. In some embodiments, the detecting comprises determining the presence or absence of DP1 or DP2 anhydro-subunit containing oligosaccharides, or both. In some embodiments, the detecting comprises determining or correlating a level of the signal. In some embodiments, the detecting comprises correlating a level of DP1 anhydro-subunit containing

oligosaccharides in the nutritional composition. In some embodiments, the detecting comprises correlating a level of DP2 anhydro-subunit containing oligosaccharides in the nutritional composition.

[0005] In one aspect, described herein is a method of performing quality control of a nutritional composition comprising a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition, the method comprising: (a) providing a first sample of the nutritional composition, (b) providing a second sample of the nutritional composition, (c) detecting a first signal of at least a portion of oligosaccharides in the first sample, (d) detecting a second signal of at least a portion of oligosaccharides in the second sample, and (e) comparing the first signal and the second signal, wherein the first signal and the second signal are independently (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the method comprises correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition of the first sample, a concentration of the synthetic oligosaccharide preparation in the nutritional composition of the second sample, or both. In some embodiments, the first signal and the second signal are each independently indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the first signal and the second signal are attributed to the same species of an anhydro-subunit containing oligosaccharide. In some embodiments, the first signal and the second signal are attributed to different species of anhydro-subunit containing oligosaccharides In some embodiments, the first sample and the second sample are taken from different batches of the nutritional composition. In some embodiments, the first signal and the second signal are each independently detected by high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, size exclusion chromatography (SEC), field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), weight determination of fractions by preparative chromatography, or any combination thereof. In some embodiments, the nutritional composition comprises a base nutritional composition. In some embodiments, the base nutritional composition comprises the naturally occurring oligosaccharide composition. In some embodiments, the base nutritional composition lacks a detectable level of anhydro-subunit containing oligosaccharides. In some embodiments, the base nutritional composition is essentially free of anhydro-subunit containing oligosaccharides. In some embodiments, the one or more

anhydro-subunit containing oligosaccharides originate from the synthetic oligosaccharide preparation. In some embodiments, the first signal, the second signal, or both are independently attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 1 (DP1). In some embodiments, the first signal, the second signal, or both are independently attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof. In some embodiments, the first signal, the second signal, or both are independently attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2). In some embodiments, the signal is attributed to anhydro-cellobiose. In some embodiments, the first signal, the second signal, or both are independently attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 3 (DP3). In some embodiments, the first signal, the second signal, or both are independently attributed to DP1, DP2, or DP3 anhydro-subunit containing oligosaccharides, or a combination thereof. In some embodiments, the detecting comprises a weight determination of one or more degree of polymerization (DP) fractions from the sample or the second sample. In some embodiments, the detecting comprises a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the first sample or the second sample. In some embodiments, the at least a portion of anhydro-subunit containing oligosaccharides have a degree of polymerization of 1, 2, or 3. In some embodiments, the one or more DP fractions of oligosaccharides or the at least a portion of anhydro-subunit containing oligosaccharides are isolated by preparative chromatography. In some embodiments, the first signal, the second signal, or both are independently detected, at least in part, by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS). In some embodiments, the first signal, the second signal, or both are independently detected, at least in part, by liquid chromatography–mass spectrometry (LC-MS)/MS. In some embodiments, the first signal, the second signal, or both are independently detected, at least in part, by GC-flame ionization detector (GC-FID) or GC-MS. In some embodiments, the first signal, the second signal, or both are independently detected, at least in part, by NMR spectroscopy. In some embodiments, the first signal, the second signal, or both are independently associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages of oligosaccharides. In some embodiments, the detecting comprises obtaining an NMR spectroscopy. In some embodiments, the first signal, the second signal, or both are associated with α-(1,2) glycosidic linkages. In some embodiments, the first signal, the second signal, or both are associated with α-(1,3) glycosidic linkages. In some embodiments, the first signal, the second signal, or both are associated with α-(1,6) glycosidic linkages. In some embodiments, the first

signal, the second signal, or both are associated with β-(1,2) glycosidic linkages. In some embodiments, the first signal, the second signal, or both are associated with β-(1,3) glycosidic linkages. In some embodiments, the first signal, the second signal, or both are associated with β-(1,4) glycosidic linkages. In some embodiments, the first signal, the second signal, or both are associated with β-(1,6) glycosidic linkages. In some embodiments, the first signal is indicative of one or more anhydro-subunit containing oligosaccharides and the second signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the detecting comprises determining the presence or absence of the first signal and the second signal. In some embodiments, the detecting comprises determining the presence or absence of DP1 or DP2 anhydro-subunit containing oligosaccharides, or both. In some embodiments, the detecting comprises determining or correlating a level of the first signal and the second signal. In some embodiments, the detecting comprises correlating a level of DP1 anhydro-subunit containing oligosaccharides in the nutritional composition. In some embodiments, the detecting comprises correlating a level of DP2 anhydro-subunit containing oligosaccharides in the nutritional composition.

[0006] In some embodiments, a method described herein comprises accepting or rejecting a batch of the nutritional composition. In some embodiments, the method comprises adjusting a level of the synthetic oligosaccharide preparation in the nutritional composition after the detecting. In some embodiments, the base nutritional composition comprises a plurality of oligosaccharides. In some embodiments, the base nutritional composition comprises starch or plant fibers. In some embodiments, a level of α-(1,2) glycosidic linkage, α-(1,3) glycosidic linkage, β-(1,2) glycosidic linkage, β-(1,3) glycosidic linkage, or β-(1,4) glycosidic linkage in the base nutritional composition is at least 10% lower than a level of the same glycosidic linkage in the synthetic oligosaccharide preparation. In some embodiments, the method comprises a derivatization step prior to the detecting. In some embodiments, the method comprises extracting oligosaccharides from the sample of the nutritional composition. In some embodiments, the method comprises filtering or clarifying the extracted oligosaccharides. In some embodiments, the method comprises concentrating the extracted oligosaccharides. In some embodiments, the concentrating comprises freeze-drying. In some embodiments, the concentrating comprises nano-filtration. In some embodiments, the method comprises introducing an internal standard into the extracted or concentrated oligosaccharides. In some embodiments, the method comprises reducing the

extracted or concentrated oligosaccharides. In some embodiments, the method comprises digesting the extracted or concentrated oligosaccharides with one or more hydrolytic enzymes. In some embodiments, the one or more hydrolytic enzymes comprise carbohydratase, protease, lipase, or any combination thereof. In some embodiments, the one or more hydrolytic enzymes comprise α-amylase, amyloglycosidase, invertase, α-galactosidase, or any combination thereof. In some embodiments, the one or more hydrolytic enzymes cleave one or more naturally occurring glycosidic bonds. In some embodiments, the method comprises isolating the oligosaccharides that are not digested. In some embodiments, the method comprises separating the extracted, concentrated, digested, or reduced oligosaccharides. In some embodiments, the oligosaccharides are separated chromatographically. In some embodiments, the method comprises isolating the separated oligosaccharides. In some embodiments, the oligosaccharides are separated or isolated by their degrees of polymerization. In some embodiments, the method comprises isolating or separating the oligosaccharides with a degree of polymerization of 1, 2, 3, 4, or 5. In some embodiments, the DP1 oligosaccharides are isolated or separated. In some embodiments, the DP2 oligosaccharides are isolated or separated. In some embodiments, the DP3 oligosaccharides are isolated or separated. In some embodiments, the method comprises isolating at least a portion of the DP1 anhydro-subunit containing oligosaccharides from the sample. In some embodiments, the method comprises isolating at least a portion of the DP2 anhydro-subunit containing oligosaccharides from the sample. In some embodiments, the method comprises isolating at least a portion of the DP3 anhydro-subunit containing oligosaccharides from the sample. In some embodiments, the isolated or separated oligosaccharides are quantified. In some embodiments, a majority of the quantified oligosaccharides originate from the synthetic oligosaccharide preparation. In some embodiments, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, more than 95%, or more than 99% by weight of the quantified oligosaccharide originate from the synthetic oligosaccharide preparation.

[0007] In one aspect, described herein is a method of correlating a synthetic oligosaccharide preparation in a nutritional composition, wherein the nutritional composition comprises (i) a synthetic oligosaccharide preparation that comprises anhydro-subunit containing oligosaccharides and (ii) a naturally occurring oligosaccharide composition, the method comprising: (a) providing a sample of the nutritional composition, (b) isolating one or more anhydro-subunit containing oligosaccharides from the sample, (c) detecting a signal that is indicative of the one or more anhydro-subunit containing oligosaccharides, wherein the detecting comprises (i) a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the sample or (ii) analyzing at least a portion of anhydro-subunit containing oligosaccharides from the sample

by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), liquid chromatography–mass spectrometry (LC-MS)/MS, or gas chromatography(GC)-MS, and (d) correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition. In some embodiments, the detecting comprises a weight determination of at least a portion of the anhydro-subunit containing oligosaccharides having a degree of polymerization of 1 (DP1) from the sample. In some embodiments, the detecting comprises a weight determination of at least a portion of the anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2) from the sample. In some embodiments, the detecting comprises analyzing at least a portion of DP1 or DP2 anhydro-subunit containing oligosaccharides from the sample by MALDI-MS. In some embodiments, the detecting comprises analyzing at least a portion of DP1 or DP2 anhydro-subunit containing oligosaccharides from the sample by LC-MS/MS. In some embodiments, the detecting comprises analyzing at least a portion of DP1 or DP2 anhydro-subunit containing oligosaccharides from the sample by GC/MS. In some embodiments, the detecting comprises analyzing at least a portion of DP3 anhydro-subunit containing oligosaccharides from the sample. In some embodiments, the isolating comprises separating the one or more anhydro-subunit containing oligosaccharides by preparative chromatography. In some embodiments, the synthetic oligosaccharide preparation is present in the nutritional composition at a concentration of from about 1 to about 5000 ppm, from about 1 to about 1000 ppm, from about 1 to about 500 ppm, from about 10 to about 5000 ppm, from about 10 to about 2000 ppm, from about 10 to about 1000 ppm, from about 10 to about 500 ppm, from about 10 to about 250 ppm, from about 10 to about 100 ppm, from about 50 to about 5000 ppm, from about 50 to about 2000 ppm, from about 50 to about 1000 ppm, from about 50 to about 500 ppm, from about 50 to about 250 ppm, or from about 50 to about 100 ppm. In some embodiments, the synthetic oligosaccharide preparation is present in the nutritional composition at a concentration of greater than 10 ppm, greater than 50 ppm, greater than 100 ppm, greater than 200 ppm, greater than 300 ppm, greater than 400 ppm, greater than 500 ppm, greater than 600 ppm, greater than 1000 ppm, or greater than 2000 ppm. In some embodiments, the nutritional composition is an animal feed composition.

[0008] In one aspect, described herein is a method of manufacturing a nutritional composition comprising: (a) combining a base nutritional composition with a synthetic oligosaccharide preparation comprising anhydro-subunit containing oligosaccharides, and (b) performing a quality control method (e.g., methods of correlating a synthetic oligosaccharide preparation in a nutritional composition) as provided herein. In some embodiments, the synthetic oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of

polymerization selected from 1 to n (DP1 to DPn fractions), wherein n is an integer greater than or equal to 3; and wherein the DP1 and DP2 fractions each independently comprises from about 0.5% to about 15% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry. . In some embodiments, the synthetic oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions), wherein n is an integer greater than or equal to 2; and wherein the DP1 and DP2 fractions each independently comprises from about 0.1% to about 15% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry. In some embodiments, the relative abundance is determined by LC-MS/MS. In some embodiments, the relative abundance of oligosaccharides in each of the n fractions decreases monotonically with its degree of polymerization.

[0009] In one aspect, provided herein is a method of quantifying an oligosaccharide preparation in a nutritional composition comprising: (a) determining a level of a signal in a sample of the nutritional composition, and (b) calculating a concentration of the oligosaccharide preparation in the nutritional composition based on the level of the signal, wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In one aspect, provided herein is a method of performing quality control of a nutritional composition comprising: (a) detecting a signal in a sample of the nutritional composition through analytical instrumentation, and (b) accepting or rejecting a batch of the nutritional composition based on the presence or absence of the signal, wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In another aspect, provided herein is a method of performing quality control of a nutritional composition comprising: (a) detecting, through analytical instrumentation, the presence or absence of a first signal in a first sample of the nutritional composition, and a second signal in a second sample of the nutritional composition, and (b) comparing the first signal and the second signal, wherein the first signal and the second

signal are (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the signal, the level of the signal, the first signal, and/or the second signal are determined or detected by high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, size exclusion chromatography (SEC), field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), or any combination thereof. In some embodiments, the nutritional composition comprises a base nutritional composition. In some embodiments, the signal, first signal, and/or the second signal is indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the one or more anhydro-subunit containing oligosaccharides originate from the oligosaccharide preparation. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to one or more anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to cellobiosan. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to anhydro-subunit containing oligosaccharides in the DP3 fraction. In some embodiments, the first signal and the second signal are attributed to the same species of an anhydro-subunit containing oligosaccharide. In some embodiments, the first signal and the second signal are attributed to different species of anhydro-subunit containing oligosaccharides. In some embodiments, the signal, the level of the signal, the first signal, and/or the second signal are determined or detected by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS). In some embodiments, the signal, the level of the signal, the first signal, and/or the second signal are determined or detected by liquid chromatography–mass spectrometry (LC-MS)/MS. In some embodiments, the signal, the level of the signal, the first signal, and/or the second signal are determined or detected by GC-flame ionization detector (GC-FID) or GC-MS. In some embodiments, the signal, the level of the signal, the first signal, and/or the second signal are

determined or detected by NMR. In some embodiments, a derivatization step is performed prior to detection. In some embodiments, the signal, the level of the signal, the first signal, and/or the second signal are determined by the weight of isolated and/or purified fractions from preparative chromatography. In some embodiments, the base nutritional composition lacks a detectable level of anhydro-subunits. In some embodiments, the base nutritional composition is essentially free of anhydro-subunits. In certain embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the signal, the first signal, and/or the second signal are detected or determined by NMR. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,2) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,3) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,6) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,2) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,3) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,4) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,6) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,1)-α glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,1)-β glycosidic linkages or β-(1,1)-α glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,1)-β glycosidic linkages. In certain embodiments, the signal, the first signal, and/or the second signal are associated with a DP distribution of oligosaccharides. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to the oligosaccharides in the DP2 fraction. In some embodiments, the method further comprises accepting or rejecting a batch of the nutritional composition. In some embodiments, the method further comprises adjusting the level of the oligosaccharide preparation after the determining or detecting. In some embodiments, the base nutritional composition comprises a

plurality of oligosaccharides. In some embodiments, the base nutritional composition comprises starch and/or plant fibers. In some embodiments, the level of α-(1,2) glycosidic linkage, α-(1,3) glycosidic linkage, β-(1,2) glycosidic linkage, β-(1,3) glycosidic linkage, or β-(1,4) glycosidic linkage in the base nutritional composition is at least 10% lower than the level of the same glycosidic linkage in the oligosaccharide preparation. In some embodiments, the level of α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides in the base nutritional composition is at least 10% lower than the level of the same glycosidic linkage in the oligosaccharide preparation. In some embodiments, the method further comprises extracting oligosaccharides from a sample of the nutritional composition. In some embodiments, the method further comprises filtering or clarifying the extracted oligosaccharides. In some embodiments, the method further comprises concentrating the extracted oligosaccharides. In some embodiments, the concentrating the extracted oligosaccharides comprises freeze-drying. In some embodiments, the method further comprises introducing an internal standard into the extracted or concentrated oligosaccharides. In some embodiments, the method further comprises reducing the extracted or concentrated oligosaccharides. In some embodiments, the method further comprises digesting the extracted or concentrated oligosaccharides with one or more hydrolytic enzymes. In some embodiments, the one or more hydrolytic enzymes comprise carbohydratase, protease, lipase, or any combination thereof. In some embodiments, the one or more hydrolytic enzymes comprise α-amylase, amyloglycosidase, invertase, α-galactosidase, or any combination thereof. In some embodiments, the one or more hydrolytic enzymes cleave one or more naturally occurring glycosidic bonds. In some embodiments, the method further comprises isolating the oligosaccharides that are not digested. In some embodiments, the method further comprises separating the extracted, concentrated, digested, or reduced oligosaccharides. In some embodiments, the oligosaccharides are separated chromatographically. In some embodiments, the method further comprises isolating the separated oligosaccharides. In some embodiments, the oligosaccharides are separated or isolated by the degree of polymerization. In some embodiments, the method comprises isolating or separating the oligosaccharides with a degree of polymerization of 1, 2, 3, 4, or 5. In some embodiments, the oligosaccharides in the DP1 fraction are isolated or separated. In some embodiments, the oligosaccharides in the DP2 fraction are isolated or separated. In some embodiments, the oligosaccharides in the DP3 fraction are isolated or separated. In some embodiments, the oligosaccharides in the DP4 fraction are isolated or separated. In some embodiments, the oligosaccharides in the DP5 fraction are isolated or separated. In some embodiments, the isolated or separated oligosaccharides are quantified. In some embodiments, the

anhydro-subunit containing oligosaccharides within the isolated or separated oligosaccharides are quantified. In some embodiments, the majority of the quantified oligosaccharides are from the oligosaccharide preparation. In some embodiments, more than 50, 60, 70, 80, 90, 95, or 99 wt% of the quantified oligosaccharide are from the oligosaccharide preparation. In some embodiments, the method comprises analyzing glycosidic linkages of the oligosaccharides by NMR, wherein the glycosidic linkages are α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages, thereby determining or detecting the signal, the level of the signal, the first signal, or the second signal associated with the corresponding glycosidic linkages. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides by mass spectrometry, thereby determining or detecting the signal, the level of the signal, the first signal, or the second signal indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides by HPLC, thereby determining or detecting the signal, the level of the signal, the first signal, or the second signal indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides by FFF or A4F, thereby determining or detecting the signal, the level of the signal, the first signal, or the second signal indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the method comprises analyzing the DP distribution of the oligosaccharides by SEC, GC, or HPLC, thereby determining or detecting the signal, the level of the signal, the first signal, or the second signal associated with the DP distribution. In some embodiments, the method comprises quantifying the oligosaccharides in the DP2 fraction by SEC, GC, or HPLC, thereby determining or detecting the signal, the level of the signal, the first signal, or the second signal associated with the DP distribution. In some embodiments, the oligosaccharide preparation is from 1 to 5000 ppm, 1 to 1000 ppm, 1 to 500 ppm, 10 to 5000 ppm, 10 to 2000 ppm, 10 to 1000 ppm, 10 to 500 ppm, 10 to 250 ppm, 10 to 100 ppm, 50 to 5000 ppm, 50 to 2000 ppm, 50 to 1000 ppm, 50 to 500 ppm, 50 to 250 ppm, or 50 to 100 ppm relative to the nutritional composition. In some embodiments, the oligosaccharide preparation is greater than 10 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 1000 ppm, or 2000 ppm relative to the nutritional composition. In some embodiments, the nutritional composition is an animal feed composition.

[0010] In one aspect, provided herein is a method of manufacturing a nutritional composition comprising: (a) combining a synthetic oligosaccharide preparation comprising anhydro-subunit

containing oligosaccharides with a base nutritional composition, and (b) performing a quality control step as provided herein. In some embodiments, the synthetic oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions), wherein n is an integer greater than or equal to 2; wherein each fraction comprises from 0.1% to 15% anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry. In some embodiments, the synthetic oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions), wherein n is an integer greater than or equal to 3; wherein each fraction comprises from 0.5% to 15% anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry. In some embodiments, the relative abundance of oligosaccharides in each of the n fractions decreases monotonically with its degree of polymerization.

[0011] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0012] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawing (also "figure" and "FIG." herein), of which:

[0014] FIG. 1 illustrates a ^{1}H, ^{13}C- HSQC NMR spectrum of the oligosaccharide preparation of Example 9.7.

[0015] FIG. 2 illustrates an MALDI-MS spectrum of an oligosaccharide preparation from Example 9 that demonstrates the presence of anhydro-subunits.

[0016] FIG. 3 illustrates a 1D ^{1}H- proton NMR spectrum of an anhydro DP1 fraction isolated from an oligosaccharide of Example 9.

[0017] FIG. 4 illustrates a 1D APT ^{13}C- NMR spectrum of an anhydro DP1 fraction isolated from an oligosaccharide of Example 9.

[0018] FIG. 5 illustrates the structures of two anhydro DP1 compounds (1,6-anhydro-beta-D-glucofuranose and 1,6-anhydro-beta-D-glucopyranose) and their NMR assignments.

[0019] FIG. 6 illustrates an enlargement of the GC-MS chromatogram (TIC and XIC (m/z 229) plots) for the oligosaccharide preparation of Example 9.7 following derivatization.

[0020] FIG. 7 illustrates MALDI-MS spectra comparing the oligosaccharide preparation from Example 9 versus a conventional dextran.

[0021] FIG. 8 illustrates LC-MS/MS detection of the anhydro DP2 species at concentration of 1 – 80 μg/mL of an oligosaccharide preparation in water.

[0022] FIG. 9 illustrates a linear calibration curve resulting from the LC-MS/MS detection of **FIG. 8**.

[0023] FIG. 10 illustrates the quantification of the anhdro-DP2 content of various control and treated diet compositions.

[0024] FIG. 11 illustrates a 2D-1H JRES NMR spectrum of an anhydro-subunit containing gluco-oligosaccharides sample.

[0025] FIG. 12 is a representative ^{1}H, ^{13}C- HSQC NMR spectrum of an anhydro-subunit containing gluco-oligosaccharides sample with relevant resonances and assignments used for linkage distribution.

[0026] FIG. 13 illustrates an overlay of 1H DOSY spectra of three anhydro-subunit containing oligosaccharides.

[0027] FIG. 14 illustrates a comparison of 1,6-Anhydro-ß-D-glucose (DP1-18), 1,6-Anhydro-ß-D-cellobiose (DP2-18), and an anhydro-subunit containing oligosaccharides sample.

[0028] FIG. 15 illustrates mass chromatograms of anhydro-subunit containing oligosaccharides (top) and digested anhydro-subunit containing oligosaccharides (bottom) at selected multiple reaction monitoring (MRM).

[0029] FIG. 16 illustrates mass chromatograms of (1) feed that contains anhydro-subunit containing oligosaccharides, (2) digested feed that contains anhydro-subunit containing oligosaccharides, and (3) blank digested feed at selected MRM.

[0030] FIG. 17 illustrates a representative workflow for the analysis of oligosaccharide preparations in animal feed.

[0031] FIG. 18 illustrates two DP1 and one DP2 anhydro-subunit containing oligosaccharides.

[0032] FIG. 19 illustrates an anhydro-subunit containing oligosaccharide (cellotriosan).

[0033] FIG. 20A illustrates a MALDI-MS spectrum of an oligosaccharide preparation from Example 2 that demonstrates the presence of anhydro-subunits; **FIG. 20B** illustrates an enlargement of a part of the MALDI-MS spectrum shown in **FIG. 20A**.

[0034] FIG. 21A illustrates LC-MS/MS detection of the anhydro DP2 species of an oligosaccharide preparation of Example 1; **FIG. 21B** illustrates LC-MS/MS detection of the anhydro DP1 species of an oligosaccharide preparation of Example 1; **FIG. 21C** illustrates LC-MS/MS detection of the DP2 species of an oligosaccharide preparation of Example 1.

[0035] FIG. 22A illustrates LC-MS/MS detection of the anhydro DP2 species of an oligosaccharide preparation of Example 3; **FIG. 22B** illustrates LC-MS/MS detection of the anhydro DP1 species of an oligosaccharide preparation of Example 3; **FIG. 22C** illustrates LC-MS/MS detection of the DP2 species of an oligosaccharide preparation of Example 3.

[0036] FIG. 23A illustrates LC-MS/MS detection of the anhydro DP2 species of an oligosaccharide preparation of Example 4; **FIG. 23B** illustrates LC-MS/MS detection of the anhydro DP1 species of an oligosaccharide preparation of Example 4; **FIG. 23C** illustrates LC-MS/MS detection of the DP2 species of an oligosaccharide preparation of Example 4.

[0037] FIG. 24A illustrates LC-MS/MS detection of the anhydro DP2 species of an oligosaccharide preparation of Example 7; **FIG. 24B** illustrates LC-MS/MS detection of the anhydro DP1 species of an oligosaccharide preparation of Example 7; **FIG. 24C** illustrates LC-MS/MS detection of the DP2 species of an oligosaccharide preparation of Example 7.

[0038] FIG. 25A illustrates GC-MS spectrum detection of the DP1, anhydro DP1, DP2 and anhydro DP2 fractions of an oligosaccharide preparation of Example 1; **FIG. 25B** illustrates an enlargement of the DP2 and anhydro DP 2 fractions as shown in **FIG. 25A**.

[0039] FIG. 26A illustrates GC-MS spectrum detection of the DP1, anhydro DP1, DP2 and anhydro DP2 fractions of an oligosaccharide preparation of Example 3; **FIG. 26B** illustrates an enlargement of the DP2 and anhydro DP 2 fractions as shown in **FIG. 26A**.

[0040] FIG. 27A illustrates GC-MS spectrum detection of the DP1, anhydro DP1, DP2 and anhydro DP2 fractions of an oligosaccharide preparation of Example 4; **FIG. 27B** illustrates an enlargement of the DP2 and anhydro DP 2 fractions as shown in **FIG. 27A**.

[0041] FIG. 28A illustrates GC-MS spectrum detection of the DP1, anhydro DP1, DP2 and anhydro DP2 fractions of an oligosaccharide preparation of Example 7; **FIG. 28B** illustrates an enlargement of the DP2 and anhydro DP 2 fractions as shown in **FIG. 28A**.

[0042] FIG. 29 illustrates the effect of reaction temperature, water content, and reaction time on the content of DP2 anhydro-subunit containing oligosaccharides in the oligosaccharide preparations, as compared to an oligosaccharide preparation according to Example 2.

[0043] FIG. 30 illustrates MALDI-MS spectra comparing the oligosaccharide preparation from Example 9 at different laser energies.

DETAILED DESCRIPTION

[0044] The following description and examples illustrate embodiments of the present disclosure in detail. It is to be understood that this present disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this present disclosure, which are encompassed within its scope.

[0045] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0046] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0047] Although various features of the present disclosure may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the present disclosure may be described herein in the context of separate embodiments for clarity, the present disclosure may also be implemented in a single embodiment.

[0048] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

I. Definitions

[0049] As used herein the term "administering" includes providing a synthetic oligosaccharide preparation, a nutritional composition, a liquid, or an animal feed composition described herein, to an animal such that the animal may ingest the synthetic oligosaccharide preparation, the nutritional composition, the liquid, or the animal feed composition. In such embodiments, the animal ingests some portion of the synthetic oligosaccharide preparation, the nutritional composition, or the animal feed composition. In some embodiments, the animal ingests some portion of the synthetic oligosaccharide preparation, the nutritional composition, the liquid, or the animal feed composition in every 24-hour period or every other 24-hour period for at least 7 days, 14 days, 21 days, 30 days, 45 days, 60 days, 75 days, 90 days or 120 days. In some embodiments, the oligosaccharide preparation may be dissolved in water or another liquid, and the animal ingests some portion of the oligosaccharide preparation by drinking the liquid. In certain embodiments, the oligosaccharide is provided to the animal via its drinking water. In certain embodiments, the oligosaccharide preparation, nutritional composition, liquid, or animal feed composition is consumed at will.

[0050] As used herein, the term "inclusion level" or "dose" refers to the concentration of an oligosaccharide preparation in a nutritional composition, a liquid, a diet, or an animal feed composition provided to the animal. In some embodiments, the inclusion level is measured as the mass concentration of the oligosaccharide preparation in the final nutritional composition, liquid, diet, or animal feed. For example, the inclusion level may be measured in units of parts per million (ppm) of the oligosaccharide on a dry solids weight basis per the total weight of the final nutritional composition, liquid, diet, or animal feed. In certain embodiments, the dry solids mass of the oligosaccharide preparation is measured as the dry-basis mass of DP1+ species. In other embodiments, the dry solids mass of the oligosaccharide preparation is measured as the dry-basis mass of DP2+ species.

[0051] As used herein, the term "specific dose" refers to the quantity of an oligosaccharide preparation consumed by an animal per unit of time and relative to its body mass. In some embodiments, the specific dose may be measured in units of mg of oligosaccharide preparation (on a dry solids-basis) per kg of body weight of the animal per day (i.e., mg/kg/day).

[0052] As used herein, the term "anhydro-subunit" refers to a product of thermal dehydration of a monosaccharide (or monosaccharide subunit) or a sugar caramelization product. For example, an "anhydro-subunit" can be an anhydro-monosaccharide such as anhydro-glucose. As another

example, an "anhydro-subunit" can be linked with one or more regular or anhydro-monosaccharide subunits via glycosidic linkage.

[0053] As used herein, the term an "anhydro DPn oligosaccharide," an "anhydro DPn species," or a "DPn anhydro-subunit containing oligosaccharide" refers to an oligosaccharide that has a degree of polymerization of n and comprises one or more anhydro-subunits. As such, an anhydro-glucose is a DP1 anhydro-subunit containing oligosaccharide and an cellotriosan is a DP3 anhydro-subunit containing oligosaccharide.

[0054] As used herein the term "feed conversion ratio (FCR)," refers to the ratio of feed mass input (for example consumed by the animal) to the animal output, wherein the animal output is the target animal product. For example, the animal output for dairy animals is milk, whereas the animal output for animals raised for meat is body mass.

[0055] The term "oligosaccharide" refers to a monosaccharide or a compound containing two or more monosaccharide subunits linked by glycosidic bonds. As such, an oligosaccharide includes a regular monosaccharide; an anhydro-monosaccharide; or a compound containing two or more monosaccharide subunits, wherein one or more monosaccharide subunits are optionally, independently replaced by one or more anhydro-subunits. An oligosaccharide can be functionalized. As used herein, the term oligosaccharide encompasses all species of the oligosaccharide, wherein each of the monosaccharide subunit in the oligosaccharide is independently and optionally functionalized and/or replaced with its corresponding anhydro-monosaccharide subunit.

[0056] As used herein, the term "oligosaccharide preparation" refers to a preparation that comprises at least one oligosaccharide.

[0057] As used herein, the term "gluco-oligosaccharide" refers to a glucose or a compound containing two or more glucose monosaccharide subunits linked by glycosidic bonds. As such, a gluco-oligosaccharide includes a glucose; an anhydro-glucose; or a compound containing two or more glucose monosaccharide subunits linked by glycosidic bonds, wherein one or more of said glucose monosaccharide subunits are each optionally and independently replaced with an anhydro-glucose subunit.

[0058] As used herein, the term "galacto-oligosaccharide" refers to a galactose or a compound containing two or more galactose monosaccharide subunits linked by glycosidic bonds. As such, a galacto-oligosaccharide includes a galactose; an anhydro-galactose or a compound containing two or more galactose monosaccharide subunits linked by glycosidic bonds, wherein one or more of said galactose monosaccharide subunits are each optionally and independently replaced with an anhydro-galactose subunit.

[0059] As used herein, the term "gluco-galacto-oligosaccharide preparation" refers to a composition that is produced from a complete or incomplete sugar condensation reaction of glucose and galactose. Accordingly, in some embodiments, a gluco-galactose-oligosaccharide preparation comprises gluco-oligosaccharides, galacto-oligosaccharides, compounds containing one or more glucose monosaccharide subunits and one or more galactose monosaccharide subunits linked by glycosidic bonds, or a combination thereof. In some embodiments, a gluco-galactose-oligosaccharide preparation comprises gluco-oligosaccharides and compounds containing one or more glucose monosaccharide subunits and one or more galactose monosaccharide subunits linked by glycosidic bonds. In some embodiments, a gluco-galactose-oligosaccharide preparation comprises galacto-oligosaccharides and compounds containing one or more glucose monosaccharide subunits and one or more galactose monosaccharide subunits linked by glycosidic bonds. In some embodiments, a gluco-galactose-oligosaccharide preparation comprises compounds containing one or more glucose monosaccharide subunits and one or more galactose monosaccharide subunits linked by glycosidic bonds.

[0060] As used herein, the term "monosaccharide unit" and "monosaccharide subunit" are used interchangeably. A "monosaccharide subunit" refers to a monosaccharide monomer in an oligosaccharide. For an oligosaccharide having a degree of polymerization of 1, the oligosaccharide can be referred to as a monosaccharide subunit or monosaccharide. For an oligosaccharide having a degree of polymerization of 2 or higher, its monosaccharide subunits are linked via glycosidic bonds.

[0061] As used herein, the term "regular monosaccharide" refers to a monosaccharide that does not contain an anhydro-subunit. The term "regular disaccharide" refers to a disaccharide that does not contain an anhydro-subunit. Accordingly, the term "regular subunit" refers to a subunit that is not an anhydro-subunit.

[0062] The term "relative abundance" or "abundance," as used herein, refers to the abundance of a species in terms of how common or rare the species exists. For example, a DP1 fraction comprising 10% anhydro-subunit containing oligosaccharides by relative abundance can refer to a plurality of DP1 oligosaccharides, wherein 10% of the DP1 oligosaccharides are anhydro-monosaccharides. The relative abundance, e.g., for a certain DP fraction of oligosaccharides, can be determined by suitable analytical instrumentations, for example, mass spectrometry and liquid chromatography such as LC-MS/MS, GC-MS, HPLC-MS, and MALDI-MS. In some embodiments, the relative abundance is determined by integrating the area under the peaks of the chromatographs (e.g., LC-MS/MS, GC-MS, and HPLC-MS) that correspond to the fractions of interest. In some embodiments, the relative abundance is determined by the peak intensities (e.g.,

MALDI-MS). In some embodiments, the relative abundance is determined by a combination of analytical methods such as a weight determination after separation by liquid chromatography.

[0063] As used herein, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an agent" includes a plurality of such agents, and reference to "the oligosaccharide" includes reference to one or more oligosaccharides (or to a plurality of oligosaccharides) and equivalents thereof known to those skilled in the art, and so forth.

[0064] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included. The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range, in some instances, will vary between 1% and 15% of the stated number or numerical range. In some embodiments, the term "about" means within 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, or 0.05% of a given value or range.

[0065] The term "comprising" (and related terms such as "comprise" or "comprises" or "having" or "including") is intended to include, but not necessarily be limited to the things so described.

II. Applications of the Methods

[0066] Oligosaccharide preparations are used as additives in nutritional compositions such as complete animal feed. The addition of such oligosaccharide preparations improves the health and performance (weight, growth rate, feed conversion efficiency) of animals such as poultry (broilers, layers, broiler breeders, turkeys) and pigs (nursery pigs, grower/finisher pigs, sows, etc., and other species).

[0067] The impact of the oligosaccharide preparation on animal health and performance depends on the chemical and physiochemical properties of the oligosaccharides. In particular, the chemical composition of the oligosaccharide preparation can affect the manner in which it is utilized by the microflora of the animal, in particular by the gut microbiome. The microflora of the animal, and in particular the gut microbiome, in turn affects the health and performance of the animal as a whole. Such effects include, for example, physiological and morphological effects on the digestive tract, immune system activation, stimulation of mucus production, improvement of the gut barrier function, improvement in the liberation and absorption of nutrients, modulation of the abundance of the various members of the gut microflora (e.g., suppression of pathogenic species, augmentation of beneficial commensal species), and modulation of biochemical species produced

by the gut microflora. Accordingly, oligosaccharide preparations can be added to nutritional compositions such as animal feed to act as prebiotics.

[0068] The method of producing oligosaccharide preparations suitable for use as an additive in nutritional compositions are provided herein. Oligosaccharide preparations can also be produced by other means, including enzymatic hydrolysis or acid hydrolysis of plant fiber, extraction of bacterial and yeast cell wall glycans and glycopeptides, enzymatic condensation from saccharides, and fermentation by wild type or recombinant micro-organisms.

[0069] Due to the respective structural complexities of both the nutritional compositions and the oligosaccharide preparations, a simple, selective, and sensitive technique for analyzing the presence and concentration of oligosaccharide preparations within the feed material is lacking. The nutritional compositions comprise a large quantity and diversity of carbohydrate structures (e.g., starch, plant fibers and pectins). It is therefore particularly challenging to distinguish small quantities of oligosaccharide-based feed additives from the vast sea of other carbohydrates present as base of the nutritional composition. As such, an analytical method with the required sensitivity and selectivity is needed to distinguish such oligosaccharide feed additives from natural oligomers.

[0070] It is of commercial utility to assay for the presence and/or concentration of feed additives. Such assay can be performed for the purpose of quality control, to determine whether the additive was blended consistently with the base nutritional composition to provide a final nutritional composition comprising the additive at the intended dose or level of inclusion. In certain embodiments, the manufacturing process can include a step of either accepting or rejecting a quantity (e.g., a lot or batch) of the final feed composition based on determining that the additive was included within a range of a pre-determined value.

[0071] Such oligosaccharide preparations comprise a diversity of glycosidic linkages between the various monomers in the oligosaccharide preparation. In certain embodiments, the relative abundances of glycosidic linkages, e.g., α-(1,4) linkages, easily hydrolyzed by the digestive acids and enzymes of the animal's upper digestive are low. As such, the oligosaccharides survive primary digestion by the animal and proceed through the digestive track to the lower digestive track where they interact with the gut microbiome. In some embodiments, the relative abundances of glycosidic linkages commonly encountered in plant fibers and pectins are also low. In some embodiments, the oligosaccharide preparations comprise linkages that occur only with low relative abundance in the base carbohydrates (i.e., the background carbohydrates) in the nutritional composition. In certain embodiments, the oligosaccharide preparations comprise anhydro-subunit

containing oligosaccharides. In certain embodiments, the oligosaccharide preparations comprise chemically synthesized anhydro-subunit containing oligosaccharides.

[0072] Despite the need for detecting and/or quantifying the relative abundance of the oligosaccharide preparations in nutritional compositions, existing analytical methods for quantifying various glycosidic linkages in complex carbohydrates can lack sensitivity. Certain existing analytical methods have a minimum threshold of resolving glycosidic linkages at a level of several percentages. Commercially relevant inclusion levels of feed additives are, however, typically in the range of 1 to 5000 ppm, 10 to 1000 ppm, 10 to 500 ppm, or 50 to 500 ppm. Thus, the glycosidic linkages in feed additives are not detectable via some of the existing methods.

[0073] Surprisingly, we have found that oligosaccharide preparations can be analyzed and quantified in a complex nutritional composition such as complete animal feed as provided herein.

III. Oligosaccharide Preparations

Oligosaccharides by Manufacturing Method

[0074] Provided herein are oligosaccharide preparations suitable for use in nutritional compositions. In some embodiments, oligosaccharide preparations, as provided herein, can comprise monosaccharides, oligosaccharides, polysaccharides, or any combination thereof, wherein one or more monosaccharide subunits in any of the monosaccharides, oligosaccharides, or polysaccharides can be independently functionalized. In some embodiments, the oligosaccharide preparations comprise oligosaccharides produced by hydrolysis or pyrolysis of polysaccharides such as cellulose and starch, by condensation or polymerization of monosaccharides or oligosaccharides, by enzymatic hydrolysis or acid hydrolysis of polysaccharides such as plant fiber, by extraction of bacterial and yeast cell wall glycans and glycopeptides, by enzymatic condensation from saccharides, by fermentation by wild type or recombinant micro-organisms, or by any combination thereof. In some embodiments, the oligosaccharide preparations can comprise any oligosaccharides known in the art. In some embodiments, the oligosaccharide preparations are produced chemically, naturally, or enzymatically.

[0075] In one aspect, a described oligosaccharide preparation suitable for the methods described herein is a synthetic oligosaccharide preparation. In some embodiments, a synthetic oligosaccharide preparation refers to a plurality of oligosaccharides produced by a process that does not require live organisms. In some embodiments, a synthetic oligosaccharide preparation refers to a plurality of oligosaccharides produced by a process that does not require enzymes. In some embodiments, a synthetic oligosaccharide preparation refers to a plurality of

oligosaccharides produced by a chemical process. In certain embodiments, a synthetic oligosaccharide preparation refers to a plurality of oligosaccharides produced by the condensation of sugars.

Degree of Polymerization (DP) Distribution

[0076] In some embodiments, a herein described oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions). In some embodiments, the oligosaccharide preparation comprises n fractions of oligosaccharides, each fraction having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions). In some embodiments, the DP1 fraction comprises one or more monosaccharides and/or one or more anhydro-monosaccharides. As another example, in some embodiments, the DP1 fraction comprises glucose, galactose, fructose, 1,6-anhydro-β-D-glucofuranose, 1,6-anhydro-β-D-glucopyranose, or any combination thereof. For example, in some embodiments, the DP2 fraction comprises one or more regular disaccharides and one or more anhydro-subunit containing disaccharides. In some embodiments, the DP2 fraction comprises lactose.

[0077] In some embodiments, n is at least 2, at least 3, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56, at least 57, at least 58, at least 59, at least 60, at least 61, at least 62, at least 63, at least 64, at least 65, at least 66, at least 67, at least 68, at least 69, at least 70, at least 71, at least 72, at least 73, at least 74, at least 75, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, or at least 100. In some embodiments, n is 2, 3, 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, 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, 99, or 100. In some embodiments, n is less than 10, less than 11, less than 12, less than 13, less than 14, less than 15, less than 16, less than 17, less than 18, less than 19, less than 20, less than 21, less than 22, less than 23, less than 24, less than 25, less than 26, less than 27, less than 28, less than 29, less than 30, less than

31, less than 32, less than 33, less than 34, less than 35, less than 36, less than 37, less than 38, less than 39, less than 40, less than 41, less than 42, less than 43, less than 44, less than 45, less than 46, less than 47, less than 48, less than 49, less than 50, less than 51, less than 52, less than 53, less than 54, less than 55, less than 56, less than 57, less than 58, less than 59, less than 60, less than 61, less than 62, less than 63, less than 64, less than 65, less than 66, less than 67, less than 68, less than 69, less than 70, less than 71, less than 72, less than 73, less than 74, less than 75, less than 76, less than 77, less than 78, less than 79, less than 80, less than 81, less than 82, less than 83, less than 84, less than 85, less than 86, less than 87, less than 88, less than 89, less than 90, less than 91, less than 92, less than 93, less than 94, less than 95, less than 96, less than 97, less than 98, less than 99, or less than 100. In some embodiments, n is from 2 to 100, from 5 to 90, from 10 to 90, from 10 to 80, from 10 to 70, from 10 to 60, from 10 to 50, from 10 to 40, from 10 to 30, from 15 to 60, from 15 to 50, from 15 to 45, from 15 to 40, from 15 to 35, or from 15 to 30.

[0078] A distribution of the degree of polymerization of the oligosaccharide preparation can be determined by any suitable analytical method and instrumentation, including but not limited to end group method, osmotic pressure (osmometry), ultracentrifugation, viscosity measurements, light scattering method, size exclusion chromatography (SEC), SEC-MALLS, field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), high-performance liquid chromatography (HPLC), and mass spectrometry (MS). For example, the distribution of the degree of polymerization can be determined and/or detected by mass spectrometry, such as matrix-assisted laser desorption/ionization (MALDI)-MS, liquid chromatography (LC)-MS, or gas chromatography (GC)-MS. For another example, the distribution of the degree of polymerization can be determined and/or detected by SEC, such as gel permeation chromatography (GPC). As yet another example, the distribution of the degree of polymerization can be determined and/or detected by HPLC, FFF, or A4F. In some embodiments, the distribution of the degree of polymerization is determined and/or detected by MALDI-MS. In some embodiments, the distribution of the degree of polymerization is determined and/or detected by GC-MS or LC-MS. In some embodiments, the distribution of the degree of polymerization is determined and/or detected by SEC. In some embodiments, the distribution of the degree of polymerization is determined and/or detected by HPLC. In some embodiments, the distribution of the degree of polymerization is determined and/or detected by a combination of analytical instrumentations such as MALDI-MS and SEC. In some embodiments, the degree of polymerization of the oligosaccharide preparation can be determined based on its molecular weight and molecular weight distribution. For example, FIG. 2 shows a MALDI-MS spectrum that illustrates the degrees

of polymerizations of various fractions and the presence of anhydro-subunit containing oligosaccharides (the -18 g/mol MW offset peaks) in all of the observed fractions.

[0079] In some embodiments, the relative abundance of oligosaccharides in a majority of the fractions decreases monotonically with its degree of polymerization. In some embodiments, the relative abundance of oligosaccharides of less than 6, less than 5, less than 4, less than 3, or less than 2 fractions of the oligosaccharide preparation do not decrease monotonically with its degree of polymerization.

[0080] In some embodiments, the relative abundance of oligosaccharides in at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50 DP fractions decreases monotonically with its degree of polymerization. In some embodiments, the relative abundance of oligosaccharides in at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50 consecutive DP fractions decreases monotonically with its degree of polymerization. In some embodiments, the relative abundance of oligosaccharides in at least 5, at least 10, at least 20, or at least 30 DP fractions decreases monotonically with its degree of polymerization. In some embodiments, the relative abundance of oligosaccharides in at least 5, at least 10, at least 20, or at least 30 consecutive DP fractions decreases monotonically with its degree of polymerization.

[0081] In some embodiments, the relative abundance of oligosaccharides in each of the n fractions decreases monotonically with its degree of polymerization. For example, FIG. 10 provides an example of a DP distribution where the relative abundance of oligosaccharides in each of the n fractions decrease monotonically with its DP. For example, in some embodiments, only the relative abundance of oligosaccharides in the DP3 fraction does not decrease monotonically with its degree of polymerization, i.e., the relative abundance of oligosaccharides in the DP3 fraction is lower than the relative abundance of oligosaccharides in the DP4 fraction. In some embodiments, the relative abundance of oligosaccharides in the DP2 fraction is lower than the relative abundance of oligosaccharides in the DP3 fraction.

[0082] In some embodiments, a herein described oligosaccharide preparation has a DP1 fraction content of from about 1% to about 50%, from about 1% to about 40%, from about 1% to about 35%, from about 1% to about 30%, from about 1% to about 25%, from about 1% to about 20%, from about 1% to about 15%, from about 5% to about 50%, from about 5% to about 40%, from about 5% to about 35%, from about 5% to about 30%, from about 5% to about 25%, from about 5% to about 20%, from about 5% to about 15%, from about 10% to about 50%, from about 10% to about 40%, from about 10% to about 35%, from about 10% to about 30%, from about 10% to about 25%, from about 10% to about 20%, or from about 10% to about 15% by weight or by

relative abundance. In some embodiments, the oligosaccharide preparation has a DP1 fraction content of from about 10% to about 35%, from about 10% to about 20%, or from about 10% to about 15% by weight or by relative abundance. In some embodiments, the content of the DP1 fraction is determined by mass spectrometry. In some embodiments, the content of the DP1 fraction is determined by HPLC. In some embodiments, the content of the DP1 fraction is determined by LC-MS/MS or GC-MS.

[0083] In some embodiments, a herein described oligosaccharide preparation has a DP2 fraction content of from about 1% to about 35%, from about 1% to about 30%, from about 1% to about 25%, from about 1% to about 20%, from about 1% to about 15%, from about 1% to about 10%, from about 5% to about 30%, from about 5% to about 25%, from about 5% to about 20%, from about 5% to about 15%, or from about 5% to about 10% by weight or by relative abundance. In some embodiments, the oligosaccharide preparation has a DP2 fraction content of from about 5% to about 25%, from about 5% to about 20%, from about 5% to about 15%, or from about 5% to about 10% by weight or by relative abundance. In some embodiments, the content of the DP2 fraction is determined by mass spectrometry. In some embodiments, the content of the DP2 fraction is determined by HPLC. In some embodiments, the content of the DP2 fraction is determined by LC-MS/MS or GC-MS.

[0084] In some embodiments, a herein described oligosaccharide preparation has a DP3 fraction content of from about 1% to about 30%, from about 1% to about 25%, from about 1% to about 20%, from about 1% to about 15%, from about 1% to about 10%, from about 5% to about 30%, from about 5% to about 25%, from about 5% to about 20%, from about 5% to about 15%, or from about 5% to about 10% by weight or by relative abundance. In some embodiments, the oligosaccharide preparation has a DP3 fraction content of from about 1% to about 15%, from about 1% to about 10%, from about 5% to about 15%, or from about 5% to about 10% by weight or by relative abundance. In some embodiments, the content of the DP3 fraction is determined by MALDI-MS. In some embodiments, the content of the DP3 fraction is determined by HPLC. In some embodiments, the content of the DP3 fraction is determined by LC-MS/MS or GC-MS.

[0085] In some embodiments, a herein described oligosaccharide preparation has a DP4 fraction content of from about 0.1% to about 20%, from about 0.1% to about 15%, from about 0.1% to about 10%, from about 0.1% to about 5%, from about 1% to about 20%, from about 1% to about 15%, from about 1% to about 10%, or from about 1% to about 5% by weight or by relative abundance. In some embodiments, the oligosaccharide preparation has a DP4 fraction content of from about 1% to about 15%, from about 1% to about 10%, or from about 1% to about 5% by weight or by relative abundance. In some embodiments, a herein described oligosaccharide

preparation has a DP5 fraction content of from about 0.1% to about 15%, from about 0.1% to about 10%, from about 0.1% to about 5%, from about 1% to about 15%, from about 1% to about 10%, or from about 1% to about 5% by weight or by relative abundance. In some embodiments, the oligosaccharide preparation has a DP5 fraction content of from about 1% to about 10% or from about 1% to about 5% by weight or by relative abundance. In some embodiments, the content of the DP4 and/or the DP5 fraction is determined by MALDI-MS. In some embodiments, the content of the DP4 and/or the DP5 fraction is determined by HPLC. In some embodiments, the content of the DP4 and/or the DP5 fraction is determined by LC-MS/MS or GC-MS.

[0086] In some embodiments, the ratio of DP2 fraction to DP1 fraction in the oligosaccharide preparation is from about 0.01 to about 0.8, from about 0.02 to about 0.7, from about 0.02 to about 0.6, from about 0.02 to about 0.5, from about 0.02 to about 0.4, from about 0.02 to about 0.3, from about 0.02 to about 0.2, from about 0.1 to about 0.6, from about 0.1 to about 0.5, from about 0.1 to about 0.4, or from about 0.1 to about 0.3 by their weight or relative abundance. In some embodiments, the ratio of DP2 fraction to DP1 fraction in the oligosaccharide preparation is from about 0.02 to about 0.4 by their weight or relative abundance.

[0087] In some embodiments, the ratio of DP3 fraction to DP2 fraction in the oligosaccharide preparation is from about 0.01 to about 0.7, from about 0.01 to about 0.6, from about 0.01 to about 0.5, from about 0.01 to about 0.4, from about 0.01 to about 0.3, or from about 0.01 to about 0.2 by their weight or relative abundance. In some embodiments, the ratio of DP3 fraction to DP2 fraction in the oligosaccharide preparation is from about 0.01 to about 0.3 by their weight or relative abundance.

[0088] In some embodiments, the aggregate content of DP1 and DP2 fractions in the oligosaccharide preparation is less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% by weight or by relative abundance. In some embodiments, the aggregate content of DP1 and DP2 fractions in the oligosaccharide preparation is less than 50%, less than 30%, or less than 10% by weight or by relative abundance.

[0089] In some embodiments, an oligosaccharide preparation described herein has a mean DP value within a range of 2 to 10. In some embodiments, the oligosaccharide preparation has a mean DP value of from about 2 to about 8, from about 2 to about 5, or from about 2 to about 4. In some embodiments, the oligosaccharide preparation has a mean DP value of about 3.5. The mean DP value can be determined by SEC or by elemental analysis.

Anhydro-subunit Level

[0090] In some embodiments, a herein described oligosaccharide preparation comprises one or more anhydro-subunits, i.e., the oligosaccharide preparation comprises one or more anhydro-

subunit containing oligosaccharides. In some embodiments, each of the n fractions of oligosaccharides in a herein described oligosaccharide preparation independently comprises an anhydro-subunit level. For instance, in some embodiments, the DP1 fraction comprises about 10% of anhydro-subunit containing oligosaccharides by relative abundance, and the DP2 fraction comprises about 15% of anhydro-subunit containing oligosaccharides by relative abundance. For another example, in some embodiments, DP1, DP2, and DP3 fractions each comprises about 5%, about 10%, and about 2% of anhydro-subunit containing oligosaccharides by relative abundance, respectively. In some embodiments, two or more fractions of oligosaccharides comprise similar levels of anhydro-subunit containing oligosaccharides. For example, in some embodiments, the DP1 and DP3 fractions each comprises about 5% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, a herein described oligosaccharide preparation does not comprise any anhydro-subunit, i.e., the oligosaccharide preparation does not comprise any anhydro-subunit containing oligosaccharides.

[0091] In some embodiments, each of the 1 to n fractions in a herein described oligosaccharide preparation independently comprises from about 0.1% to 15% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry, LC-MS/MS or GC-MS. In some embodiments, each of the 1 to n fractions in the oligosaccharide preparation independently comprises from about 0.5% to 15% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry, LC-MS/MS or GC-MS. In some embodiments, LC-MS/MS is used to determine the relative abundance for oligosaccharides in the DP1, DP2, and/or DP3 fractions.

[0092] In some embodiments, the presence, the type of species, and/or level of anhydro-subunits can be determined and/or detected by any suitable analytical methods, such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, HPLC, FFF, A4F, or any combination thereof. In some embodiments, the presence and level of anhydro-subunits containing oligosaccharides are determined and/or detected by MALDI-MS, as illustrated by the -18 g/mol MW offset peaks in FIG. 2. In some embodiments, the presence and the type of species of anhydro-subunits are determined and/or detected by NMR, as illustrated by Example 11, FIG. 3, and FIG. 4. In some embodiments, the presence, the type of species, and/or level of anhydro-subunits or anhydro-subunit containing oligosaccharides is determined and/or detected, at least in part, by mass spectrometry such as MALDI-MS. In some embodiments, the presence, the type of species, and/or level of anhydro-subunits or anhydro-subunit containing oligosaccharides is determined and/or detected, at least in part, by NMR. In some embodiments, the presence, the type of species, and/or level of anhydro-subunits or anhydro-subunit containing oligosaccharides

is determined and/or detected, at least in part, by HPLC. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by MALDI-MS. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by LC-MS/MS, as illustrated in FIGs. 21A-21C, 22A-22C, 23A-23C and 24A-24C. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by GC-MS, as illustrated in FIGs. 25A-25B, 26A-26B, 27A-27B and 28A-28B. In some embodiments, GC-MS or LC-MS/MS is used to determine the relative abundance for oligosaccharides in the DP1, DP2, and/or DP3 fractions. In some embodiments, MALDI-MS is used to determine the relative abundance for oligosaccharides in the DP4 fraction or in a higher DP fraction. In some embodiments, the relative abundance of a certain fraction is determined by integrating the area under the peaks of the LC-MS/MS chromatogram that are designated as corresponding to that fraction. In some embodiments, the relative abundance of a certain fraction is determined by integrating the area under the peaks of the GC-MS chromatogram that are designated as corresponding to that fraction.

[0093] In some embodiments, at least one fraction of a herein described oligosaccharide preparation comprises less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, at least one fraction of a herein described oligosaccharide preparation comprises less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, or less than 2% of anhydro-subunit containing oligosaccharides by relative abundance. In other embodiments, at least one fraction of a herein described oligosaccharide preparation comprises greater than 0.5%, greater than 0.8%, greater than 1%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, greater than 15%, greater than 16%, greater than 17%, greater than 18%, greater than 19%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, or greater than 80% of anhydro-subunit containing oligosaccharides by relative abundance. In other embodiments, at least one fraction of a herein described oligosaccharide preparation comprises greater than 20%, greater than 21%, greater than 22%, greater than 23%, greater than 24%, greater than 25%, greater than 26%, greater than 27%, greater than 28%, greater than 29%, or greater than 30% of anhydro-subunit containing

oligosaccharides by relative abundance. In some embodiments, at least one fraction (such as DP1, DP2, and/or DP3) of the oligosaccharide preparation comprises about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, or about 30% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, at least one fraction (such as DP1, DP2, and/or DP3) of the oligosaccharide preparation comprises about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0,8%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, at least one fraction (such as DP1, DP2, and/or DP3) of the oligosaccharide preparation comprises from about 0.1% to about 90%, from about 0.5% to about 90%, from about 0.5% to about 80%, from about 0.5% to about 70%, from about 0.5% to about 60%, from about 0.5% to about 50%, from about 0.5% to about 40%, from about 0.5% to about 30%, from about 0.5% to about 20%, from about 0.5% to about 10%, from about 0.5% to about 9%, from about 0.5% to about 8%, from about 0.5% to about 7%, from about 0.5% to about 6%, from about 0.5% to about 5%, from about 0.5% to about 4%, from about 0.5% to about 3%, from about 0.5% to about 2%, from about 2% to about 9%, from about 2% to about 8%, from about 2% to about 7%, from about 2% to about 6%, from about 2% to about 5%, from about 2% to about 4%, from about 2% to about 3%, or from about 5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the relative abundance is measured by mass spectrometry, LC-MS/MS, or GC-MS. In some embodiments, the DP1 and DP2 fractions each independently comprises from about 0.5% to about 15% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry or by LC-MS/MS or GC-MS.

[0094] In some embodiments, each fraction of a herein described oligosaccharide preparation comprises less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, each fraction of a herein described oligosaccharide preparation comprises less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2% anhydro-subunit containing oligosaccharides by

relative abundance. In other embodiments, each fraction of a herein described oligosaccharide preparation comprises greater than 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of anhydro-subunit containing oligosaccharides by relative abundance. In other embodiments, each fraction of a herein described oligosaccharide preparation comprises greater than 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, each fraction of a herein described oligosaccharide preparation comprises about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, or about 30% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, each fraction of a herein described oligosaccharide preparation comprises about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0,8%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, each fraction of a herein described oligosaccharide preparation comprises from about 0.1% to about 90%, from about 0.1% to about 15%, from about 0.5% to about 90%, from about 0.5% to about 80%, from about 0.5% to about 70%, from about 0.5% to about 60%, from about 0.5% to about 50%, from about 0.5% to about 40%, from about 0.5% to about 30%, from about 0.5% to about 20%, from about 0.5% to about 10%, from about 0.5% to about 9%, from about 0.5% to about 8%, from about 0.5% to about 7%, from about 0.5% to about 6%, from about 0.5% to about 5%, from about 0.5% to about 4%, from about 0.5% to about 3%, from about 0.5% to about 2%, from about 2% to about 9%, from about 1% to about 10%, from about 2% to about 8%, from about 2% to about 7%, from about 2% to about 6%, from about 2% to about 5%, from about 2% to about 4%, from about 2% to about 3%, or from about 5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance.

[0095] In some embodiments, a herein described oligosaccharide preparation comprises less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the oligosaccharide preparation comprises less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%

anhydro-subunit containing oligosaccharides by relative abundance. In other embodiments, the oligosaccharide preparation comprises greater than greater than 0.5%, greater than 0.8%, greater than 1%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, greater than 15%, greater than 16%, greater than 17%, greater than 18%, greater than 19%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, or greater than 80% of anhydro-subunit containing oligosaccharides by relative abundance. In other embodiments, the oligosaccharide preparation comprises greater than 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the oligosaccharide preparation comprises about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, or about 30% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the oligosaccharide preparation comprises about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the oligosaccharide preparation comprises from about 0.1% to about 90%, from about 0.1% to about 15%,from about 0.5% to about 90%, from about 0.5% to about 80%, from about 0.5% to about 70%, from about 0.5% to about 60%, from about 0.5% to about 50%, from about 0.5% to about 40%, from about 0.5% to about 30%, from about 0.5% to about 20%, from about 0.5% to about 10%, from about 0.5% to about 9%, from about 0.5% to about 8%, from about 0.5% to about 7%, from about 0.5% to about 6%, from about 0.5% to about 5%, from about 0.5% to about 4%, from about 0.5% to about 3%, from about 0.5% to about 2%, from about 2% to about 9%, from about 2% to about 8%, from about 2% to about 7%, from about 2% to about 6%, from about 2% to about 5%, from about 2% to about 4%, from about 2% to about 3%, or from about 5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance.

[0096] In some embodiments, the DP1 fraction of a herein described oligosaccharide preparation comprises less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%,

less than 2%, or less than 1% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP1 fraction of a herein described oligosaccharide preparation comprises greater than 0.1%, greater than 0.5%, greater than 0.8%, greater than 1%, greater than 1.5%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, or greater than 15% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP1 fraction of a herein described oligosaccharide preparation comprises about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP1 fraction of a herein described oligosaccharide preparation comprises from about 0.1% to about 15%, from about 0.1% to about 20%, from about 0.5% to about 20%, from 0.5% to about 10%, from about 0.5% to about 15%, from about 1% to about 20%, from about 1% to about 15%, from about 1% to about 10%, from about 2% to about 14%, from about 3% to about 13%, from about 4% to about 12%, from about 5% to about 11%, from about 5% to about 10%, from about 6% to about 9%, or from about 7% to about 8% of anhydro-subunit containing oligosaccharides by relative abundance, or any ranges therebetween.. In some embodiments, the DP1 fraction of a herein described oligosaccharide preparation comprises from about 5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by mass spectrometry. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by LC-MS/MS. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by GC-MS.

[0097] In some embodiments, the DP2 fraction of a herein described oligosaccharide preparation comprises less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP2 fraction of a herein described oligosaccharide preparation comprises greater than 0.1%, greater than 0.5%, greater than 0.8%, greater than 1%, greater than 1.5%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, or greater than 15% of anhydro-subunit

containing oligosaccharides by relative abundance. In some embodiments, the DP2 fraction of a herein described oligosaccharide preparation comprises about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP2 fraction of a herein described oligosaccharide preparation comprises from about 0.1% to about 15%, from about 0.1% to about 20%, from about 0.5% to about 20%, from 0.5% to about 10%, from about 0.5% to about 15%, from about 1% to about 20%, from about 1% to about 15%, from about 1% to about 10%, from about 2% to about 14%, from about 3% to about 13%, from about 4% to about 12%, from about 5% to about 11%, from about 5% to about 10%, from about 6% to about 9%, or from about 7% to about 8% of anhydro-subunit containing oligosaccharides by relative abundance, or any ranges therebetween.. In some embodiments, the DP2 fraction of a herein described oligosaccharide preparation comprises from about 5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by mass spectrometry such as MALDI-MS. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by LC-MS/MS. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by GC-MS.

[0098] In some embodiments, the DP3 fraction of a herein described oligosaccharide preparation comprises less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP3 fraction of a herein described oligosaccharide preparation comprises greater than 0.1%, greater than 0.5%, greater than 0.8%, greater than 1%, greater than 1.5%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, or greater than 15% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the DP3 fraction of a herein described oligosaccharide preparation comprises about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20% of anhydro-subunit containing oligosaccharides by relative abundance. In some

embodiments, the DP3 fraction of a herein described oligosaccharide preparation comprises from about 0.1% to about 15%, from about 0.1% to about 20%, from about 0.5% to about 20%, from 0.5% to about 10%, from about 0.5% to about 15%, from about 1% to about 20%, from about 1% to about 15%, from about 1% to about 10%, from about 2% to about 14%, from about 3% to about 13%, from about 4% to about 12%, from about 5% to about 11%, from about 5% to about 10%, from about 6% to about 9%, or from about 7% to about 8% of anhydro-subunit containing oligosaccharides by relative abundance, or any ranges therebetween.. In some embodiments, the DP3 fraction of a herein described oligosaccharide preparation comprises from about 5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by mass spectrometry such as MALDI-MS. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by LC-MS/MS. In some embodiments, the relative abundance of anhydro-subunit containing oligosaccharides is determined by GC-MS.

[0099] In some embodiments, an anhydro-subunit containing oligosaccharide comprises one or more anhydro-subunits. For instance, a DP1 anhydro-subunit containing oligosaccharide comprises one anhydro-subunit. In some embodiments, a DPn anhydro-subunit containing oligosaccharide can comprise from 1 to n anhydro-subunits. For example, in some embodiments, a DP2 anhydro-subunit containing oligosaccharide comprises one or two anhydro-subunits. In some embodiments, each oligosaccharide in the oligosaccharide preparation independently comprises zero, one, or two anhydro-subunits. In some embodiments, more than 99%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, or 30% of the anhydro-subunit containing oligosaccharides have only one anhydro-subunit. In some embodiments, more than 99%, 95%, 90%, 85%, or 80% of the anhydro-subunit containing oligosaccharides have only one anhydro-subunit.

[00100] In some embodiments, one or more oligosaccharides in the oligosaccharide preparation or in each fraction of the oligosaccharide preparation comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 anhydro-subunits each linked via a glycosidic bond, wherein the glycosidic bond linking each anhydro-subunit are independently chosen. In some embodiments, one or more oligosaccharides in the oligosaccharide preparation or in each fraction of the oligosaccharide preparation comprise 1, 2, or 3 anhydro-subunits each linked via a glycosidic bond, wherein the glycosidic bond linking each anhydro-subunit are independently chosen. In some embodiments, greater than 50%, 60%, 70%, 80%, 90%, or 99% of oligosaccharides in the oligosaccharide preparation or in each fraction comprise 1, 2, or 3 anhydro-subunits each linked via a glycosidic bond, wherein the glycosidic

bond linking each anhydro-subunit are independently chosen. In some embodiments, one or more oligosaccharides in the oligosaccharide preparation or in each fraction comprise 1 anhydro-subunit linked via a glycosidic bond. In some embodiments, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, or greater than 99% of oligosaccharides in the oligosaccharide preparation or in each fraction comprise 1 anhydro-subunit linked via a glycosidic bond.

Anhydro-subunit Species

[00101] In some embodiments, the oligosaccharide preparation comprises different species of anhydro-subunits. In some embodiments, exemplary anhydro-subunit containing oligosaccharides are illustrated in FIG. 5, FIG. 18, and FIG. 19. In some embodiments, the oligosaccharide preparation comprises one or more anhydro-subunits that are products of thermal dehydration of monosaccharides, i.e., anhydro-monosaccharide subunits. In some embodiments, the oligosaccharide preparation comprises one or more anhydro-subunits that are products of reversible thermal dehydration of monosaccharides.

[00102] It is to be understood that an anhydro-monosaccharide (or an anhydro-monosaccharide subunit) refers to one or more species of the thermal dehydration products of the monosaccharide. For example, in some embodiments, an anhydro-glucose refers to 1,6-anhydro-β-D-glucopyranose (levoglucosan) or 1,6-anhydro-β-D-glucofuranose. In some embodiments, a plurality of anhydro-glucose refer to a plurality of 1,6-anhydro-β-D-glucopyranose (levoglucosan), a plurality of 1,6-anhydro-β-D-glucofuranose, a plurality of other thermal dehydration products of glucose, or any combination thereof. Similarly, in some embodiments, a plurality of anhydro-galactose refers to a plurality of any thermal dehydration products of galactose, or any combination thereof.

[00103] In some embodiments, an oligosaccharide preparation as described herein comprises one or more anhydro-glucose, anhydro-galactose, anhydro-mannose, anhydro-allose, anhydro-altrose, anhydro-gulose, anhydro-indose, anhydro-talose, anhydro-fructose, anhydro-ribose, anhydro-arabinose, anhydro-rhamnose, anhydro-lyxose, anhydro-xylose, or any combination of these subunits. In some embodiments, the oligosaccharide preparation comprises one or more anhydro-glucose, anhydro-galactose, anhydro-mannose, or anhydro-fructose subunits. In some embodiments, an oligosaccharide preparation as described herein comprises one or more of: 1,6-anhydro-3-O-β-D-glucopyranosyl-β-D-glucopyranose, 1,6-anhydro-3-O-α-D-glucopyranosyl-β-D-glucopyranose, 1,6-anhydro-2-O-β-D-glucopyranosyl-β-D-glucopyranose, 1,6-anhydro-2-O-α-D-glucopyranosyl-β-D-glucopyranose, 1,6-anhydro-β-D-cellobiose (cellobiosan), 1,6-anhydro-

β-D-cellotriose (cellotriosan), 1,6-anhydro-β-D-cellotetraose (cellotetraosan), 1,6-anhydro-β-D-cellopentaose (cellopentaosan), and 1,6-anhydro-β-D-maltose (maltosan).

[00104] In some embodiments, the oligosaccharide preparation comprises one or more 1,6-anhydro-β-D-glucofuranose subunits. In some embodiments, the oligosaccharide preparation comprises one or more 1,6-anhydro-β-D-glucopyranose (levoglucosan) subunits. For example, FIG. 18 illustrates two DP1 anhydro-subunit containing oligosaccharides (levoglucosan and 1,6-anhydro-β-D-glucofuranose) and a DP2 anhydro-subunit containing oligosaccharide (anhydro-cellobiose).

[00105] The presence and the level of a species of anhydro-subunit can vary based on the feed sugars used to manufacture the oligosaccharide. For instance, in some embodiments, gluco-oligosaccharides comprise anhydro-glucose subunits, galacto-oligosaccharides comprise anhydro-galactose subunits, and gluco-galacto-oligosaccharides comprise anhydro-glucose and anhydro-galactose subunits.

[00106] In some embodiments, the oligosaccharide preparation comprises both 1,6-anhydro-β-D-glucofuranose and 1,6-anhydro-β-D-glucopyranose anhydro-subunits. In some embodiments, at least 0.1%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% of anhydro-subunits are selected from a group consisting of 1,6-anhydro-β-D-glucofuranose and 1,6-anhydro-β-D-glucopyranose. In some embodiments, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of anhydro-subunits are 1,6-anhydro-β-D-glucofuranose. In some embodiments, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, or 60% of anhydro-subunits are 1,6-anhydro-β-D-glucopyranose.

[00107] In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is from about 10:1 to 1:10, 9:1 to 1:10, 8:1 to 1:10, 7:1 to 1:10, 6:1 to 1:10, 5:1 to 1:10, 4:1 to 1:10, 3:1 to 1:10, 2:1 to 1:10, 10:1 to 1:9, 10:1 to 1:8, 10:1 to 1:7, 10:1 to 1:6, 10:1 to 1:5, 10:1 to 1:4, 10:1 to 1:3, 10:1 to 1:2, or 1:1 to 3:1 in the preparation. In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:8, 1:9, or 1:10 in the preparation. In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about 2:1 in the preparation.

[00108] In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about from 10:1 to 1:10, 9:1 to 1:10, 8:1 to 1:10, 7:1 to 1:10, 6:1 to 1:10, 5:1 to 1:10, 4:1 to 1:10, 3:1 to 1:10, 2:1 to 1:10, 10:1 to 1:9, 10:1 to 1:8, 10:1 to 1:7, 10:1 to 1:6, 10:1 to 1:5, 10:1 to 1:4, 10:1 to 1:3, 10:1 to 1:2, or 1:1 to 3:1 in each fraction. In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about 10:1, 9:1, 8:1,

7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:8, 1:9, or 1:10 in each fraction. In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about 2:1 in each fraction.

[00109] In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about from 10:1 to 1:10, 9:1 to 1:10, 8:1 to 1:10, 7:1 to 1:10, 6:1 to 1:10, 5:1 to 1:10, 4:1 to 1:10, 3:1 to 1:10, 2:1 to 1:10, 10:1 to 1:9, 10:1 to 1:8, 10:1 to 1:7, 10:1 to 1:6, 10:1 to 1:5, 10:1 to 1:4, 10:1 to 1:3, 10:1 to 1:2, or 1:1 to 3:1 in at least one fraction. In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:8, 1:9, or 1:10 in at least one fraction. In some embodiments, the ratio of 1,6-anhydro-β-D-glucofuranose to 1,6-anhydro-β-D-glucopyranose is about 2:1 in at least one fraction.

[00110] In some embodiments, a herein described oligosaccharide preparation comprises anhydro-subunit containing DP2 oligosaccharides. In some embodiments, the oligosaccharide preparation comprises anhydro-lactose, anhydro-sucrose, anhydro-cellobiose, or a combination thereof. In some embodiment, the oligosaccharide preparation comprises from about 2 to 20, 2 to 15, 5 to 20, 5 to 15, or 5 to 10 species of DP2 anhydro-subunit containing oligosaccharides. In some embodiments, an oligosaccharide preparation described herein does not comprise cellobiosan or does not comprise a detectable level of cellobiosan.

[00111] In some embodiments, a herein described oligosaccharide preparation comprises one or more anhydro-subunits that are sugar caramelization products. In some embodiments, the oligosaccharide preparation comprises one or more anhydro-subunits are sugar caramelization products selected from the group consisting of: methanol; ethanol; furan; methyl glyoxal; 2-methyl furan; vinyl acetate; glycolaldehyde; acetic acid; acetol; furfural; 2-furanmethanol; 3-furanmethanol; 2-hydroxy cyclopent-2-en-1-one; 5-methyl furfural; 2(5H)-furanone; 2 methyl cyclopentenolone; levoglucosenone; cyclic hydroxyl lactone; 1,4,3,6-dianhydro-α-D-glucopyranose; dianhydro glucopyranose; and 5-hydroxy methyl furfural (5-hmf).

[00112] In some embodiments, in the oligosaccharide preparation or in at least one of the DP fractions, the anhydro-subunits that are caramelization products are less abundant than the anhydro-subunits that are products of reversible thermal dehydration of a monosaccharide. In some embodiments, in the oligosaccharide preparation or in at least one of the fractions, the anhydro-subunits that are caramelization products are more abundant than the anhydro-subunits that are products of reversible thermal dehydration of a monosaccharide. In some embodiments, in the oligosaccharide preparation or in at least one of the fractions, anhydro-subunits that are

caramelization products and anhydro-subunits that are products of reversible thermal dehydration of a monosaccharide have similar abundance.

[00113] In some embodiments, from about 0.01% to about 50%, from about 0.01% to about 40%, from about 0.01% to about 30%, from about 0.01% to about 20%, from about 0.01% to about 10%, from about 0.01% to about 5%, from about 0.01% to about 4%, from about 0.01% to about 3%, from about 0.01% to about 2%, from about 0.01% to about 1%, from about 0.01% to about 0.5%, from about 0.1% to about 50%, from about 0.1% to about 40%, from about 0.1% to about 30%, from about 0.1% to about 20%, from about 0.1% to about 10%, from about 0.1% to about 5%, from about 0.1% to about 4%, from about 0.1% to about 3%, from about 0.1% to about 2%, from about 0.1% to about 1%, or from about 0.1% to about 0.5% of the anhydro-subunits in a herein described oligosaccharide preparation are caramelization products. In some embodiments, from about 0.1% to about 5%, from about 0.1% to about 2 %, or from about 0.1% to about 1% of the anhydro-subunits in the oligosaccharide preparation are caramelization products. In some embodiments, less than 50%, less than 40%, less than 30%, less than 25%, less than 20%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of the anhydro-subunits in the oligosaccharide preparation are caramelization products.

[00114] In some embodiments, from about 0.01% to about 50%, from about 0.01% to about 40%, from about 0.01% to about 30%, from about 0.01% to about 20%, from about 0.01% to about 10%, from about 0.01% to about 5%, from about 0.01% to about 4%, from about 0.01% to about 3%, from about 0.01% to about 2%, from about 0.01% to about 1%, from about 0.01% to about 0.5%, from about 0.1% to about 50%, from about 0.1% to about 40%, from about 0.1% to about 30%, from about 0.1% to about 20%, from about 0.1% to about 10%, from about 0.1% to about 5%, from about 0.1% to about 4%, from about 0.1% to about 3%, from about 0.1% to about 2%, from about 0.1% to about 1%, or from about 0.1% to about 0.5% of the anhydro-subunits in at least one fraction (e.g., DP1, DP2 and/or DP3) of a herein described preparation are caramelization products. In some embodiments, from about 0.1% to about 5%, from about 0.1% to about 2 %, or from about 0.1% to about 1% of the anhydro-subunits in at least one fraction (e.g., DP1, DP2 and/or DP3) of the preparation are caramelization products. In some embodiments, less than 50%, 40%, 30%, 25%, 20%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the anhydro-subunits in at least one fraction of the preparation are caramelization products. In some embodiments, less than 20%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than

5%, less than 4%, less than 3%, less than 2%, or less than 1% of the anhydro-subunits in the DP1, DP2, and/or DP3 fractions of a herein described oligosaccharide preparation are caramelization products.

[00115] In some embodiments, from about 0.01% to about 50%, from about 0.01% to about 40%, from about 0.01% to about 30%, from about 0.01% to about 20%, from about 0.01% to about 10%, from about 0.01% to about 5%, from about 0.01% to about 4%, from about 0.01% to about 3%, from about 0.01% to about 2%, from about 0.01% to about 1%, from about 0.01% to about 0.5%, from about 0.1% to about 50%, from about 0.1% to about 40%, from about 0.1% to about 30%, from about 0.1% to about 20%, from about 0.1% to about 10%, from about 0.1% to about 5%, from about 0.1% to about 4%, from about 0.1% to about 3%, from about 0.1% to about 2%, from about 0.1% to about 1%, or from about 0.1% to about 0.5% of the anhydro-subunits in each fraction of a herein described oligosaccharide preparation are caramelization products. In some embodiments, from about 0.1% to about 5%, from about 0.1% to about 2 %, or from about 0.1% to about 1% of the anhydro-subunits in each fraction of the preparation are caramelization products. In some embodiments, less than 50%, less than 40%, less than 30%, less than 20%, less than 25%, less than 20%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of the anhydro-subunits in each fraction of the preparation are caramelization products.

[00116] In some embodiments, each of the oligosaccharides in a herein described oligosaccharide preparation independently and optionally comprises an anhydro-subunit. In some embodiments, two or more independent oligosaccharides comprise the same or different anhydro-subunits. In some embodiments, two or more independent oligosaccharides comprise different anhydro-subunits. For example, in some embodiments, the oligosaccharide preparation comprise a DP1 anhydro-subunit containing oligosaccharide that comprises a 1,6-anhydro-β-D-glucopyranose and a DP2 anhydro-subunit containing oligosaccharide that comprises a 1,6-anhydro-β-D-glucofuranose subunit. In some embodiments, one or more oligosaccharides in the oligosaccharide preparation comprise two or more the same or different anhydro-subunits.

[00117] In some embodiments, in any fraction of the oligosaccharide preparation that has a degree of polymerization equal or greater than 2 (i.e., DP2 to DPn fractions), an anhydro-subunit can be linked to one or more regular or anhydro-subunits. In some embodiments, in the DP2 to DPn fractions, at least one anhydro-subunit is linked to one, two, or three other regular or anhydro-subunits. In some embodiments, in the DP2 to DPn fractions, at least one anhydro-subunit is linked to one or two regular subunits. In some embodiments, in the DP2 to DPn fractions, at least one

anhydro-subunit is linked to one regular subunit. In some embodiments, in any of the DP2 to DPn fractions, more than 99%, 90%, 80%, 70%, 60%, 50%, 40%, or 30% of anhydro-subunits are linked to one regular subunit. In some embodiments, in each of the DP2 to DPn fraction, more than 99%, 90%, 80%, 70%, 60%, 50%, 40%, or 30% of anhydro-subunits are linked to one regular subunit.

[00118] In some embodiments, in any fraction of the oligosaccharide preparation that has a degree of polymerization equal or greater than 2 (i.e., DP2 to DPn fractions), an anhydro-subunit can be located at a chain-end of an oligosaccharide. In some embodiments, in any fraction of the oligosaccharide preparation that has a degree of polymerization equal or greater than 3 (i.e., DP3 to DPn fractions), an anhydro-subunit can be located at a position that is not a chain-end of an oligosaccharide. In some embodiments, greater than 99%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, or 30% of the anhydro-subunits in the DP2 to DPn fractions are located at the chain-end of the oligosaccharides. In some embodiments, greater than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the anhydro-subunit containing oligosaccharides comprise a chain-end anhydro-subunit. In some embodiments, greater than 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the anhydro-subunit containing oligosaccharides comprise a chain-end anhydro-subunit.

[00119] In some embodiments, greater than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the anhydro-subunit containing oligosaccharides comprise a chain-end anhydro-subunit. In some embodiments, greater than 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the anhydro-subunit containing oligosaccharides comprise a chain-end anhydro-subunit.

Glycosidic Linkages

[00120] In some embodiments, a herein described oligosaccharide preparation used in the methods described herein comprises a variety of glycosidic linkages. The type and distribution of the glycosidic linkages can depend on the source and manufacturing method of the oligosaccharide preparation. In some embodiments, the type and distribution of various glycosidic linkages can be determined and/or detected by any suitable methods known in the art such as NMR. For example, in some embodiments, the glycosidic linkages are determined and/or detected by ^{1}H NMR, ^{13}C NMR, 2D NMR such as 2D JRES, HSQC, HMBC, DOSY, COSY, ECOSY, TOCSY, NOESY, or ROESY, or any combination thereof. In some embodiments, the glycosidic linkages are determined and/or detected, at least in part, by proton NMR. In some embodiments, the glycosidic linkages are determined and/or detected, at least in part, by ^{13}C NMR. In some

embodiments, the glycosidic linkages are determined and/or detected, at least in part, by 2D ^{1}H, ^{13}C- HSQC NMR.

[00121] In some embodiments, a herein described oligosaccharide preparation comprises one or more α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,4) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-β glycosidic linkages, or any combination thereof.

[00122] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of about from 0 to about 60 mol%, from about 5% to about 55 mol%, from about 5% to about 50 mol%, from about 5% to about 45 mol%, from about 5% to about 40 mol%, from about 5% to about 35 mol%, from about 5% to about 30 mol%, from about 5% to about 25 mol%, from about 10% to about 60 mol%, from about 10% to about 55 mol%, from about 10% to about 50 mol%, from about 10% to about 45 mol%, from about 10% to about 40 mol%, from about 10% to about 35 mol%, from about 15% to about 60 mol%, from about 15% to about 55 mol%, from about 15% to about 50 mol%, from about 15% to about 45 mol%, from about 15% to about 40 mol%, from about 15% to about 35 mol%, from about 20% to about 60 mol%, from about 20% to about 55 mol%, from about 20% to about 50 mol%, from about 20% to about 45 mol%, from about 20% to about 40 mol%, from about 20% to about 35 mol%, from about 25% to about 60 mol%, from about 25% to about 55 mol%, from about 25% to about 50 mol%, from about 25% to about 45 mol%, from about 25% to about 40 mol%, or from about 25% to about 35 mol% of α-(1,6) glycosidic linkages.

[00123] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 50 mol%, from about 0 to about 40 mol%, from about 0 to about 35 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 5% to about 40 mol%, from about 5% to about 35 mol%, from about 5% to about 30 mol%, from about 5% to about 25 mol%, from about 5% to about 20 mol%, from about 10% to about 40 mol%, from about 10% to about 35 mol%, from about 10% to about 20 mol%, from about 15% to about 40 mol%, from about 15% to about 35 mol%, from about 15% to about 30 mol%, from about 15% to about 25 mol%, or from about 15% to about 20 mol% of α-(1,3) glycosidic linkages.

[00124] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 40 mol%, from about 0 to about 35 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 0 to about 15 mol%, from about 0 to about 10 mol%, from about 2% to about 30 mol%, from about

2% to about 25 mol%, from about 2% to about 20 mol%, from about 2% to about 15 mol%, from about 2% to about 10 mol%, from about 3% to about 30 mol%, from about 3% to about 25 mol%, from about 3% to about 20 mol%, from about 3% to about 15 mol%, from about 3% to about 10 mol%, from about 5% to about 30 mol%, from about 5% to about 25 mol%, from about 5% to about 20 mol%, from about 5% to about 15 mol%, or from about 5% to about 10 mol% of α-(1,2) glycosidic linkages.

[00125] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 40 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 0 to about 15 mol%, from about 0 to about 10 mol%, or from about 0 to about 5 mol% of α-(1,4) glycosidic linkages. In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of less than 40 mol%, less than 30 mol%, less than 20 mol%, less than 15 mol%, less than 10 mol%, less than 9 mol%, less than 8 mol%, less than 7 mol%, less than 6 mol%, less than 5 mol%, less than 4 mol%, less than 3 mol%, or less than 2 mol% of α-(1,4) glycosidic linkages.

[00126] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 40 mol%, from about 0 to about 35 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 0 to about 15 mol%, from about 0 to about 10 mol%, from about 2% to about 30 mol%, from about 2% to about 25 mol%, from about 2% to about 20 mol%, from about 2% to about 15 mol%, from about 2% to about 10 mol%, from about 5% to about 30 mol%, from about 5% to about 25 mol%, from about 5% to about 20 mol%, from about 5% to about 15 mol%, from about 5% to about 10 mol%, from about 8% to about 30 mol%, from about 8% to about 25 mol%, from about 8% to about 20 mol%, from about 8% to about 15 mol%, or from about 10% to about 15 mol% of β-(1,6) glycosidic linkages.

[00127] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 40 mol%, from about 0 to about 35 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 0 to about 15 mol%, from about 0 to about 10 mol%, from about 2% to about 30 mol%, from about 2% to about 25 mol%, from about 2% to about 20 mol%, from about 2% to about 15 mol%, from about 2% to about 10 mol%, from about 3% to about 30 mol%, from about 3% to about 25 mol%, from about 3% to about 20 mol%, from about 3% to about 15 mol%, from about 3% to about 10 mol%, from about 5% to about 30 mol%, from about 5% to about 25 mol%, from about 5% to about 20 mol%, from about 5% to about 15 mol%, or from about 5% to about 10 mol% of β-(1,4) glycosidic linkages.

[00128] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 40 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 0 to about 15 mol%, from about 0 to about 10 mol%, from about 0 to about 5 mol%, from about 1% to about 20 mol%, from about 1% to about 15 mol%, from about 1% to about 10 mol%, from about 1% to about 5 mol%, from about 2% to about 20 mol%, from about 2% to about 15 mol%, from about 2% to about 10 mol%, or from about 2% to about 5 mol% of β-(1,2) glycosidic linkages. In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of less than 40 mol%, less than 30 mol%, less than 20 mol%, less than 15 mol%, less than 10 mol%, less than 9 mol%, less than 8 mol%, less than 7 mol%, less than 6 mol%, less than 5 mol%, less than 4 mol%, less than 3 mol%, or less than 2 mol% of β-(1,2) glycosidic linkages.

[00129] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of from about 0 to about 40 mol%, from about 0 to about 30 mol%, from about 0 to about 25 mol%, from about 0 to about 20 mol%, from about 0 to about 15 mol%, from about 0 to about 10 mol%, from about 0 to about 5 mol%, from about 1% to about 20 mol%, from about 1% to about 15 mol%, from about 1% to about 10 mol%, from about 1% to about 5 mol%, from about 2% to about 20 mol%, from about 2% to about 15 mol%, from about 2% to about 10 mol%, or from about 2% to about 5 mol% of β-(1,3) glycosidic linkages. In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution of less than 40 mol%, less than 30 mol%, less than 20 mol%, less than 15 mol%, less than 10 mol%, less than 9 mol%, less than 8 mol%, less than 7 mol%, less than 6 mol%, less than 5 mol%, less than 4 mol%, less than 3 mol%, or less than 2 mol% of β-(1,3) glycosidic linkages.

[00130] In some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution that is different from a glycosidic bond type distribution of non-synthetic oligosaccharide preparations. For example, in some embodiments, the oligosaccharide preparations have a glycosidic bond type distribution that is different from that of the base nutritional compositions. In some embodiments, the base nutritional compositions comprise a natural carbohydrate source, such as starch and plant fibers. Some of the natural carbohydrate sources have a high percentage of α-(1,4), α-(1,6), and/or β-(1,6) glycosidic linkages. Accordingly, in some embodiments, the oligosaccharide preparations have a lower percentage of α-(1,4) glycosidic linkages than the base nutritional composition. In some embodiments, the oligosaccharide preparations have a lower percentage of α-(1,6) glycosidic linkages than the base nutritional composition. In other embodiments, the oligosaccharide preparations have a higher percentage of α-(1,6) glycosidic linkages than the base nutritional composition. In some

embodiments, the oligosaccharide preparations have a lower percentage of β-(1,6) glycosidic linkages than the base nutritional composition. In some embodiments, the oligosaccharide preparation comprises glycosidic linkages that are not readily digestible or hydrolysable by enzymes.

[00131] Specifically, in some embodiments, the α-(1,2), α-(1,3), α-(1,4), α-(1,6), β-(1,2), β-(1,3), β-(1,4), and/or β-(1,6) glycosidic linkages in the glycosidic bond type distribution of a herein described oligosaccharide preparations is at least 50 mol%, at least 40 mol%, at least 30 mol%, at least 20 mol%, at least 15 mol%, at least 10 mol%, at least 5 mol%, at least 2 mol%, or at least 1 mol% lower than that of the base nutritional composition. In some embodiments, the α-(1,2), α-(1,3), α-(1,4), α-(1,6), β-(1,2), β-(1,3), β-(1,4), and/or β-(1,6) glycosidic linkages in the glycosidic bond type distribution of the oligosaccharide preparations is at least 50 mol%, at least 40 mol%, at least 30 mol%, at least 20 mol%, at least 15 mol%, at least 10 mol%, at least 5 mol%, at least 2 mol%, or at least 1 mol% higher than that of the base nutritional composition.

[00132] It should be understood by one of skill in the art that certain types of glycosidic linkages may not be applicable to oligosaccharides comprising certain type of monosaccharides. For example, in some embodiments, the oligosaccharide preparation comprises α-(1,2) glycosidic linkages and α-(1,6) glycosidic linkages. In other embodiments, the oligosaccharide preparation comprises α-(1,2) glycosidic linkages and β-(1,3) glycosidic linkages. In some embodiments, the oligosaccharide preparation comprises α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, and β-(1,6) glycosidic linkages. In some embodiments, the oligosaccharide preparation comprises α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,4) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, and β-(1,6) glycosidic linkages.

Molecular Weight

[00133] The molecular weight and molecular weight distribution of the herein described oligosaccharide preparations can be determined by any suitable analytical means and instrumentation, such as end group method, osmotic pressure (osmometry), ultracentrifugation, viscosity measurements, light scattering method, SEC, SEC-MALLS, FFF, A4F, HPLC, and mass spectrometry. In some embodiments, the molecular weight and molecular weight distribution are determined by mass spectrometry, such as MALDI-MS, LC-MS, or GC-MS. In some embodiments, the molecular weight and molecular weight distribution are determined by size exclusion chromatography (SEC), such as gel permeation chromatography (GPC). In other embodiments, the molecular weight and molecular weight distribution are determined by HPLC.

In some embodiments, the molecular weight and molecular weight distribution are determined by MALDI-MS.

[00134] In some embodiments, a herein described oligosaccharide preparation has a weight average molecular weight of from about 100 to about 10000 g/mol, from about 200 to about 8000 g/mol, from about 300 to about 5000 g/mol, from about 500 to about 5000 g/mol, from about 700 to about 5000 g/mol, from about 900 to about 5000 g/mol, from about 1100 to about 5000 g/mol, from about 1300 to about 5000 g/mol, from about 1500 to about 5000 g/mol, from about 1700 to about 5000 g/mol, from about 300 to about 4500 g/mol, from about 500 to about 4500 g/mol, from about 700 to about 4500 g/mol, from about 900 to about 4500 g/mol, from about 1100 to about 4500 g/mol, from about 1300 to about 4500 g/mol, from about 1500 to about 4500 g/mol, from about 1700 to about 4500 g/mol, from about 1900 to about 4500 g/mol, from about 300 to about 4000 g/mol, from about 500 to about 4000 g/mol, from about 700 to about 4000 g/mol, from about 900 to about 4000 g/mol, from about 1100 to about 4000 g/mol, from about 1300 to about 4000 g/mol, from about 1500 to about 4000 g/mol, from about 1700 to about 4000 g/mol, from about 1900 to about 4000 g/mol, from about 300 to about 3000 g/mol, from about 500 to about 3000 g/mol, from about 700 to about 3000 g/mol, from about 900 to about 3000 g/mol, from about 1100 to about 3000 g/mol, from about 1300 to about 3000 g/mol, from about 1500 to about 3000 g/mol, from about 1700 to about 3000 g/mol, from about 1900 to about 3000 g/mol, from about 2100 to about 3000 g/mol, from about 300 to about 2500 g/mol, from about 500 to about 2500 g/mol, from about 700 to about 2500 g/mol, from about 900 to about 2500 g/mol, from about 1100 to about 2500 g/mol, from about 1300 to about 2500 g/mol, from about 1500 to about 2500 g/mol, from about 1700 to about 2500 g/mol, from about 1900 to about 2500 g/mol, from about 2100 to about 2500 g/mol, from about 300 to about 1500 g/mol, from about 500 to about 1500 g/mol, from about 700 to about 1500 g/mol, from about 900 to about 1500 g/mol, from about 1100 to about 1500 g/mol, from about 1300 to about 1500 g/mol, from about 2000 to about 2800 g/mol, from about 2100 to about 2700 g/mol, from about 2200 to about 2600 g/mol, from about 2300 to about 2500 g/mol, or from about 2320 to about 2420 g/mol. In some embodiments, the weight average molecular weight of the oligosaccharide preparation is from about 2000 to about 2800 g/mol, from about 2100 to about 2700 g/mol, from about 2200 to about 2600 g/mol, from about 2300 to about 2500 g/mol, or from about 2320 to about 2420 g/mol. In some embodiments, the oligosaccharide preparation has a weight average molecular weight in a range from at least 500 g/mol, 750 g/mol, 1000 g/mol, or 1500 g/mol to at most 1750 g/mol, 2000 g/mol, 2250 g/mol, 2500 g/mol, or 3000 g/mol. In some embodiments, the weight average molecular weight of a herein described oligosaccharide preparation is determined by HPLC according to Example 9.

[00135] In some embodiments, a herein described oligosaccharide preparation has a number average molecular weight of from about 100 to about 10000 g/mol, from about 200 to about 8000 g/mol, from about 300 to about 5000 g/mol, from about 500 to about 5000 g/mol, from about 700 to about 5000 g/mol, from about 900 to about 5000 g/mol, from about 1100 to about 5000 g/mol, from about 1300 to about 5000 g/mol, from about 1500 to about 5000 g/mol, from about 1700 to about 5000 g/mol, from about 300 to about 4500 g/mol, from about 500 to about 4500 g/mol, from about 700 to about 4500 g/mol, from about 900 to about 4500 g/mol, from about 1100 to about 4500 g/mol, from about 1300 to about 4500 g/mol, from about 1500 to about 4500 g/mol, from about 1700 to about 4500 g/mol, from about 1900 to about 4500 g/mol, from about 300 to about 4000 g/mol, from about 500 to about 4000 g/mol, from about 700 to about 4000 g/mol, from about 900 to about 4000 g/mol, from about 1100 to about 4000 g/mol, from about 1300 to about 4000 g/mol, from about 1500 to about 4000 g/mol, from about 1700 to about 4000 g/mol, from about 1900 to about 4000 g/mol, from about 300 to about 3000 g/mol, from about 500 to about 3000 g/mol, from about 700 to about 3000 g/mol, from about 900 to about 3000 g/mol, from about 1100 to about 3000 g/mol, from about 1300 to about 3000 g/mol, from about 1500 to about 3000 g/mol, from about 1700 to about 3000 g/mol, from about 1900 to about 3000 g/mol, from about 2100 to about 3000 g/mol, from about 300 to about 2500 g/mol, from about 500 to about 2500 g/mol, from about 700 to about 2500 g/mol, from about 900 to about 2500 g/mol, from about 1100 to about 2500 g/mol, from about 1300 to about 2500 g/mol, from about 1500 to about 2500 g/mol, from about 1700 to about 2500 g/mol, from about 1900 to about 2500 g/mol, from about 2100 to about 2500 g/mol, from about 300 to about 2000 g/mol, from about 500 to about 300 to 2000 g/mol, from about 700 to about 2000 g/mol, from about 900 to about 2000 g/mol, from about 1100 to about 2000 g/mol, from about 300 to about 1500 g/mol, from about 500 to about 1500 g/mol, from about 700 to about 1500 g/mol, from about 900 to about 1500 g/mol, from about 1100 to about 1500 g/mol, from about 1300 to about 1500 g/mol, from about 1000 to about 2000 g/mol, from about 1100 to about 1900 g/mol, from about 1200 to about 1800 g/mol, from about 1300 to about 1700 g/mol, from about 1400 to about 1600 g/mol, or from about 1450 to about 1550 g/mol. In some embodiments, the number average molecular weight of the oligosaccharide preparation is from about 1000 to about 2000 g/mol, from about 1100 to about 1900 g/mol, from about 1200 to about 1800 g/mol, from about 1300 to about 1700 g/mol, 1400 to 1600 g/mol, or 1450-1550 g/mol. In some embodiments, the oligosaccharide preparation has a number average molecular weight in a range from at least 500 g/mol, 750 g/mol, 1000 g/mol, or 1500 g/mol to at most 1750 g/mol, 2000 g/mol, 2250 g/mol, 2500 g/mol, or 3000 g/mol. In some embodiments, the number average

molecular weight of a herein described oligosaccharide preparation is determined by HPLC according to Example 9.

Types of Oligosaccharides

[00136] In some embodiments, a herein described oligosaccharide preparations comprises one or more species of monosaccharide subunits. In some embodiments, the oligosaccharide preparation can comprise oligosaccharides with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more different species of monosaccharides subunits.

[00137] In some embodiments, a herein described oligosaccharide preparation comprises one or more monosaccharide subunits selected from a group consisting of: triose, tetrose, pentose, hexose, heptose, and any combination thereof, wherein each of the said triose, tetrose, pentose, hexose, or heptose subunit is independently and optionally functionalized and/or replaced with one of its corresponding anhydro-subunits. In some embodiments, the corresponding anhydro-subunit is a product of reversible thermal dehydration of the monosaccharide subunit. In some embodiments, the corresponding anhydro-subunit is a caramelization product of the monosaccharide subunit.

[00138] In some embodiments, a herein described oligosaccharide preparation comprises pentose subunits, hexose subunits, or any combination thereof, wherein each of the said pentose or hexose subunit is independently and optionally functionalized and/or replaced with one of its corresponding anhydro-subunits. In some embodiments, the oligosaccharide preparation comprises hexose subunits, wherein each of the said hexose subunits is independently and optionally replaced with one of its corresponding anhydro-subunits.

[00139] As used herein, a tetrose refers to a monosaccharide with four carbon atoms, such as erythrose, threose, and erythrulose. As used herein, a pentose refers to a monosaccharide with five carbon atoms, such as arabinose, lyxose, ribose, and xylose. As used herein, a hexose refers to a monosaccharide with six carbon atoms, such as allose, altrose, glucose, mannose, gulose, idose, galactose, talose, psicose, fructose, sorbose, and tagatose. As used herein, a heptose refers to a monosaccharide with seven carbon atoms, such as sedoheptulose and mannoheptulose.

[00140] In some embodiments, a herein described oligosaccharide preparation comprises glucose subunit, wherein at least one glucose subunit is optionally replaced with an anhydro-glucose subunit. In some embodiments, a herein described oligosaccharide preparation comprises galactose subunit, wherein at least one galactose subunit is optionally replaced with anhydro-galactose subunit. In some embodiments, a herein described oligosaccharide preparation comprises galactose and glucose subunits, wherein at least one galactose subunit or at least one glucose subunit is optionally replaced with one of its corresponding anhydro-subunits. In some

embodiments, a herein described oligosaccharide preparation comprises fructose and glucose subunits, wherein at least one fructose subunit or at least one glucose subunit is optionally replaced with one of its corresponding anhydro-subunits. In some embodiments, a herein described oligosaccharide preparation comprises mannose and glucose subunit, wherein at least one mannose subunit or at least one glucose subunit is optionally replaced with one of its corresponding anhydro-subunits.

[00141] In some embodiments, a herein described oligosaccharide preparation comprises a gluco-galactose-oligosaccharide preparation, a gluco-oligosaccharide preparation, a galacto-oligosaccharide preparation, a fructo-oligosaccharide preparation, a manno-oligosaccharide preparation, an arabino-oligosaccharide preparation, a xylo-oligosaccharide preparation, a gluco-fructo-oligosaccharide preparation, a gluco-manno-oligosaccharide preparation, a gluco-arabino-oligosaccharide preparation, a gluco-xylo-oligosaccharide preparation, a galacto-fructo-oligosaccharide preparation, a galacto-manno-oligosaccharide preparation, a galacto-arabino-oligosaccharide preparation, a galacto-xylo-oligosaccharide preparation, a fructo-manno-oligosaccharide preparation, a fructo-arabino-oligosaccharide preparation, a fructo-xylo-oligosaccharide preparation, a manno-arabino-oligosaccharide preparation, a manno-xylo-oligosaccharide preparation, an arabino-xylo-oligosaccharide preparation, a galacto-arabino-xylo-oligosaccharide preparation, a fructo-galacto-xylo-oligosaccharide preparation, an arabino-fructo-manno-xylo-oligosaccharide preparation, a gluco-fructo-galacto-arabino-oligosaccharide preparation, a fructo-gluco-arabino-manno-xylo oligosaccharide preparation, a gluco-galacto-fructo-manno-arabinoxylo-oligosaccharide preparation, or any combinations thereof; wherein each of the monosaccharide subunit within the preparation is independently and optionally functionalized and/or replaced with one of its corresponding anhydro-subunits.

[00142] In certain embodiments, a herein described oligosaccharide preparation comprises more than 99% of glucose subunits by weight. In some embodiments, the oligosaccharide preparation comprises only glucose subunits.

[00143] In some embodiments, a herein described oligosaccharide preparation comprises about 45% to 55% of glucose subunits and about 55% to 45% of galactose subunits by weight. In some embodiments, the oligosaccharide preparation comprises about 50% glucose and 50% galactose subunits by weight.

[00144] In some embodiments, a herein described oligosaccharide preparation comprises about 80% to 95% of glucose subunits and about 20% to 5% of mannose subunits by weight. In some embodiments, the oligosaccharide preparation comprises about 85 % to 90% of glucose subunits and about 15% to 10% of mannose subunits by weight.

[00145] In some embodiments, a herein described oligosaccharide preparation comprises about 80% to 95% of glucose subunits and about 20% to 5% of galactose subunits by weight. In some embodiments, the oligosaccharide preparation comprises about 85 % to 90% of glucose subunits and about 15% to 10% of galactose subunits by weight.

[00146] In some embodiments, a herein described oligosaccharide preparation comprises about 80% to 95% of glucose subunits, 0% to 8% of galactose subunits, and 5% to 20% of mannose subunits by weight. In some embodiments, the oligosaccharide preparation comprises about 80 % to 90% of glucose subunits, 1% to 5% of galactose subunits, and 10% to 15% of mannose subunits by weight.

[00147] In some embodiments, an oligosaccharide preparation described herein comprises from about 1 wt% to about 100 wt%, from about 50 wt% to about 100 wt%, from about 80 wt% to about 98 wt%, or from about 85 wt% to about 95 wt% of glucose subunits, or any ranges therebetween. In some embodiments, galactose subunits are present in an oligosaccharide preparation described herein at an amount of from about 0 wt% to about 90 wt%, from about 1 wt% to about 50 wt%, from about 2 wt% to about 20wt%, or from about 5 wt% to about 15 wt%, or any ranges therebetween. In some embodiments, mannose subunits are present in an oligosaccharide preparation described herein at an amount of from about 0 wt% to about 90 wt%, from about 1 wt% to about 50 wt%, from about 2 wt% to about 20wt%, or from about 5 wt% to about 15 wt%, or any ranges therebetween.

D- vs. L- Form

[00148] In some embodiments, at least one monosaccharide subunit in an oligosaccharide is in L-form. In some embodiments, at least one monosaccharides subunit in an oligosaccharide is in D-form. In some embodiments, the monosaccharide subunits in a herein described oligosaccharide preparation are in their naturally-abundant form, for example, D-glucose, D-xylose, and L-arabinose.

[00149] In some embodiments, a herein described oligosaccharide preparation comprises a mixture of L- and D-forms of monosaccharide subunits. In some embodiments, the ratio of monosaccharide subunits in L- to D- or in D- to L- form is about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:12, about 1:14, about 1:16, about 1:18, about 1:20, about 1:25, about 1:30, about 1:35, about 1:40, about 1:45, about 1:50, about 1:55, about 1:60, about 1:65, about 1:70, about 1:75, about 1:80, about 1:85, about 1:90, about 1:100 or about 1:150.

Functionalized Oligosaccharides

[00150] In some embodiments, one or more oligosaccharides in a herein described oligosaccharide preparation are independently functionalized. Functionalized oligosaccharides can be produced by, for example, combining one or more sugars with one or more functionalizing compounds in the presence of a catalyst. Methods of producing functionalized oligosaccharides are described in WO 2012/118767, WO 2014/031956, and WO/2016/122887, which are hereby incorporated by reference in their entirety and for their disclosure.

[00151] In some embodiments, the functionalizing compound comprises one or more acid groups (e.g., -COOH), hydroxyl groups, or N-containing groups (e.g., –CN, $-NO_2$, and $-N(R_a)_2$, wherein R_a is hydrogen, alkyl, alkenyl, alkynyl, haloalkyl, heteroalkyl, cycloalkyl, aryl, heterocycloalkyl, or heteroaryl groups), S-containing groups (e.g., thiol and sulfates), halides (e.g., -Cl), P-containing groups (e.g., phosphate), or any combination thereof. In some embodiments, the functionalizing compound is linked to at least one monosaccharide subunit via an ether, ester, oxygen-sulfur, amine, or oxygen-phosphorous bond. In some embodiments, one or more functionalizing compounds are linked to a monosaccharide subunit via a single linkage. In some embodiments, at least one functionalizing compound is linked to one or two oligosaccharides via two or more linkages.

[00152] It is to be understood that for each oligosaccharide in the oligosaccharide preparation, each of the described embodiments is independent and can be combined as if each and every combination were listed separately; thus, any combination of the embodiments, are encompassed by the present disclosure. For instance, the various embodiments can be grouped into several categories that include but are not limited to (i) the presence or absence of anhydro-subunit; (ii) the number and level of anhydro-subunit, (iii) the type of species of anhydro-subunit, (iv) the location of anhydro-subunit, (v) the degree of polymerization, (vi) the molecular weight, (vii) the presence or absence of any functional groups, (viii) the type of the oligosaccharide, (ix) the type of glycosidic linkage, and (x) the L- versus D-form. Accordingly, the described oligosaccharide preparation comprises a plurality of oligosaccharides of different species. In some embodiments, In some embodiments, a herein described oligosaccharide preparation comprises at least 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or 10^{10} different oligosaccharide species. In some embodiments, the preparation comprises at least 10^3, 10^4, 10^5, 10^6, or 10^9 different oligosaccharide species. In some embodiments, the preparation comprises at least 10^3 different oligosaccharide species.

IV. Methods of Manufacturing Oligosaccharide Preparations

[00153] In one aspect, provided herein are methods of manufacturing oligosaccharide preparations. In some embodiments, provided herein are methods of manufacturing oligosaccharide preparations suitable for use in a nutritional composition, such as an animal feed

composition, or being fed directly to an animal. In one aspect, provided herein are methods of manufacturing an oligosaccharide preparation, the method comprising heating an aqueous composition comprising one or more feed sugars and a catalyst to a temperature and for a time sufficient to induce polymerization, wherein the catalyst is selected from the group consisting of: (+)-camphor-10-sulfonic acid; 2-pyridinesulfonic acid; 3-pyridinesulfonic acid; 8-hydroxy-5-quinolinesulfonic acid hydrate; α-hydroxy-2-pyridinemethanesulfonic acid; (β)-camphor-10-sulfonic acid; butylphosphonic acid; diphenylphosphinic acid; hexylphosphonic acid; methylphosphonic acid; phenylphosphinic acid; phenylphosphonic acid; tert-butylphosphonic acid; SS)-VAPOL hydrogenphosphate; 6-quinolinesulfonic acid, 3-(1-pyridinio)-1-propanesulfonate; 2-(2-pyridinyl)ethanesulfonic acid; 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate; 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate; bis(4-methoxyphenyl)phosphinic acid; phenyl(3,5-xylyl)phosphinic acid; L-cysteic acid monohydrate; poly(styrene sulfonic acid -co- divinylbenzene); lysine; Ethanedisulfonic acid; Ethanesulfonic acid; Isethionic acid; Homocysteic acid; HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); 2-Hydroxy-3-morpholinopropanesulfonic acid; 2-(N-morpholino)ethanesulfonic acid; Methanesulfonic acid; Methaniazide; Naphthalene-1-sulfonic acid; Naphthalene-2-sulfonic acid; Perfluorobutanesulfonic acid; 6-sulfoquinovose; Triflic acid; 2-aminoethanesulfonic acid; Benzoic acid; Chloroacetic acid; Trifluoroacetic acid; Caproic acid; Enanthic acid; Caprylic acid; Pelargonic acid; Lauric acid; Pamitic acid; Stearic acid; Arachidic acid; Aspartic acid; Glutamic acid; Serine; Threonine; Glutamine; Cysteine; Glycine; Proline; Alanine; Valine; Isoleucine; Leucine; Methionine; Phenylalanine; Tyrosine; Tryptophan,, and wherein the oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 (DP1 fraction) to n (DPn fraction), wherein n is an integer greater than or equal to 2. In some embodiments, n is an integer greater than or equal to 3. In some embodiments, n is an integer within a range of 1 to 100, such as 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, 40, or 50. In some embodiments, the polymerization of the feed sugars is achieved by a step-growth polymerization. In some embodiments, the polymerization of the feed sugars is achieved by polycondensation.

Feed Sugar

[00154] In some embodiments, a method of manufacturing oligosaccharide preparations described herein comprises heating one or more types of feed sugars. In some embodiments, the one or more feed sugars comprise monosaccharides, disaccharides, trisaccharides, tetrasaccharides, or any mixtures thereof.

[00155] In some embodiments, the one or more feed sugars comprise glucose. In some embodiments, the one or more feed sugars comprise glucose and galactose. In some embodiments, the one or more feed sugars comprise glucose, xylose, and galactose. In some embodiments, the one or more feed sugars comprise glucose and mannose. In some embodiments, the one or more feed sugars comprise glucose and fructose. In some embodiments, the one or more feed sugars comprise glucose, fructose, and galactose. In some embodiments, the one or more feed sugars comprise glucose, galactose, and mannose.

[00156] In some embodiments, the one or more feed sugars comprise disaccharides such as lactose, sucrose and cellobiose. In some embodiments, the one or more feed sugars comprise trisaccharides, such as maltotriose or raffinose. In certain embodiments, the one or more feed sugar comprise glucose, mannose, galactose, xylose, malto-dextrin, arabinose, or galactose, or any combinations thereof. In certain embodiments, the one or more feed sugars comprise sugar syrup such as corn syrup. In some embodiments, the one or more feed sugars comprise glucose and lactose. In some embodiments, the one or more feed sugars comprise glucose and sucrose.

[00157] In some embodiments, the type of feed sugars can impact the resulting manufactured oligosaccharide preparations. For example, in some variations where the one or more feed sugars are all glucose, the resulting oligosaccharide preparations comprise gluco-oligosaccharides preparations. In other embodiments, where the one or more feed sugars are all mannose, the resulting oligosaccharide preparations comprise manno-oligosaccharide preparations. In some embodiments, wherein the one or more feed sugars comprise glucose and galactose, the resulting oligosaccharide preparations comprise gluco-galacto-oligosaccharide preparations. In yet other embodiments, where the one or more feed sugars comprise xylose, glucose and galactose, the resulting oligosaccharide preparations comprise gluco-galacto-xylo-oligosaccharide preparations.

[00158] In some embodiments, each of the one or more feed sugars can be independently in its de-hydrate or hydrate form. In some embodiments, the one or more feed sugars comprise glucose, galactose, fructose, mannose, or any combination thereof, and wherein each of the glucose, galactose, fructose, or mannose is independently in its mono-hydrate or de-hydrate form. In some embodiments, the one or more feed sugars comprise a monosaccharide mono-hydrate such as glucose monohydrate. In some embodiments, the one or more feed sugars comprise a saccharide di-hydrate such as trehalose di-hydrate. In some embodiments, the one or more feed sugars comprise at least one sugar in its de-hydrate form and at least one sugar in its hydrate form.

[00159] In some embodiments, the one or more feed sugars can be provided as a sugar solution, in which the sugars are combined with water and fed into the reactor. In some embodiments, the sugars can be fed into the reactor in a solid form and combined with water in the reactor. In some

embodiments, the one or more feed sugars are combined and mixed before the addition of water. In some embodiments, the one or more feed sugars are combined into water and mixed thereafter.

[00160] In some embodiments, the method comprises combining two or more feed sugars with the catalyst to produce an oligosaccharide preparation. In some embodiments, the two or more feed sugars comprise from glucose, galactose, fructose, mannose, lactose, or any combination thereof. In some embodiments, the method comprises combining a mixture of sugars (e.g., monosaccharides, disaccharides, and/or trisaccharides) with the catalyst to produce an oligosaccharide preparation. In other embodiments, the method comprises combining a mixture of sugars and sugar alcohols with the catalyst to produce an oligosaccharide preparation.

[00161] In some embodiments, the one or more feed sugars comprise functionalized or modified sugars. Functionalized or modified sugars can comprise amino sugars, sugar acids, sugar alcohols, sugar amides, sugar ethers, or any combination thereof. In some embodiments, amino sugars refer to sugar molecules in which a hydroxyl group is replaced with an amine group. Exemplary amino sugars include, but are not limited to, N-Acetyl-d-glucosamine, mannosamine, neuraminic acid, muramic acid, N-acetyl-neuramin, N-acetyl-muramic, N-acetyl-galactosamine, N-acetyl-mannosa, N-glycolylneuram, acarviosin, D-glucosamine, and D-galactosamine.

[00162] In some embodiments, sugar acids refer to sugars with a carboxyl group. Exemplary sugar acids include, but are not limited to, aldonic acids (such as glyceric acid, xylonic acid, gluconic acid, and ascorbic acid), ulosonic acids (such as neuraminic acid and ketodeoxyoctulosonic acid), uronic acids (such as glucuronic acid, galacturonic acid, and iduronic acid), and aldaric acids (such as tartaric acid, mucic acid, and saccharic acid).

[00163] In some embodiments, sugar alcohols refer to sugar-derived polyols. Exemplary sugar alcohols include, but are not limited to, ethylene glycol, arabitol, glycerol, erythritol, threitol, xylitol, ribitol, mannitol, sorbitol, galactitol, fucitol, iditol, inositol, and volemitol.

[00164] In embodiments, sugar amides refer to sugar molecules that contain a -C(=O)-N- group. In embodiments, sugar ethers refer to sugar molecules that contain an ether bond, such as glucosides.

[00165] In some embodiments, the functionalized or modified sugar acids comprise glucosamine, N-acetylglucosamine, glucuronic acid, galacturonic acid, glucitol, xylitol, mannitol, sorbitol. In some embodiments, the one of more feed sugars comprise deoxysugars, such as fucose, rhamnose, deoxyribose, or fuculose.

[00166] In some embodiments, a herein described method of manufacturing oligosaccharide preparation is performed at gram scale. In some embodiments, a herein described method of manufacturing oligosaccharide preparation is performed at kilogram or higher scale. Accordingly,

in some embodiments, the method comprises heating an aqueous composition comprising one or more feed sugars at a quantity of more than 0.5, more than 1, more than 2, more than 3, more than 4, more than 5, more than 6, more than 7, more than 9, more than 10, more than 100, or more than 1000 kg. In some embodiments, the method comprises heating an aqueous composition comprising one or more feed sugars at a quantity of no more than 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 10, 100, 1000, or 1500 kg. In some embodiments, the method comprises heating an aqueous composition comprising one or more feed sugars at a quantity of more than 1 kg.

Catalyst

[00167] In some embodiments, a herein described method of manufacturing oligosaccharide preparation comprises the addition of one or more catalysts. In some embodiments, the catalyst provided herein comprises one or more acids. In some embodiments, the catalyst provided herein comprises mineral acid, carboxylic acid; amino acid; sulfonic acid; boronic acid; phosphonic acid; phosphinic acid; sulfuric acid; phosphoric acid; poly(styrene sulfonic acid-co-vinylbenzyl-imidazolium sulfate-co-divinylbenzene); poly(styrene sulfonic acid-co-divinylbenzene); (+)-camphor-10-sulfonic acid; 2-pyridinesulfonic acid; 3-pyridinesulfonic acid; 8-hydroxy-5-quinolinesulfonic acid hydrate; α-hydroxy-2-pyridinemethanesulfonic acid; (β)-camphor-10-sulfonic acid; butylphosphonic acid; diphenylphosphinic acid; hexylphosphonic acid; methylphosphonic acid; phenylphosphinic acid; phenylphosphonic acid; tert-butylphosphonic acid; SS)-VAPOL hydrogenphosphate; 6-quinolinesulfonic acid; 3-(1-pyridinio)-1-propanesulfonate; 2-(2-pyridinyl)ethanesulfonic acid; 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate; 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate; bis(4-methoxyphenyl)phosphinic acid; phenyl(3,5-xylyl)phosphinic acid; L-cysteic acid monohydrate; acetic acid; propionic acid; butanoic acid; glutamic acid; lysine; Ethanedisulfonic acid; Ethanesulfonic acid; Isethionic acid; Homocysteic acid; HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); 2-Hydroxy-3-morpholinopropanesulfonic acid; 2-(N-morpholino)ethanesulfonic acid; Methanesulfonic acid; Methaniazide; Naphthalene-1-sulfonic acid; Naphthalene-2-sulfonic acid; Perfluorobutanesulfonic acid; 6-sulfoquinovose; Triflic acid; 2-aminoethanesulfonic acid; Benzoic acid; Chloroacetic acid; Trifluoroacetic acid; Caproic acid; Enanthic acid; Caprylic acid; Pelargonic acid; Lauric acid; Pamitic acid; Stearic acid; Arachidic acid; Aspartic acid; Glutamic acid; Serine; Threonine; Glutamine; Cysteine; Glycine; Proline; Alanine; Valine; Isoleucine; Leucine; Methionine; Phenylalanine; Tyrosine; Tryptophan; polymeric acid; carbon-supported acid; or any combination thereof.

[00168] In some embodiments, the catalyst provided herein comprises: (+)-camphor-10-sulfonic acid; 2-pyridinesulfonic acid; 3-pyridinesulfonic acid; 8-hydroxy-5-quinolinesulfonic acid hydrate; α-hydroxy-2-pyridinemethanesulfonic acid; (β)-camphor-10-sulfonic acid; butylphosphonic acid; diphenylphosphinic acid; hexylphosphonic acid; methylphosphonic acid; phenylphosphinic acid; phenylphosphonic acid; tert-butylphosphonic acid; SS)-VAPOL hydrogenphosphate; 6-quinolinesulfonic acid, 3-(1-pyridinio)-1-propanesulfonate; 2-(2-pyridinyl)ethanesulfonic acid; 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate; 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate; bis(4-methoxyphenyl)phosphinic acid; phenyl(3,5-xylyl)phosphinic acid; L-cysteic acid monohydrate; poly(styrene sulfonic acid -co- divinylbenzene); lysine; Ethanedisulfonic acid; Ethanesulfonic acid; Isethionic acid; Homocysteic acid; HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); 2-Hydroxy-3-morpholinopropanesulfonic acid; 2-(N-morpholino)ethanesulfonic acid; Methanesulfonic acid; Methaniazide; Naphthalene-1-sulfonic acid; Naphthalene-2-sulfonic acid; Perfluorobutanesulfonic acid; 6-sulfoquinovose; Triflic acid; 2-aminoethanesulfonic acid; Benzoic acid; Chloroacetic acid; Trifluoroacetic acid; Caproic acid; Enanthic acid; Caprylic acid; Pelargonic acid; Lauric acid; Pamitic acid; Stearic acid; Arachidic acid; Aspartic acid; Glutamic acid; Serine; Threonine; Glutamine; Cysteine; Glycine; Proline; Alanine; Valine; Isoleucine; Leucine; Methionine; Phenylalanine; Tyrosine; Tryptophan; or any combination thereof.

[00169] In some embodiments, the catalyst provided herein is (+)-camphor-10-sulfonic acid. In some embodiments, the catalyst provided herein is 2-pyridinesulfonic acid. In some embodiments, the catalyst provided herein is 3-pyridinesulfonic acid. In some embodiments, the catalyst provided herein is 8-hydroxy-5-quinolinesulfonic acid hydrate. In some embodiments, the catalyst provided herein is α-hydroxy-2-pyridinemethanesulfonic acid. In some embodiments, the catalyst provided herein is (β)-camphor-10-sulfonic acid. In some embodiments, the catalyst provided herein is butylphosphonic acid. In some embodiments, the catalyst provided herein is diphenylphosphinic acid. In some embodiments, the catalyst provided herein is hexylphosphonic acid. In some embodiments, the catalyst provided herein is methylphosphonic acid. In some embodiments, the catalyst provided herein is phenylphosphinic acid. In some embodiments, the catalyst provided herein is phenylphosphonic acid. In some embodiments, the catalyst provided herein is tert-butylphosphonic acid. In some embodiments, the catalyst provided herein is SS)-VAPOL hydrogenphosphate. In some embodiments, the catalyst provided herein is 6-quinolinesulfonic acid. In some embodiments, the catalyst provided herein is 3-(1-pyridinio)-1-propanesulfonate. In some embodiments, the catalyst provided herein is 2-(2-

pyridinyl)ethanesulfonic acid. In some embodiments, the catalyst provided herein is 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate. In some embodiments, the catalyst provided herein is 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate. In some embodiments, the catalyst provided herein is bis(4-methoxyphenyl)phosphinic acid. In some embodiments, the catalyst provided herein is phenyl(3,5-xylyl)phosphinic acid. In some embodiments, the catalyst provided herein is L-cysteic acid monohydrate. In some embodiments, the catalyst provided herein is poly(styrene sulfonic acid -co- divinylbenzene). In some embodiments, the catalyst provided herein is lysine.

[00170] In some embodiments, the catalyst is Ethanedisulfonic acid. In some embodiments, the catalyst is Ethanesulfonic acid. In some embodiments, the catalyst is Isethionic acid. In some embodiments, the catalyst is Homocysteic acid. In some embodiments, the catalyst is HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)). In some embodiments, the catalyst is HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). In some embodiments, the catalyst is 2-Hydroxy-3-morpholinopropanesulfonic acid. In some embodiments, the catalyst is 2-(N-morpholino) ethanesulfonic acid. In some embodiments, the catalyst is Methanesulfonic acid. In embodiments, the catalyst is Naphthalene-1-sulfonic acid. In some embodiments, the catalyst is some embodiments, the catalyst is Methaniazide. In some Naphthalene-2-sulfonic acid. In some embodiments, the catalyst is Perfluorobutanesulfonic acid. In some embodiments, the catalyst is 6-sulfoquinovose. In some embodiments, the catalyst is Triflic acid. In some embodiments, the catalyst is 2-aminoethanesulfonic acid. In some embodiments, the catalyst is Benzoic acid. In some embodiments, the catalyst is Chloroacetic acid. In some embodiments, the catalyst is Trifluoroacetic acid. In some embodiments, the catalyst is Caproic acid. In some embodiments, the catalyst is Enanthic acid. In some embodiments, the catalyst is Caprylic acid. In some embodiments, the catalyst is Pelargonic acid. In some embodiments, the catalyst is Lauric acid. In some embodiments, the catalyst is Pamitic acid. In some embodiments, the catalyst is Stearic acid. In some embodiments, the catalyst is Arachidic acid. In some embodiments, the catalyst is Aspartic acid. In some embodiments, the catalyst is Glutamic acid. In some embodiments, the catalyst is Serine. In some embodiments, the catalyst is Threonine. In some embodiments, the catalyst is Glutamine. In some embodiments, the catalyst is Cysteine. In some embodiments, the catalyst is Glycine. In some embodiments, the catalyst is Proline. In some embodiments, the catalyst is Alanine. In some embodiments, the catalyst is Valine. In some embodiments, the catalyst is Isoleucine. In some embodiments, the catalyst is Leucine. In some embodiments, the catalyst is Methionine. In some embodiments, the catalyst is Phenylalanine. In some embodiments, the catalyst is Tyrosine. In some embodiments, the catalyst is Tryptophan. In some

embodiments, the catalyst provided herein is a polymeric catalyst or a carbon-supported catalyst disclosed in WO 2016122887, which is hereby incorporated by reference in its entirety and for its disclosure.

[00171] In some embodiments, the catalyst provided herein is present in an amount of from about 0.01% to about 5%, from about 0.02% to about 4%, from about 0.03% to about 3%, or from about 0.05% to about 2% of the one or more feed sugars by dry weight. In some embodiments, the catalyst provided herein is present in an amount of from about 1% to 2% of the one or more feed sugars by dry weight. In some embodiments, the catalyst provided herein is present in an amount of about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3.0% of the one or more feed sugars by dry weight.

[00172] In some embodiments, the catalyst provided herein is present in an amount of from about 0.01% to about 5%, from about 0.02% to about 4%, from about 0.03% to about 3%, or from about 0.05% to about 2% of the aqueous composition by dry weight. In some embodiments, the catalyst provided herein is present in an amount of from about 1% to 2% of the aqueous composition by dry weight. In some embodiments, the catalyst provided herein is present in an amount of about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3.0% of the aqueous composition by dry weight.

[00173] In some embodiments, the catalyst provided herein is a combination of two or more different catalysts. In some embodiments, the catalyst comprises a recyclable catalyst such as resins and polymeric catalysts and a non-recyclable catalyst. In some embodiments, where the catalyst comprises at least two different catalysts, each of the catalyst is present in an amount provided herein. In other embodiments, where the catalyst comprises at least two different catalysts, the at least two different catalysts are present in aggregate in an amount provided herein.

[00174] In some embodiments, the catalyst is added into the aqueous composition in a dry form. In other embodiments, the catalyst is added into the aqueous composition in a wet form such as in an aqueous solution. In some embodiment, the catalyst is combined with the one or more feed sugars before the addition of water. In other embodiments, the catalyst is dissolved into water before its combining with the one or more feed sugars. In some embodiments, the method provided herein comprises producing an aqueous composition by combining the one or more feed sugars in the de-hydrate form and the catalyst in a wet form (e.g., as an aqueous solution).

Addition of Water

[00175] In some embodiments, a herein described method of manufacturing oligosaccharide preparations comprises adding water to form an aqueous composition. In some embodiments, all or part of the water in the aqueous composition is added as free water. In other embodiments, all of the water in the aqueous composition is added as bonded water, for example, in saccharide mono- or di-hydrate. In some embodiments, all of the water in the aqueous composition is added as bonded water in monosaccharide mono-hydrate, such as glucose mono-hydrate. In certain embodiments, all or part of the water in the aqueous composition is added with the catalyst, i.e., via a catalyst solution.

Water Content

[00176] As the methods of manufacturing the oligosaccharide preparations proceed, water can be produced through reaction. For example, in some embodiments, water is produced (i) with the formation of a glycosidic bond, (ii) with the formation of an anhydro-subunit, or (iii) through other mechanisms or sources. As the sugar condensation and dehydration reactions both involve water, in some embodiments, the water content influences the composition of the oligosaccharide preparation.

[00177] Further, in some embodiments, water content influences the viscosity of the aqueous composition, which in turn can affect the effectiveness of mixing of the aqueous composition. For example, in some embodiments, an overly viscous aqueous composition can lead to an undesirable heterogeneous catalyst distribution in the aqueous composition. Moreover, in some embodiments, very low water content can lead to the solidification of the aqueous composition, which prevents effective mixing. On the other hand, in some other embodiments, exceedingly high water content can impede sugar condensation reaction and lower the level of the anhydro-subunits. Accordingly, the present disclosure describes suitable water content for the manufacturing of oligosaccharide preparations.

[00178] In some embodiments, a herein described method of manufacturing oligosaccharide preparation comprises forming and/or heating an aqueous composition. In some embodiments, the aqueous composition comprises from about 0% to about 80%, from about 0% to about 70%, from about 0% to about 60%, from about 0% to about 50%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, from about 0% to about 25%, from about 0% to about 20%, from about 0% to about 19%, from about 0% to about 18%, from about 0% to about 17%, from about 0% to about 16%, from about 0% to about 15%, from about 0% to about 14%, from about 0% to about 13%, from about 0% to about 12%, from about 0% to about 11%, from about 0% to about 10%, from about 0% to about 9%, from about 0% to about 8%, from about 0%

to about 7%, from about 0% to about 6%, from about 0% to about 5%, from about 0% to about 4%, from about 0% to about 3%, from about 0% to about 2%, or from about 0% to about 1% of water by total weight. In some embodiments, the aqueous composition comprises from about 1% to about 20%, from about 1% to about 18%, from about 1% to about 16%, from about 1% to about 14%, from about 1% to about 12%, from about 1% to about 10%, from about 1% to about 8%, from about 1% to about 6%, or from about 1% to about 4% of water by total weight. In some embodiments, the aqueous composition comprises from about 3% to about 16%, from about 3% to about 14%, from about 3% to about 12%, from about 3% to about 10%, from about 3% to about 8%, from about 3% to about 6%, from about 5% to about 16%, from about 5% to about 14%, from about 5% to about 12%, from about 5% to about 10%, from about 7% to about 16%, from about 7% to about 14%, from about 7% to about 12%, from about 7% to about 10%, or from about 8% to about 10% of water by total weight. In some embodiments, the aqueous composition comprises about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% of water by total weight. In some embodiments, the aqueous composition comprises about 9% water by total weight. It should be understood, however, that the amount of water in the aqueous composition can be adjusted based on the reaction conditions and specific catalyst used. In some embodiments, the water content in the aqueous composition as disclosed above is measured at the beginning of the reaction, for example, before heating the feed sugars. In some embodiments, the water content in the aqueous composition as disclosed above is measured at the end of the polymerization or condensation reaction. In some embodiments, the water content in the aqueous composition as disclosed above is measured as an average water content of the beginning of the reaction and at the end of the reaction.

[00179] In certain embodiments, a method described herein can further comprise monitoring the content of water present in the aqueous composition and/or the ratio of water to sugars or catalyst over a period of time. In some embodiments, the method further comprises removing at least a portion of water in the aqueous composition, for example, by distillation. Any method known in the art can be used to remove water from the aqueous composition, including, for example, by vacuum filtration, vacuum distillation, heating, steam, hot air, and/or evaporation.

[00180] In some embodiments, herein described oligosaccharide preparations are hygroscopic. Thus, in some embodiments, the hygroscopicity of the feed sugars and the oligosaccharides formed in the polymerization can affect the rate by which the water can be removed from the aqueous composition.

[00181] In some embodiments, a herein described method comprises removing at least a portion of water in the aqueous composition such that the water content in the aqueous composition is from about 1% to about 20%, from about 1% to about 18%, from about 1% to about 16%, from about 1% to about 14%, from about 1% to about 12%, from about 1% to about 10%, from about 1% to about 8%, from about 2% to about 16%, from about 2% to about 14%, from about 2% to about 12%, from about 2% to about 10%, from about 2% to about 8%, from about 2% to about 6%, from about 4% to about 16%, from about 4% to about 14%, from about 4% to about 12%, from about 4% to about 10%, from about 4% to about 8%, from about 6% to about 16%, from about 6% to about 12%, from about 6% to about 10%, or from about 6% to about 8% by total weight. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that the water content in the aqueous composition is from about 2% to about 10%, from about 2% to about 8%, or from about 4% to about 8% by total weight. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that the water content in the aqueous composition is about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% by total weight. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that the water content in the aqueous composition is from about 4% to about 8 % by total weight. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that, at the end of the polymerization and/or condensation reaction, the water content in the aqueous composition is a water content as disclosed above. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that, at the beginning of the polymerization and/or condensation reaction, the water content in the aqueous composition is a water content as disclosed above. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that, the average water content in the aqueous composition at the beginning and the end of the polymerization and/or condensation reaction is within a range as disclosed above. In some embodiments, the method comprises removing at least a portion of water in the aqueous composition such that, throughout the polymerization and/or condensation reaction, the water content in the aqueous composition remains within a range as disclosed above.

[00182] In some embodiments, a herein described method comprises adding at least a portion of water in the aqueous composition such that the water content in the aqueous composition is from about 1% to about 20%, from about 1% to about 18%, from about 1% to about 16%, from about 1% to about 14%, from about 1% to about 12%, from about 1% to about 10%, from about 1% to about 8%, from about 2% to about 16%, from about 2% to about 14%, from about 2% to about

12%, from about 2% to about 10%, from about 2% to about 8%, from about 2% to about 6%, from about 4% to about 16%, from about 4% to about 14%, from about 4% to about 12%, from about 4% to about 10%, from about 4% to about 8%, from about 6% to about 16%, from about 6% to about 12%, from about 6% to about 10%, or from about 6% to about 8% by total weight. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that the water content in the aqueous composition is from about 2% to about 10%, from about 2% to about 8%, or from about 4% to about 8% by total weight. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that the water content in the aqueous is about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% by total weight. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that the water content in the aqueous composition is from about 4% to about 8% by total weight. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that, at the end of the polymerization and/or condensation reaction, the water content in the aqueous composition is a water content as disclosed above. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that, at the beginning of the polymerization and/or condensation reaction, the water content in the aqueous composition is a water content as disclosed above. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that, the average water content in the aqueous composition at the beginning and the end of the polymerization and/or condensation reaction is within a range as disclosed above. In some embodiments, the method comprises adding at least a portion of water in the aqueous composition such that, throughout the polymerization and/or condensation reaction, the water content in the aqueous composition remains within a range as disclosed above.

[00183] In some embodiments, the degrees of polymerization of the oligosaccharides and/or the amount and type of the anhydro-subunits within the oligosaccharide preparation can be regulated by adjusting or controlling the content of water present in the aqueous composition throughout the manufacturing process. For example, in some embodiments, the degrees of polymerization of the oligosaccharides and the amount of the anhydro-subunits are increased by decreasing the water content.

[00184] Accordingly, in some embodiments, a herein described method comprises in-process control (IPC) of the water content, which can comprise monitoring water content, maintaining water content, increasing water content, decreasing water content, or any combination thereof. In some embodiments, an IPC process comprises maintaining the water content while the aqueous composition is heated to a temperature described herein. In some embodiments, the method

comprises maintaining the water content for the time sufficient to induce polymerization. In some embodiments, the method comprises maintaining the water content within a disclosed range by either adding water or removing water from the aqueous composition, or both. In some embodiments, the method comprises maintaining the water content within a disclosed range by distillation. In some embodiments, the method comprises maintaining the water content within a disclosed range by vacuum distillation. In some embodiments, the method comprises maintaining the water content within a disclosed range by distillation under atmosphere pressure.

[00185] In some embodiments, the water content of the aqueous composition is maintained within a range of from about 1% to about 20%, from about 1% to about 18%, from about 1% to about 16%, from about 1% to about 14%, from about 1% to about 12%, from about 1% to about 10%, from about 1% to about 8%, from about 2% to about 16%, from about 2% to about 14%, from about 2% to about 12%, from about 2% to about 10%, from about 2% to about 8%, from about 2% to about 6%, from about 4% to about 16%, from about 4% to about 14%, from about 4% to about 12%, from about 4% to about 10%, from about 4% to about 8%, from about 6% to about 16%, from about 6% to about 12%, from about 6% to about 10%, or from about 6% to about 8% by total weight. In some embodiments, the water content of the aqueous composition is maintained within a range of from about 2% to about 10%, from about 2% to about 8%, or from about 4% to about 8% by total weight. In some embodiments, the water content of the aqueous composition is maintained within a range of from about 2% to about 8% by total weight.

[00186] The water content of the aqueous composition can be determined by a variety of analytical methods and instruments. In some embodiments, the water content is determined by an evaporation method (e.g., loss on drying technique), a distillation method, or a chemical reaction method (e.g., Karl Fischer titration). In some embodiments, the water content is determined by an analytical instrument such as a moisture analyzer. In some embodiments, the water content is determined by Karl Fischer titration.

[00187] In some embodiments, the water content of the aqueous composition is measured during the reaction and used to implement in-process control (IPC) of the water content. In certain embodiments, the water content of the reaction is measured by Karl-Fisher titration, IR spectroscopy, NIR spectroscopy, conductivity, viscosity, density, mixing torque, or mixing energy. In some embodiments, the measurement of the water content of the reaction is used to control an apparatus that actively adjusts the water content of the reaction, such as a water addition pump or flow valve.

[00188] Without being bound by theory, it is believed that water content during the sugar polymerization and/or condensation reaction can affect the level of the anhydro-subunits in a

herein described oligosaccharide preparation. For example, as illustrated in FIG. 29, in some embodiments, a higher water content correlates with a lower level of anhydro-subunits. In some embodiments, a lower reaction temperature can correlate with a lower level of anhydro-subunits content.

Temperature

[00189] In some embodiments, the degrees of polymerization of the oligosaccharides and/or the amount and type of the anhydro-subunits within the oligosaccharide preparation can be regulated by adjusting the temperature, to which the aqueous composition is heated. In some embodiments, a herein described method of manufacturing an oligosaccharide preparation comprises heating the aqueous composition to a temperature of from about 80 °C to about 250 °C, from about 90°C to about 200 °C, from about 100 °C to about 200 °C, from about 100 °C to about 180 °C, from about 110 °C to about 170 °C, from about 120 °C to about 160 °C, from about 130 °C to about 150 °C, or from about 135 °C to about 145 °C. In some embodiments, the method of manufacturing an oligosaccharide preparation comprises heating the aqueous composition to a temperature of from about 100 °C to about 200 °C, from about 100 °C to about 180 °C, from about 110 °C to about 170 °C, from about 120 °C to about 160 °C, from about 130 °C to about 150 °C, or from about 135 °C to about 145 °C. In some embodiments, the method of manufacturing an oligosaccharide preparation comprises heating the aqueous composition to a temperature of from about 135 °C to about 145 °C. In other embodiments, the method of manufacturing an oligosaccharide preparation comprises heating the aqueous composition to a temperature of from about 125 °C to about 135 °C.

Reaction Time

[00190] In some embodiments, a herein described method of manufacturing an oligosaccharide preparation comprises heating the aqueous composition for a sufficient time. In some embodiments, the degrees of polymerization of the oligosaccharides manufactured according to the methods described herein can be regulated by the reaction time.

[00191] In some embodiments, the sufficient time is prescribed by a number of hours. For example, in some embodiments, the sufficient time is at least 30 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hour, at least 8 hours, at least 9 hours, or at least 10 hours. In some embodiments, the sufficient time is from about 1 to about 24 hours, from about 1 to about 16 hours, from about 1 to about 8 hours, from about 1 to about 4 hours, from about 1 to about 3 hours, from about 1 to about 2 hours, from about 2 to about 12 hours, from about 2 to about 10 hours, from about 2 to about 8 hours, from about 2

to about 6 hours, from about 2 to about 4 hours, from about 3 to about 8 hours, from about 3 to about 6 hours, from about 3 to about 5 hours, or from about 3 to about 4 hours.

[00192] In some embodiments, the sufficient time is determined by measuring one or more chemical or physical properties of the oligosaccharide preparation, for example, water content, viscosity, molecular weight, anhydro-subunit content, and/or the distribution of degree of polymerization.

[00193] In some embodiments, the molecular weight of the oligosaccharide preparation is monitored during polymerization. In some embodiments, the method comprises heating the aqueous composition for a time sufficient for the aqueous composition to reach a number average molecular weight or weight average molecular weight as described herein. In certain embodiments, the method comprises heating the aqueous composition for a time sufficient for the aqueous composition to reach a number average molecular weight within a range of from about 300 to about 5000 g/mol, from about 500 to about 5000 g/mol, from about 700 to about 5000 g/mol, from about 500 to about 2000 g/mol, from about 700 to about 2000 g/mol, from about 700 to about 1500 g/mol, from about 300 to about 1500 g/mol, from about 300 to about 2000 g/mol, from about 400 to about 1000 g/mol, from about 400 to about 900 g/mol, from about 400 to about 800 g/mol, from about 500 to about 900 g/mol, or from about 500 to about 800 g/mol. In certain embodiments, the method comprises heating the aqueous composition for a time sufficient for the aqueous composition to reach a number average molecular weight of from about 500 to about 2000 g/mol. In certain embodiments, the method comprises heating the aqueous composition for a time sufficient for the aqueous composition to reach a weight average molecular weight within a range of from about 300 to about 5000 g/mol, from about 500 to about 5000 g/mol, from about 700 to about 5000 g/mol, from about 500 to about 2000 g/mol, from about 700 to about 2000 g/mol, from about 700 to about 1500 g/mol, from about 300 to about 1500 g/mol, from about 300 to about 2000 g/mol, from about 400 to about 1300 g/mol, from about 400 to about 1200 g/mol, from about 400 to about 1100 g/mol, from about 500 to about 1300 g/mol, from about 500 to about 1200 g/mol, from about 500 to about 1100 g/mol, from about 600 to about 1300 g/mol, from about 600 to about 1200 g/mol, or from about 600 to about 1100 g/mol. In certain embodiments, the method comprises heating the aqueous composition for a time sufficient for the aqueous composition to reach a weight average molecular weight of from about 700 to about 3000 g/mol.

[00194] In some embodiments, the sufficient time is the time required for the aqueous composition to reach reaction equilibrium at the respective reaction temperature. Accordingly, in some embodiments, the method comprises heating the aqueous composition for a time sufficient for the aqueous composition to reach equilibrium. For example, in some embodiments, the equilibrium

is determined by measuring the molecular weight, viscosity, or DP distribution of the aqueous composition.

[00195] In certain embodiments, the equilibrium is determined by measuring the number average or weight average molecular weight of the aqueous composition. In some embodiments, the equilibrium is determined by the number or weight average molecular weight of the aqueous composition that remains essentially unchanged over time. In some embodiments, the equilibrium is determined by a change of the number or weight average molecular weight of the aqueous composition that is less than certain percentage over a period of time. In some embodiments, the molecular weight of the aqueous composition is measured by HPLC or SEC.

[00196] In some embodiments, the equilibrium is determined by a change of the number or weight average molecular weight of the aqueous composition of less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% over a period of time. In some embodiments, the equilibrium is determined by a change of the number or weight average molecular weight of the aqueous composition over a period of 3 hours, 2 hours, 1 hour, 30 minutes, 20 minutes, or 10 minutes. In some embodiments, the equilibrium is determined by a change of the weight average molecular weight of the aqueous composition of less than 15% over the period of 1 hour.

[00197] In certain embodiments, the equilibrium is determined by measuring the viscosity of the aqueous composition. In some embodiments, the equilibrium is determined by the viscosity of the aqueous composition that remains essentially unchanged over time. In some embodiments, the equilibrium is determined by a change of the viscosity of the aqueous composition that is less than certain percentage over a period of time. In some embodiments, the viscosity of the aqueous composition is measured by a viscometer or rheometer.

[00198] In some embodiments, the equilibrium is determined by a change of the viscosity of the aqueous composition of less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% over a period of time. In some embodiments, the equilibrium is determined by a change of the viscosity of the aqueous composition over a period of 3 hours, 2 hours, 1 hour, 30 minutes, 20 minutes, or 10 minutes. In some embodiments, the equilibrium is determined by a change of the viscosity of the aqueous composition of less than 15% over the period of 1 hour.

[00199] In certain embodiments, the equilibrium is determined by measuring the DP distribution of the aqueous composition. In some embodiments, the equilibrium is determined by the DP distribution of the aqueous composition that remains essentially unchanged over time. In some embodiments, a change of the DP distribution of the aqueous composition is determined by calculating a series of Km, wherein $Km = \frac{[DP_m][\mathrm{H_2O}]}{[DP_{m-1}][DP1]}$, wherein [$H_2O$] represents the molar water concentration (mol/L), and [DP1], [DPm-1], and [DPm] represent the molar concentrations of

oligosaccharides (mol/L) in the DP1, DPm-1, and DPm fractions respectively. For example, K2 equals [DP2][H_2O]/[DP1][DP1] according to the above formula. In some embodiments, m is an integer larger than 1 and smaller than n. In other embodiments, m equals n. In some embodiment, m is an integer larger than 1 and less than or equal to n. . In some embodiments, m is 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00200] In some embodiments, the concentration of the oligosaccharides in the DP1, DPm-1, and DPm fractions are determined by SEC, HPLC, FFF, A4F, mass spectrometry, or any other suitable method. In some embodiments, the concentration of the oligosaccharides in the DP1, DPm-1, and DPm fractions are determined by SEC such as GPC. In some embodiments, the concentration of the oligosaccharides in the DP1, DPm-1, and DPm fractions are determined by mass spectrometry such as GC-MS, LC-MS/MS, and MALDI-MS. In some embodiments, the concentration of the oligosaccharides in the DP1, DPm-1, and DPm fractions are determined by HPLC. In some embodiments, the water concentration is determined by an evaporation method (e.g., loss on drying technique), a distillation method, or by a chemical reaction method (e.g., Karl Fischer titration). In some embodiments, the water concentration is determined by any suitable analytical instrument such as a moisture analyzer.

[00201] In some embodiments, the method comprises calculating a series of at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 30, at least 40, or at least 50 Km numbers. In some embodiments, the method comprises calculating a series of at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 15 Km numbers. In some embodiments, the method comprises calculating about 3, 4, 5, 6, 7, 8, 9, 10, or 15 Km numbers. In some embodiments, the method comprises calculating K2 to K4, K2 to K5, K2 to K6, K2 to K7, K2 to K8, K2 to K9, K2 to K10, K2 to K11, K2 to K12, K2 to K13, K2 to K14, K2 to K15, K3 to K5, K3 to K6, K3 to K7, K3 to K8, K3 to K9, K3 to K10, K3 to K11, K3 to K12, K3 to K13, K3 to K14, or K3 to K15. In certain embodiments, the method comprises calculating K2 to K4 or K3 to K5.

[00202] In some embodiments, the value of Km depends on the temperature, water concentration, and/or the amount and type of the feed sugars. In some embodiments, Km is from about 0.1 to about 100, from about 0.1 to about 90, from about 0.1 to about 80, from about 0.1 to about 70, from about 0.1 to about 60, from about 0.1 to about 50, from about 0.1 to about 40, from about 0.1 to about 30, from about 0.1 to about 25, from about 0.1 to about 20, or from about 0.1 to about 15. In some embodiments, Km is from about 1 to about 100, from about 1 to about 90, from about 1 to about 80, from about 1 to about 70, from about 1 to about 60, from about 1 to about 50, from about 1 to about 40, from about 1 to about 30, from about 1 to about 25, from about 1 to about 20,

from about 1 to about 15, from about 1 to about 10, from about 5 to about 50, from about 5 to about 40, from about 5 to about 30, from about 5 to about 20, from about 5 to about 15, or from about 5 to about 10. In some embodiments, Km is from about 1 to about 15 or from about 5 to about 15.

[00203] In some embodiments, an average, a standard deviation, and/or a relative standard deviation are determined for the series of Km calculated. As used herein, a relative standard deviation is expressed in percentage, and is obtained by multiplying the standard deviation by 100 and dividing this product by the average.

[00204] In some embodiments, the equilibrium is determined by the relative standard deviation of the series of Km of less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%. In some embodiments, the equilibrium is determined by the relative standard deviation of the series of Km of less than 15%, less than 10%, or less than 5%.

Post-Reaction Steps

[00205] In some embodiments, a herein described method of manufacturing oligosaccharide preparations further comprises one or more additional processing steps after heating the aqueous composition at a temperature and for a sufficient time. In some embodiments, the additional processing steps comprise, for example, separation (such as chromatographic separation), dilution, concentration, drying, filtration, demineralization, extraction, decolorization, or any combination thereof. For example, in some embodiments, the method comprises a dilution step and a decolorization step. In some embodiments, the method comprises a filtration step and a drying step.

[00206] In some embodiments, the method comprises a dilution step, where water is added into the oligosaccharide preparation to make a syrup of oligosaccharide preparation. In some embodiments, the concentration of oligosaccharide preparation in the syrup is from about 5% to about 80%, from about 10% to about 70%, from about 10% to about 60%, from about 10% to about 50%, from about 10% to about 40%, from about 10% to about 30%, or from about 15% to about 25%. In other embodiments, the method does not comprise a dilution step, but rather, the oligosaccharide preparation is allowed to solidify. In some embodiments, the method comprises a filtration step. In some embodiments, the method comprises recycling the catalyst by filtration.

[00207] In some embodiments, the method described comprises a decolorization step. In some embodiments, the oligosaccharide preparation can undergo a decolorization step using any method known in the art, including, for example, treatment with an absorbent, activated carbon,

chromatography (e.g., using ion exchange resin), hydrogenation, and/or filtration (e.g., microfiltration).

[00208] In some embodiments, the oligosaccharide preparation is contacted with a material to remove salts, minerals, and/or other ionic species. In certain embodiments, the oligosaccharide preparation is flowed through an anionic/cationic exchange column pair. In one embodiment, the anionic exchange column contains a weak base exchange resin in a hydroxide form and the cationic exchange column contains a strong acid exchange resin in a protonated form.

[00209] In some embodiments, the method comprises a concentration step. In some embodiments, the centration step produces an oligosaccharide preparation with increased concentration. For example, in some embodiments, the concentration step comprises evaporation (e.g., vacuum evaporation), drying (e.g., freeze-drying and spray drying) or any combination thereof.

[00210] In some embodiments, the method comprises an isolation step, wherein at least a portion of the oligosaccharide preparation is separated. In some embodiments, the isolation step comprises crystallization, precipitation, filtration (e.g., vacuum filtration), and centrifugation, or any combination thereof.

[00211] In some embodiments, the method comprises a separation step. In some embodiments, the separation step comprises separating at least a portion of the oligosaccharide preparation from at least a portion of the catalyst, from at least a portion of the unreacted feed sugars, or from both. In some embodiments, the separation step comprises filtration, chromatography, differential solubility, precipitation, extraction, or centrifugation.

Reactors

[00212] The methods described herein can comprise the use of one or more reactors suitable for sugar condensation, considering the reaction temperature, pH, pressure, and other factors. In some embodiments, the one or more suitable reactors comprise a fed-batch stirred reactor, a batch stirred reactor, a continuous flow stirred reactor, a continuous plug-flow column reactor, an attrition reactor, or a reactor with stirring induced by an electromagnetic field. In some embodiments, the one or more suitable reactors comprise a reactor described in Ryu, S. K., and Lee, J. M., Bioconversion of waste cellulose by using an attrition bioreactor, Biotechnol. Bioeng. 25: 53-65(1983); Gusakov, A. V., and Sinitsyn, A. P., Kinetics of the enzymatic hydrolysis of cellulose: 1. A mathematical model for a batch reactor process, Enz. Microb. TechnoL, 7: 346-352 (1985); Gusakov, A. V., Sinitsyn, A. P., Davydkin, I. Y., Davydkin, V. Y., Protas, O. V., Enhancement of enzymatic cellulose hydrolysis using a novel type of bioreactor with intensive stirring induced by electromagnetic field, Appl. Biochem. Biotechnol., 56: 141-153(1996); or Fernanda de Castilhos

Corazza, Flavio Faria de Moraes, Gisella Maria Zanin and Ivo Neitzel, Optimal control in fed-batch reactor for the cellobiose hydrolysis, Acta Scientiarum. Technology, 25: 33-38 (2003).

[00213] In some embodiments, the one or more suitable reactors comprise fluidized bed, upflow blanket, immobilized, or extruder type reactors for hydrolysis and/or fermentation. In some embodiments, the one or more suitable reactors comprise an open reactor, a closed reactor, or both. In some embodiments, where the method comprises a continuous process, the one or more suitable reactors can include a continuous mixer such as a screw mixer.

Process

[00214] In some embodiments, a herein described method of manufacturing oligosaccharide preparations comprises a batch process, a continuous process, or both. In some embodiments, the method of manufacturing the oligosaccharide preparation comprises a batch process. For example, in some embodiments of the batch process, manufacturing of subsequent batches of the oligosaccharide preparation does not start until the completion of the current batch. In some embodiments, during the batch process, all or a substantial amount of oligosaccharide preparation is removed from the reactor. In some embodiments, during the batch process, all the feed sugars and the catalyst are combined in a reactor before the aqueous composition is heated to the described temperature or before the polymerization is induced. In some embodiments, during the batch process, the feed sugars are added before, after, or simultaneous with the addition of the catalyst.

[00215] In some embodiments, the batch process is a fed-batch process, wherein all the feed sugars are not added into the reactor at the same time. In some embodiments of the fed-batch process, at least a portion of the feed sugars are added into the reactor during polymerization or after the aqueous composition is heated to the described temperature. In some embodiments of the fed-batch process, at least 10%, 20%, 30%, 40%, 50%, or 60% by weight of the feed sugars are added into the reactor during polymerization or after the aqueous composition is heated to the described temperature.

[00216] In some embodiments, the method of manufacturing the oligosaccharide preparation comprises a continuous process. For example, in some embodiments of the continuous process, the contents of the reactor continuously flow through the reactor. In some embodiments, the combination of the feed sugars with the catalyst and the removal of at least a portion of the oligosaccharide preparation are performed concurrently.

[00217] In some embodiments, the method of manufacturing the oligosaccharide preparation comprises a single-pot or multi-pot process. For example, in some embodiments of the single-pot process, the polymerization is performed in a single reactor. For another example, in some

embodiments of the multi-pot process, the polymerization is performed in more than one reactor. In some embodiments of the multi-pot process, the method comprises 2, 3, or more reactors. In some embodiments of the multi-pot process, the method comprises a combination step, where the polymerization products from two or more reactors are combined.

V. Nutritional Composition Comprising Anhydro-subunit

[00218] Provided herein are nutritional compositions comprising an oligosaccharide preparation. In certain embodiments, provided herein are nutritional compositions comprising a described oligosaccharide preparation, wherein the presence and/or concentration of the oligosaccharide preparation within the nutritional compositions can be selectively determined and/or detected. Oligosaccharide preparations, which exhibit complex functional modulation of a microbial community, can be important components of nutritional compositions. Thus, the presence and/or concentration of an oligosaccharide preparation within nutritional compositions can be one of the factors that need to be measured in the quality control and manufacturing process of the nutritional compositions. Accordingly, the provided nutritional compositions are advantageous in terms of quality control and manufacturing purposes as the presence and/or concentration of the oligosaccharide preparation can be selectively determined and/or detected. For example, in some embodiments, the presence and concentration of the oligosaccharide preparation can be determined and/or detected by measuring a signal associated with the anhydro-subunit containing oligosaccharides.

[00219] In some embodiments, the nutritional composition is an animal feed composition. In some embodiments, the nutritional composition comprises a base nutritional composition.

Base Nutritional Compositions

[00220] In some embodiments, a herein described nutritional composition comprises a base nutritional composition and a disclosed oligosaccharide preparation. In some embodiments, the base nutritional composition comprises a carbohydrate source that is different from the oligosaccharide preparation. For example, in some embodiments, the base nutritional composition comprises a naturally occurring carbohydrate source (or naturally occurring oligosaccharide composition) such as starch and plant fibers. In some embodiments, the base nutritional composition comprises starch. In some embodiments, the base nutritional composition comprises plant fibers.

[00221] In some embodiments, the base nutritional composition comprises one or more carbohydrate sources that are derived from: seeds, roots, tubers, corn, tapioca, arrowroot, wheat, rice, potatoes, sweet potato, sago, beans (e.g., favas, lentils, mung beans, peas, and chickpeas.),

maize, cassava, or other starchy foods (e.g., acorns, arrowroot, arracacha, bananas, barley, breadfruit, buckwheat, canna, colacasia, katakuri, kudzu, malanga, millet, oats, oca, polynesian arrowroot, sorghum, rye, taro, chestnuts, water chestnuts, and yams).

[00222] In some embodiments, the base nutritional composition comprises one or more carbohydrate sources that are derived from: legumes (e.g., peas, soybeans, lupins, green beans, and other beans), oats, rye, chia, barley, fruits (e.g., figs, avocados, plums, prunes, berries, bananas, apple skin, quinces, and pears), vegetables (e.g., broccoli, carrots, cauliflower, zucchini, celery, nopal, and Jerusalem artichokes), root tubers, root vegetables (e.g., sweet potatoes and onions), psyllium seed husks, seeds (e.g., flax seeds), nuts (e.g., almonds), whole grain foods, wheat, corn bran, lignans, or any combination thereof. In some embodiments, the base nutritional composition comprises one or more plant fibers derived from wheat bran, sugar beet pulp, fuzzy cottonseeds, soy hulls, or any combination thereof.

[00223] In some embodiments, the base nutritional composition comprises less than 500 ppm, less than 400 ppm, less than 300 ppm, less than 200 ppm, less than 100 ppm, less than 50 ppm, less than 10 ppm, less than 5 ppm, or less than 1 ppm anhydro-subunits or anhydro-subunit containing oligosaccharides. In some embodiments, the base nutritional composition comprises less than 50 ppm, less than 10 ppm, less than 5 ppm, or less than 1 ppm anhydro-subunits or anhydro-subunit containing oligosaccharides. In some embodiments, the base nutritional composition is essentially free of anhydro-subunits.

[00224] In some embodiments, the base nutritional composition lacks a detectable level of anhydro-subunits. Depending on the methods of detecting or determination, an anhydro-subunit level below a certain threshold can be undetectable. For example, in some embodiments, a detectable level of anhydro-subunit can refer to at least 1000 ppm, at least 500ppm, at least 400 ppm, at least 300 ppm, at least 200 ppm, at least 100 ppm, at least 50 ppm, at least 10 ppm, at least 5 ppm, or at least 1 ppm of anhydro-subunit or anhydro-subunit containing oligosaccharides in the base nutritional composition.

[00225] In some embodiments, the base nutritional composition comprises a plurality of oligosaccharides. In some embodiments, the base nutritional composition comprises a glycosidic bond type distribution that is different from the oligosaccharide preparation. For example, in some embodiments, the base nutritional composition comprises a higher percentage of α-(1,4) glycosidic linkages than the oligosaccharide preparation. In some embodiments, the glycosidic linkages such as the α-(1,4) glycosidic linkages in the base nutritional compositions are digestible by one or more enzymes. In some embodiments, the glycosidic linkages in the base nutritional

composition are more readily digestible and/or hydrolysable than the glycosidic linkages in the oligosaccharide preparation.

[00226] In some embodiments, the level of α-(1,2) glycosidic linkage, α-(1,3) glycosidic linkage, α-(1,6) glycosidic linkage, β-(1,2) glycosidic linkage, β-(1,3) glycosidic linkage, β-(1,4) glycosidic linkage, or β-(1,6) glycosidic linkage in the base nutritional composition is at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, or at least 15% lower than the level of the respective glycosidic linkage in the oligosaccharide preparation. In some embodiments, the level of α-(1,2) glycosidic linkage, α-(1,3) glycosidic linkage, α-(1,6) glycosidic linkage, β-(1,2) glycosidic linkage, β-(1,3) glycosidic linkage, β-(1,4) glycosidic linkage, or β-(1,6) glycosidic linkage in the base nutritional composition is at least 10% lower than the level of the respective glycosidic linkage in the oligosaccharide preparation.

[00227] In some embodiments, the level of α-(1,4) glycosidic linkage in the base nutritional composition is at least 50%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, at least 5%, or at least 2% higher than the level of α-(1,4) glycosidic linkage in the oligosaccharide preparation. In some embodiments, the level of α-(1,4) glycosidic linkage in the base nutritional composition is at least 10% higher than the level of α-(1,4) glycosidic linkage in the oligosaccharide preparation.

Animal Feed Composition

[00228] Depending on the type and age of an animal, a nutritional composition can comprise the oligosaccharide preparation and the base nutritional composition at different ratio. For example, the oligosaccharide preparation can be combined with the base nutritional composition at various ratios suitable for the type and age of an animal. In some embodiments, the oligosaccharide preparation is present in the nutritional composition at a concentration of from about 1 to about 10000 ppm, from about 1 to about 5000 ppm, from about 1 to about 3000 ppm, from about 1 to about 2000 ppm, from about 1 to about 1500 ppm, from about 1 to about 1000 ppm, from about 1 to about 500 ppm, from about 1 to about 250 ppm, from about 1 to about 100 ppm, from about 10 to about 5000 ppm, from about 10 to about 3000 ppm, from about 10 to about 2000 ppm, from about 10 to about 1500 ppm, from about 10 to about 1000 ppm, from about 10 to about 500 ppm, from about 10 to about 250 ppm, from about 10 to about 100 ppm, from about 50 to about 5000 ppm, from about 50 to about 3000 ppm, from about 50 to about 2000 ppm, from about 50 to about 1500 ppm, from about 50 to about 1000 ppm, from about 50 to about 500 ppm, from about 50 to about 250 ppm, from about 50 to about 100 ppm, from about 100 to about 5000 ppm, from about 100 to about 3000 ppm, from about 100 to about 2000 ppm, from about 100 to about 1500 ppm,

from about 100 to about 1000 ppm, from about 100 to about 500 ppm, from about 100 to about 400 ppm, from about 100 to about 300 ppm, from about 100 to about 200 ppm, from about 200 to about 5000 ppm, from about 200 to about 3000 ppm, from about 200 to about 2500 ppm, from about 200 to about 2000 ppm, from about 200 to about 1500 ppm, from about 200 to about 1000 ppm, from about 200 to about 500 ppm, from about 500 to about 5000 ppm, from about 500 to about 3000 ppm, from about 500 to about 2500 ppm, from about 500 to about 2000 ppm, from about 500 to about 1500 ppm, or from about 500 to about 1000 ppm. In some embodiments, the oligosaccharide preparation is present in the nutritional composition at a concentration of from about 1 to about 5000 ppm, from about 1 to about 1000 ppm, from about 1 to about 500 ppm, from about 10 to about 5000 ppm, from about 10 to about 2000 ppm, from about 10 to about 1000 ppm, from about 10 to about 500 ppm, from about 10 to about 250 ppm, from about 10 to about 100 ppm, from about 50 to about 5000 ppm, from about 50 to about 2000 ppm, from about 50 to about 1000 ppm, from about 50 to about 500 ppm, from about 50 to about 250 ppm, or from about 50 to about 100 ppm. In some embodiments, the oligosaccharide preparation is present in the nutritional composition at a concentration of from about 1 to about 5000 ppm, from about 10 to about 1000 ppm, from about 10 to about 500 ppm, or from about 50 to about 500 ppm.

[00229] In some embodiments, the oligosaccharide preparation is present in the nutritional composition at a concentration of greater than 10 ppm, greater than 50 ppm, greater than 100 ppm, greater than 200 ppm, greater than 300 ppm, greater than 400 ppm, greater than 500 ppm, greater than 600 ppm, greater than 1000 ppm, or greater than 2000 ppm. In some embodiments, the oligosaccharide preparation is present in the nutritional composition at a concentration of greater than 10 ppm, greater than 50 ppm, greater than 100 ppm, greater than 200 ppm, or greater than 500 ppm.

[00230] In some embodiments, depending on the type and age of an animal, the nutritional composition can further comprise proteins, minerals (such as copper, calcium, and zinc), salts, essential amino acids, vitamins, and/or antibiotics.

[00231] In some embodiments, described herein are methods of manufacturing nutritional compositions for animal feed. In some embodiments, the animal is selected from cattle (e.g., beef cattle and dairy cattle), swine, aquatic animal, poultry, and human. In some embodiments, the animal is swine, such as sows, piglets, and hogs. In other embodiments, the animal is poultry such as chicken, duck, turkey, goose, quail, and hen. In embodiments, the poultry is a broiler, a breeder, or a layer. In some embodiments, the animal is an aquatic animal such salmon, catfish, bass, eel, tilapia, flounder, shrimp, and crab. In some embodiments, the nutritional composition is administered to an animal in a dry form, a liquid form, a paste, or a combination thereof. In some

embodiments, the form of administration, the feeding rate, and the feeding schedule can vary depending on the type and age of the animal.

Methods of Producing Nutritional Compositions

[00232] Provided herein are methods of manufacturing a nutritional composition comprising: combining an oligosaccharide preparation with a base nutritional composition. In some embodiments, the oligosaccharide preparation comprises anhydro-subunit containing oligosaccharides. In some embodiments, the oligosaccharide preparation comprises a glycosidic bond type distribution that is different from that of the base nutritional composition.

[00233] In some embodiments, the oligosaccharide preparation is a synthetic oligosaccharide preparation. In some embodiments, the synthetic oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions). In some embodiments, n is an integer greater than or equal to 2. In some embodiments, n is an integer greater than 2. In some embodiments, n is an integer greater than or equal to 3. In some embodiments, n is an integer within a range of 1 to 100, such as 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, 40, or 50.In some embodiments, each of the DP1 to DPn fraction comprises from 0.1% to 90% anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry. In some embodiments, the DP1 and DP2 fractions of the oligosaccharide preparation each independently comprises from about 0.1% to about 15% or from about 0.5% to about 10% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry. In some embodiments, the DP1 and DP2 fractions of the oligosaccharide preparation each independently comprises anhydro-subunit containing oligosaccharides within a range of from about 0.1%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, or 1.5% to about 8%, 9%, 10%, 11%, 12%, 15% or 20% by relative abundance as measured by mass spectrometry. In some embodiments, the relative abundance of oligosaccharides in each of the n fractions decreases monotonically with its degree of polymerization. In some embodiments, the relative abundance of oligosaccharides in at least 5, 10, 20, or 30 DP fractions decreases monotonically with its degree of polymerization.

[00234] In some embodiments, the method of manufacturing a nutritional composition comprises mixing the oligosaccharide preparation with the base nutritional composition. For example, in some embodiments, the mixing can be performed by an industrial blender and/or mixer such as drum blender, double cone blender, ribbon blender, V blender, shear mixer, and paddle mixer.

[00235] In some embodiments, the method of manufacturing a nutritional composition further comprises a herein described quality control step. In some embodiments, the herein described

quality control step comprises determining a level of a signal in a sample of the nutritional composition and calculating a concentration of the oligosaccharide preparation in the nutritional composition based on the level of the signal. In some embodiments, the herein described quality control step comprises detecting a signal in a sample of the nutritional composition through analytical instrumentation, and accepting or rejecting a batch of the nutritional composition based on the presence or absence of the signal. In some embodiments, the herein described quality control step comprises detecting, through analytical instrumentation, the presence or absence of a first signal in a first sample of the nutritional composition, and a second signal in a second sample of the nutritional composition, and comparing the first signal and the second signal. In some embodiments, the signal, the first signal, and/or the second signal is/are (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

[00236] Additionally, in some embodiments, the method of manufacturing a nutritional composition comprises, after performing the quality control step, further mixing the oligosaccharide preparation with the base nutritional composition, adjusting the level of the oligosaccharide preparation, or a combination thereof. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adding additional oligosaccharide preparation into the nutritional composition or removing a portion of the oligosaccharide preparation from the nutritional composition. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adding additional base nutritional composition into the nutritional composition or removing a portion of the base nutritional composition from the nutritional composition. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adding additional oligosaccharide preparation into the nutritional composition.

VI. Method of Correlating Oligosaccharide Preparation

[00237] Provided herein are methods of correlating the presence, absence, and/or concentration of a described oligosaccharide preparation in a nutritional composition. Provided herein are methods of performing a quality control method in the manufacturing process of nutritional compositions comprising an oligosaccharide preparation and a base nutritional composition. Provided herein are methods of determining the quality of nutritional compositions comprising an

oligosaccharide preparation and a base nutritional composition. In some embodiments, described herein are methods of correlating a synthetic oligosaccharide preparation in a nutritional composition. In some embodiments, a method of correlating can refer to the establishing, relating, and/or connecting a relationship between two things, and it can also refer to comparing the presence and amount of two things and evaluating a ratio of two things. In some embodiments, described herein are methods of detecting the concentration, the presence, and/or absence of a synthetic oligosaccharide preparation in a nutritional composition. In some embodiments, the nutritional composition comprises a described synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition (e.g., base nutritional composition).

[00238] In some embodiments, as used herein, the term "quality" can refer to the level of an oligosaccharide preparation (e.g., synthetic oligosaccharide preparation) in the nutritional composition; for example, whether the level is within a specified range such as from 1 to 5000 ppm, 10 to 1000 ppm, 10 to 500 ppm, or 50 to 500 ppm. In some embodiments, the term "quality" can refer to the level of an oligosaccharide preparation in the nutritional composition as evidenced by a signal shown by an analytical instrument; for example, whether a specified peak is present or absent on an NMR, GC-MS, LC-MS/MS, MALDI-MS, or HPLC chromatogram or spectrum and/or a weight determination.

[00239] In some embodiments, the term "quality" can refer to the distribution of an oligosaccharide preparation (e.g., synthetic oligosaccharide preparation) in the nutritional composition, e.g., whether the oligosaccharide preparation is distributed consistently and homogeneously in the nutritional composition. Accordingly, in some embodiments, the quality of a batch of nutritional composition can be determined by comparing the levels and/or the signals of the oligosaccharide preparation in two or more samples of the nutritional composition taken from the same batch. In some embodiments, the quality of a batch of nutritional composition can be determined by comparing the levels and/or the signals of the oligosaccharide preparation in two or more samples of the nutritional composition taken from different batches.

[00240] Thus, provided herein is a method of quantifying an oligosaccharide preparation in a nutritional composition comprising: determining a level of a signal in a sample of the nutritional composition, and calculating a concentration of the oligosaccharide preparation in the nutritional composition based on the level of the signal. Provided herein is a method of performing quality control of a nutritional composition comprising: detecting a signal in a sample of the nutritional composition through analytical instrumentation, and accepting or rejecting a batch of the nutritional composition based on the presence or absence of the signal. Further provided herein is a method of performing quality control of a nutritional composition comprising: detecting,

through analytical instrumentation, the presence or absence of a first signal in a first sample of the nutritional composition, and a second signal in a second sample of the nutritional composition, and comparing the first signal and the second signal. In some embodiments, the signal, the first signal, and/or the second signal is/are (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

[00241] In some embodiments, described herein is a method of quantifying a synthetic oligosaccharide preparation in a nutritional composition, wherein the nutritional composition comprises the synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition, the method comprising: (a) determining a level of a signal in a sample of the nutritional composition, and (b) correlating a concentration of the oligosaccharide preparation in the nutritional composition based on the level of the signal, wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, described herein is a method of performing quality control of a nutritional composition comprising: (a) providing a batch of a nutritional composition, wherein the nutritional composition comprises a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition, (b) obtaining a sample of the nutritional composition from the batch, (c) detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, and (d) accepting or rejecting the batch of the nutritional composition, wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments,

described herein is a method of performing quality control of a nutritional composition comprising: (a) providing a sample of a nutritional composition, wherein the nutritional composition comprises a naturally occurring oligosaccharide composition, (b) detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, and wherein the signal is indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the signal is indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, described herein is a method of performing quality control of a nutritional composition comprising a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition, the method comprising: (a) detecting, through analytical instrumentation, the presence or absence of a first signal in a first sample of the nutritional composition, and a second signal in a second sample of the nutritional composition, and (b) comparing the first signal and the second signal, wherein the first signal and the second signal are (i) indicative of one or more anhydro-subunit containing oligosaccharides, (ii) associated with a degree of polymerization (DP) distribution of oligosaccharides, or (iii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides. In some embodiments, the first signal and/or the second signal are indicative of one or more anhydro-subunit containing oligosaccharides.

[00242] Depending on their types, the signal (e.g., the level of the signal), the first signal, and/or the second signal can be determined or detected by any suitable analytical methods, including without limitation, NMR, HPLC, SEC, FFF, A4F, GC, LC, GC-MS, LC-MS/MS, MALDI-MS, IR, or CD spectroscopy. The level of the signal, the first signal, and/or the second signal can be determined or detected by purifying the sample using LC and weighing the sample. In some embodiments, a signal of a described oligosaccharide preparation is determined or detected by MALDI-MS. In some embodiments, a signal of a described oligosaccharide preparation is determined or detected by LC-MS/MS. In some embodiments, a signal of a described oligosaccharide preparation is determined or detected by GC-MS. In some embodiments, a signal

of a described oligosaccharide preparation is determined or detected by NMR such as 2D HSQC NMR. In some embodiments, the relative abundance or the concentration of a described oligosaccharide preparation, or both, are calculated based on the level of a signal of the oligosaccharide preparation, e.g., the relative abundance of one or more peaks on a mass spectrum or the intensity of one or more peaks on an NMR spectrum.

Anhydro-subunit Signal

[00243] In some embodiments, a method described herein comprises the detecting of a signal of an oligosaccharide preparation. In some embodiments, a herein described signal of an oligosaccharide preparation is indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, a method described herein comprises the detecting of two or more signals, i.e., a first signal, a second signal, etc. In some embodiments, at least one of the first signal and the second signal is indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, both the first signal and the second signal are indicative of one or more anhydro-subunit containing oligosaccharides.

[00244] In some embodiments, a signal that is indicative of one or more anhydro-subunit containing oligosaccharides can be determined or detected by any suitable analytical instrumentation, including without limitation, liquid chromatography (such as HPLC), FFF, A4F, NMR, SEC, mass spectrometry such as LC-MS/MS, GC-MS, and MALDI-MS. As used herein, in some embodiments, a signal that is indicative of one or more anhydro-subunit containing oligosaccharides can comprise a signal is attributed to the one or more anhydro-subunit containing oligosaccharides.

[00245] In some embodiments, a signal that is indicative of one or more anhydro-subunit containing oligosaccharides comprises one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination that are attributed to anhydro-subunit containing oligosaccharides. In some embodiments, the one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination are attributed to anhydro-subunit containing oligosaccharides that originate from the oligosaccharide preparation. In some embodiments, the one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination are attributed to anhydro-subunit containing oligosaccharides in any of the DP1 to DPn fraction in the oligosaccharide preparation. In some embodiments, the one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, the one or more peaks on mass

spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination are attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof.

[00246] In some embodiments, a signal that is indicative of one or more anhydro-subunit containing oligosaccharides comprises one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination that are attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, the one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination are attributed to anhydro-cellobiose. In some embodiments, the one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC) and/or a weight determination are attributed to anhydro-subunit containing oligosaccharides in the DP3, DP4, DP5, or DP6 fraction.

[00247] In some embodiments, the one or more peaks on mass spectrum, NMR, or liquid chromatography (such as HPLC), and/or a weight determination are attributed to anhydro-subunit containing oligosaccharides from more than one fraction. For example, in some embodiments, the signal is attributed to anhydro-subunit containing oligosaccharides from DP1 and DP2 fractions. In some embodiments, the signal is attributed to anhydro-subunit containing oligosaccharides from DP1, DP2, and DP3 fractions.

[00248] For example, in some embodiments, the signal that is indicative of one or more anhydro-subunit containing oligosaccharides comprises one or more peaks on mass spectrum that are attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, the signal that is indicative of one or more anhydro-subunit containing oligosaccharides comprises one or more peaks on mass spectrum that are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, the signal that is indicative of one or more anhydro-subunit containing oligosaccharides comprises a weight determination of DP2 anhydro-subunit containing oligosaccharides. In some embodiments, the weight determination is performed for at least a portion of DP2 anhydro-subunit containing oligosaccharides isolated and/or purified by preparative chromatography.

[00249] In some embodiments, a method described herein comprises the detecting of two or more signals, i.e., a first signal, a second signal, a third signal, etc. In some embodiments, the first signal and the second signal are attributed to anhydro-subunit containing oligosaccharides in the same fraction. For example, in some embodiments, the first signal is attributed to levoglucosan and the second signal is attributed to 1,6-anhydro-β-D-glucofuranose. In some embodiments, the first signal and the second signal are attributed to the same species of an anhydro-subunit containing oligosaccharide. For example, in some embodiments, the first signal and the second signal are

attributed to 1,6-anhydro-β-D-glucofuranose. In some embodiments, the first signal and the second signal are attributed to anhydro-subunit containing oligosaccharides from different fractions. In some embodiments, the first signal and the second signal are attributed to different species of anhydro-subunit containing oligosaccharides. For example, in some embodiments, the first signal is attributed to levoglucosan and the second signal is attributed to anhydro-cellobiose. In some embodiments, at least one of the first signal and the second signal that are indicative of one or more anhydro-subunit containing oligosaccharides comprises one or more peaks on mass spectrum that are attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, at least one of the first signal and the second signal that are indicative of one or more anhydro-subunit containing oligosaccharides comprises one or more peaks on mass spectrum that are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, at least one of the first signal and the second signal that are indicative of one or more anhydro-subunit containing oligosaccharides comprises a weight determination of DP1 and/or DP2 anhydro-subunit containing oligosaccharides. In some embodiments, both the first signal and the second signal that are indicative of one or more anhydro-subunit containing oligosaccharides comprise one or more peaks on mass spectrum that are attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction such as anhydro-cellobiose. In some embodiments, both the first signal and the second signal that are indicative of one or more anhydro-subunit containing oligosaccharides comprise one or more peaks on mass spectrum that are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction such as levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof.

[00250] In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to anhydro-

cellobiose. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed anhydro-cellobiose. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to anhydro-subunit containing oligosaccharides in the DP3 fraction. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed to anhydro-subunit containing oligosaccharides in the DP3 fraction.

[00251] In some embodiments, a level of a described signal can be determined by any suitable analytical instrumentation, including without limitation, a balance, liquid chromatography (such as HPLC), NMR, SEC, mass spectrometry such as LC-MS/MS, GC-FID, GC-MS, and MALDI-MS. For example, in some embodiments, the level of a signal is the relative abundance of one or more species of anhydro-subunit containing oligosaccharides represented by the signal. In some embodiments, the level of a signal is the concentration of one or more species of anhydro-subunit containing oligosaccharides represented by the signal. In some embodiments, the level of a signal is the weight of at least a portion of an isolated fraction of the described oligosaccharide preparation (e.g., DP2 anhydro-subunit containing oligosaccharides).

Glycosidic Linkages Signal

[00252] In some embodiments, a herein described signal is associated with one or more glycosidic linkages. For example, in some embodiments, the signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,4) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, or any combination thereof. In some embodiments, the signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages.

[00253] In some embodiments, a method described herein comprises the detecting of two or more signals, i.e., a first signal, a second signal, a third signal, etc. In some embodiments, at least one of the first signal and the second signal is associated with one or more glycosidic linkages. For example, in some embodiments, at least one of the first signal and the second signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,4) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, or any combination thereof. In some embodiments, at least one of the first signal and the second signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages.

[00254] In some embodiments, both the first signal and the second signal are associated with one or more glycosidic linkages. For example, in some embodiments, both the first signal and the second signal are associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,4) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, or any combination thereof. In some embodiments, both the first signal and the second signal are associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages.

[00255] In some embodiments, a signal that is associated with one or more glycosidic linkages can be determined or detected by any suitable analytical instrumentation such as NMR, including without limitation, 1D ^{1}H NMR, 1D ^{13}C NMR, 2D NMR such as 2D JRES, HSQC, DOSY, HMBC, COSY, ECOSY, TOCSY, NOESY, or ROESY, or any combination thereof. In some embodiments, a signal that is associated with one or more glycosidic linkages can refer that the signal is attributed to the one or more glycosidic linkages.

[00256] In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,2) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, all of them are associated with α-(1,2) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,3) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, all of them are associated with α-(1,3) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with α-(1,6) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, all of them are associated with α-(1,6) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1, 2) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, all of them are associated with β-(1, 2) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,3) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, all of them are associated with β-(1,3) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1,4) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, all of them are associated with β-(1,4) glycosidic linkages. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are associated with β-(1, 6) glycosidic linkages. In some embodiments, among the

signal, the first signal, and the second signal, all of them are associated with β-(1, 6) glycosidic linkages.

[00257] In some embodiments, a signal that is associated with one or more glycosidic linkages comprises one or more peaks on an NMR spectrum that are attributed to the one or more glycosidic linkages. In some embodiments, the location, intensity, shape, and other characteristics of the signal can vary according to the type of NMR spectrum and the conditions of performing the NMR. In some embodiments, the presence or absence of a signal that is associated with one or more glycosidic linkages refers to the presence or absence of one or more peaks on an NMR spectrum that are attributed to the one or more glycosidic linkages. In some embodiments, the level of a signal refers to the intensity of the one or more peaks on an NMR spectrum that are attributed to the one or more glycosidic linkages, wherein the relative abundance and/or concentration of the one or more glycosidic linkages may or may not be calculated based on the intensity.

DP Distribution Signal

[00258] In some embodiments, a herein described signal is associated with a DP distribution of oligosaccharides. In some embodiments, a method described herein comprises the detecting of two or more signals, i.e., a first signal, a second signal, a third signal, etc. In some embodiments, at least one of the first signal and the second signal is associated with a DP distribution of oligosaccharides. In some embodiments, both the first signal and the second signal are associated with a DP distribution of oligosaccharides. In some embodiments, the DP distribution of oligosaccharide are mainly attributed to the oligosaccharide preparation.

[00259] In some embodiments, a signal that is associated with a DP distribution of oligosaccharides can be determined or detected by any suitable analytical instrumentation, including without limitation, HPLC, SEC, FFF, A4F, mass spectrometry such as LC-MS/MS, GC-MS, and MALDI-MS. In some embodiments, a signal that is associated with a DP distribution of oligosaccharides can be determined based on the molecular weight distribution of the oligosaccharides.

[00260] In some embodiments, a signal that is associated with a DP distribution of oligosaccharides can refer to a signal provided by a suitable analytical instrumentation that is attributed to any one or more of the DP1 to DPn fractions. In some embodiments, a signal that is associated with a DP distribution of oligosaccharides is attributed to the oligosaccharides in the DP1, DP2, DP3, DP4, or DP5 fraction.

[00261] In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to the oligosaccharides in the DP1 fraction. In some embodiments,

among the signal, the first signal, and the second signal, all of them are attributed to the oligosaccharides in the DP1 fraction. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to the oligosaccharides in the DP2 fraction. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed to the oligosaccharides in the DP2 fraction. In some embodiments, among the signal, the first signal, and the second signal, one or more of them are attributed to the oligosaccharides in the DP3 fraction. In some embodiments, among the signal, the first signal, and the second signal, all of them are attributed to the oligosaccharides in the DP3 fraction.

[00262] Depending on the type of the analytical instrument, in some embodiments, the concentration and/or relative abundance of the oligosaccharides that are associated with the signal can be determined based on a level of the signal. In some embodiments, a signal that is associated with a DP distribution of oligosaccharides is attributed to the DP2 fraction and determined or detected by SEC, wherein the quantity of the DP2 fraction in the oligosaccharides can be determined by SEC. In some embodiments, a signal that is associated with a DP distribution of oligosaccharides is attributed to the DP2 fraction and determined or detected by HPLC, wherein the quantity of the DP2 fraction in the oligosaccharides can be determined by HPLC. In some embodiments, a signal that is associated with a DP distribution of oligosaccharides is attributed to the DP2 fraction and determined or detected by LC-MS/MS, wherein the quantity of the DP2 fraction in the oligosaccharides can be determined by LC-MS/MS.

[00263] It is to be understood that each of the signals (e.g., the first signal and the second signal) can be independently chosen, determined, and/or detected. For example, in some embodiments, the first signal is associated with a DP distribution and determined by SEC, while the second signal is indicative of anhydro-subunit containing oligosaccharides in the DP2 fraction and detected by LC-MS/MS. In some embodiments, the first signal is associated with α-(1,6) glycosidic linkages and detected by 2D ^{1}H, ^{13}C- HSQC, while the second signal is indicative of anhydro-subunit containing oligosaccharides in the DP1 fraction and determined or detected by LC-MS/MS. In some embodiments, the first signal and the second signal are both indicative of and attributed to anhydro-subunit containing oligosaccharides in the DP1 fraction and determined or detected by mass spectrometry. In some embodiments, the first signal and the second signal are both indicative of and attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction and determined or detected by mass spectrometry. In some embodiments, the first signal and the second signal are both indicative of and attributed to anhydro-subunit containing oligosaccharides in the DP2 fraction and determined or detected by a weight determination after isolation by preparative chromatography.

Extraction

[00264] In some embodiments, a method described herein comprises extracting at least a portion of oligosaccharides from a sample of the nutritional composition. In some embodiments, the extractant comprises one or more solvents that can dissolve or partially dissolve oligosaccharides. In some embodiments, the extractant comprises water, alcohol (e.g., ethanol, methanol, or propanol), buffers, one or more organic solvents, or any combination thereof. In some embodiments, buffers suitable for extraction can comprise citric acid, acetic acid, phosphate, CHES, borate, diethyl barbituric acid, carbonic acid, bicarbonate, hydrochloric acid, sodium hydroxide, sodium acetate, imidazole, sodium carbonate, any combination thereof, or any other buffers known in the art. In some embodiments, the buffer has a pH lower than 7. In other embodiments, the buffer has a pH equal or greater than 7. In some embodiments, the extractant comprises water and ethanol at a ratio of about 5 to 95, about 10 to 90, about 20 to 80, about 30 to 70, about 40 to 60, about 50 to 50, about 60 to 40, about 70 to 30, about 80 to 20, about 90 to 10, or about 95 to 5 by weight or by volume. In some embodiments, the extractant comprises water and ethanol at a ratio of about 50 to 50 by weight. In some embodiments, the extractant comprises water and ethanol at a ratio of about 50 to 50 by volume. In some embodiments, the extractant comprises greater than 30 wt%, greater than 40wt%, greater than 50 wt%, greater than 60 wt%, greater than 70 wt%, greater than 80 wt%, greater than 90 wt%, greater than 95 wt%, or greater than 99 wt% of water. In some embodiments, the extractant is water.

[00265] In some embodiments, a method described herein comprises various extraction time and extraction temperature. One of ordinary skill in the art may choose a suitable extractant, extraction time, and extraction temperature based on the chemical and physical properties of the oligosaccharides and the nutritional composition. For example, in some embodiments, the extraction temperature is from about 20 to about 100 °C, from about 30 to about 95 °C, from about 40 to about 95 °C, from about 50 to about 95 °C, from about 60 to about 95 °C, from about 70 to about 95 °C, from about 80 to about 95 °C, from about 60 to about 90 °C, from about 70 to about 90 °C, or from about 75 to about 85 °C. In some embodiments, the extraction temperature is from about 60 to about 90 °C. In some embodiments, the extraction temperature is about 80 °C. For example, in certain embodiments, the extractant is heated to the desired extraction temperature.

[00266] Depending on the rate of dissolution, some extractant and/or oligosaccharides can require longer extraction time. In some embodiments, the extraction time is from about 5 minutes to 24 hours, 5 minutes to 10 hours, 5 minutes to 5 hours, 5 minutes to 2 hours, 5 minutes to 1 hour, 10 minutes to 10 hours, 10 minutes to 5 hours, 10 minutes to 2 hours, or 10 minutes to 1 hour. In

some embodiments, the extraction time is about from 5 minutes to 1 hour. In some embodiments, the extraction time is about 30 minutes.

[00267] In some embodiments, the extraction comprises breaking down a sample of the nutritional composition into smaller pieces physically, such as by grounding with a mill. In some embodiments, the method comprises filtering the extracted oligosaccharides, e.g., to remove solids from the solution. In some embodiments, the filtering step can be performed via a filter paper, ultrafiltration, microfiltration, centrifugation, precipitation, or any other separation techniques. In some embodiments, the method comprises clarifying the extracted oligosaccharides. In certain embodiments, clarifying the extracted oligosaccharides comprises contacting the extracted oligosaccharides solution with a color-absorbing material, such as activated carbon and ion exchange resin. In some embodiments, the method comprises multiple extraction steps. For example, the method can comprise a solid-liquid extraction step, and one or more liquid-liquid extraction and/or solid-liquid extraction steps.

Concentration

[00268] In some embodiments, a method described herein comprises concentrating at least a portion of the extracted oligosaccharides. In some embodiments, the concentration of the extracted oligosaccharides is increased more than 1000 times, more than 500 times, more than 100 times, more than 10 times, or more than 5 times after the concentration step. In some embodiments, the concentration of the extracted oligosaccharides is increased more than 100 times after the concentration step.

[00269] In some embodiments, the concentrating step comprises freeze-drying, vacuum distillation, membrane separation, solvent extraction, evaporating the solvent in the oligosaccharide solution at ambient or elevated temperature, or any combination thereof. In some embodiments, the concentrating step comprises freeze-drying. In some embodiments, the concentrating step comprises evaporating the solvent in the oligosaccharide solution under vacuum and/or at elevated temperature. In some embodiments, the concentrating step comprises nano-filtration. In some embodiments, the concentrating step comprises using nano-filtration to concentrate DP1 anhydro-subunit containing oligosaccharides. In some embodiments, the concentrating step comprises using nano-filtration to concentrate DP2 anhydro-subunit containing oligosaccharides. In some embodiments, the concentrating step comprises using nano-filtration to concentrate DP3 anhydro-subunit containing oligosaccharides.

[00270] In some embodiments, the extracted and concentrated oligosaccharides have an enriched DP1, DP2, DP3, DP4, or DP5 fraction. In some embodiments, the extracted and concentrated oligosaccharides have enriched anhydro-subunit containing oligosaccharides.

[00271] In some embodiments, a method described herein comprises introducing an internal standard into the extracted or concentrated oligosaccharides. For example, an internal standard can be introduced for MS quantification purpose. In some embodiments, the internal standard is an isotopically labeled material such as a synthetic oligosaccharide preparation comprising ^{13}C. In some embodiments, the internal standard is gluco-oligosaccharides synthesized from ^{13}C-glucose.

Digestion

[00272] In some embodiments, a method described herein comprises a step that selectively breaks down the carbohydrate source in the base nutritional composition. For example, a naturally occurring carbohydrate source in the base nutritional composition can be more susceptible to enzymatic hydrolysis than a synthetic oligosaccharide preparation. Accordingly, in some embodiments, the method described herein comprises digesting at least a portion of the extracted or concentrated oligosaccharides with one or more hydrolytic enzymes. In some embodiments, the one or more hydrolytic enzymes can comprise any enzyme that facilitates the hydrolysis of naturally occurring polysaccharides. In some embodiments, the one or more hydrolytic enzymes can comprise any enzyme that cleaves one or more naturally occurring glycosidic bonds. In some embodiments, the one or more hydrolytic enzymes selectively cleave α-(1,4) glycosidic linkages or naturally occurring α-(1,4) glycosidic linkages. In some embodiments, the one or more hydrolytic enzymes comprise carbohydratase, protease, lipase, amylase (e.g., α-amylase and β-amylase), amyloglycosidase, invertase, α-galactosidase, cellulase, xylanase, chitinases, lysozymes, glucoamylase, pullulanase, or any combination thereof. In some embodiments, the one or more hydrolytic enzymes comprise carbohydratase, protease, lipase, or any combination thereof. In some embodiments, the one or more hydrolytic enzymes comprise α-amylase, amyloglycosidase, invertase, α-galactosidase, or any combination thereof.

[00273] In some embodiments, the concentration of the one or more hydrolytic enzymes is from about 0.1 to 40 U/mL, 0.1 to 20 U/mL, 0.5 to 20 U/mL, 0.5 to 15 U/mL, 0.5 to 10 U/mL, 0.5 to 9 U/mL, 0.5 to 8 U/mL, 0.5 to 7 U/mL, 0.5 to 6 U/mL, 0.5 to 5 U/mL, 0.5 to 4 U/mL, 1 to 10 U/mL, 1 to 9 U/mL, 1 to 8 U/mL, 1 to 7 U/mL, 1 to 6 U/mL, 1 to 5 U/mL, 2 to 5 U/mL, or 3 to 4 U/mL for each enzyme. In some embodiments, the concentration of the one or more hydrolytic enzymes is from about 1 to 10 U/mL for each enzyme. In some embodiments, the concentration of the one or more hydrolytic enzymes is from about 3 to 4 U/mL for each enzyme.

[00274] Depending on the type and concentration of the enzymes and other digestion conditions, the digestion time can vary. In some embodiments, the digestion time is from about 10 minutes to 24 hours, 30 minutes to 12 hours, 1 hour to 12 hours, 2 hours to 11 hours, 3 hours to 10 hours, 4

hours to 12 hours, 4 hours to 11 hours, 4 hours to 10 hours, 4 hours to 9 hours, 2 hours to 10 hours, 2 hours to 8 hours, 2 hours to 6 hours, 3 hours to 8 hours, or 3 hours to 5 hours. In some embodiments, the digestion time is from about 4 hours to 12 hours. In some embodiments, the digestion time is about 4 hours.

[00275] In certain embodiments, a change in digestion temperature can affect the digestion rate of the enzymes. In some embodiments, the digestion temperature is from about 20 to 100 °C, 30 to 90 °C, 40 to 80 °C, 50 to 70 °C, or 55 to 65 °C. In some embodiments, the digestion temperature is from about 40 to 80 °C. In some embodiments, the digestion temperature is about 60 °C.

[00276] In some embodiments, the method comprises multiple steps of digestion. For example, the digesting step can be repeated for multiple times until all or substantially all carbohydrate sources from the base nutritional composition are hydrolyzed.

[00277] In certain embodiments, the method comprises reducing the extracted or concentrated oligosaccharides. In some embodiments, reducing the oligosaccharides to the respective alditols can result in less complex chromatograms, sharper peaks and thus a more sensitive detection. In some embodiments, the extracted or concentrated oligosaccharides are reduced by one or more reducing agents such as sodium borohydride ($NaBH_4$), iron sulfate, sulfur dioxide, dithionates, thiosulfates, iodides, hydrazine, and ascorbic acid. In some embodiments, the extracted or concentrated oligosaccharides are reduced by sodium borohydride.

Separation, Isolation, and Quantification

[00278] In some embodiments, a method described herein comprises a separation and/or isolation step. In some embodiments, the method described herein comprises separating at least a portion of the extracted, concentrated, digested, or reduced oligosaccharides. In some embodiments, the method described herein comprises separating the extracted oligosaccharides. In some embodiments, the method described herein comprises separating the concentrated oligosaccharides. In some embodiments, the method described herein comprises separating the digested oligosaccharides. In some embodiments, the method described herein comprises separating the reduced oligosaccharides.

[00279] The oligosaccharides can be separated by any suitable means. For example, in some embodiments, the oligosaccharides are separated by flash chromatography, low-, medium- or high-pressure liquid chromatography, ultrafiltration, membrane separation, reverse osmosis, or any combination thereof. In some embodiments, the oligosaccharides are separated by flash chromatography. In some embodiments, the oligosaccharides are separated chromatographically. In some embodiments, the oligosaccharides are separated by precipitation. In some embodiments, the oligosaccharides are separated by nano-filtration. In some embodiments, DP1 anhydro-subunit

containing oligosaccharides or DP1 oligosaccharides are separated by nano-filtration. In some embodiments, DP2 anhydro-subunit containing oligosaccharides or DP1 oligosaccharides are separated by nano-filtration. In some embodiments, DP3 anhydro-subunit containing oligosaccharides or DP1 oligosaccharides are separated by nano-filtration.

[00280] In some embodiments, the method described herein comprises isolating at least a portion of the extracted, concentrated, undigested, reduced, or separated oligosaccharides. In some embodiments, the method described herein comprises isolating the undigested oligosaccharides. In some embodiments, the method described herein comprises isolating the separated oligosaccharides. In some embodiments, the method described herein comprises isolating each fraction of the separated oligosaccharides. In some embodiments, the method comprises isolating anhydro-subunit containing oligosaccharides. In some embodiments, the method comprises isolating certain fractions of anhydro-subunit containing oligosaccharides such as DP1 or DP2 anhydro-subunit containing oligosaccharides. In some embodiments, isolating oligosaccharides can comprise concentrating and/or combining oligosaccharides with similar characteristics, such as degree of polymerization.

[00281] In certain embodiments, the oligosaccharides are separated and/or isolated by the degree of polymerization. In some embodiments, the method comprises isolating and/or separating the oligosaccharides with a degree of polymerization of 1, 2, 3, 4, or 5. In some embodiments, at least a portion of the oligosaccharides in the DP1 fraction are isolated and/or separated. In some embodiments, at least a portion of the oligosaccharides in the DP2 fraction are isolated and/or separated. In some embodiments, at least a portion of the oligosaccharides in the DP3 fraction are isolated and/or separated. In some embodiments, at least a portion of the oligosaccharides in the DP4 fraction are isolated and/or separated. In some embodiments, at least a portion of the oligosaccharides in the DP5 fraction are isolated and/or separated. In some embodiments, two or more fractions of the oligosaccharides are isolated and/or separated. In some embodiments, the oligosaccharides in the DP1 and DP2 fractions are isolated and/or separated. In some embodiments, the oligosaccharides in the DP1, DP2, and DP3 fractions are isolated and/or separated.

[00282] In certain embodiments, the oligosaccharides are separated and/or isolated by whether they comprise anhydro-subunits. In some embodiments, the method comprises isolating and/or separating anhydro-subunit containing oligosaccharides with a degree of polymerization of 1, 2, 3, 4, or 5. In some embodiments, at least a portion of the anhydro-subunit containing oligosaccharides in the DP1 fraction are isolated and/or separated. In some embodiments, at least a portion of the anhydro-subunit containing oligosaccharides in the DP2 fraction are isolated

and/or separated. In some embodiments, at least a portion of the anhydro-subunit containing oligosaccharides in the DP3 fraction are isolated and/or separated. In some embodiments, at least a portion of the anhydro-subunit containing oligosaccharides in the DP4 fraction are isolated and/or separated. In some embodiments, at least a portion of the anhydro-subunit containing oligosaccharides in the DP5 fraction are isolated and/or separated. In some embodiments, two or more fractions of the anhydro-subunit containing oligosaccharides are isolated and/or separated.

[00283] In certain embodiments, the method described herein comprises a quantification step. In some embodiments, at least a portion of the digested, undigested, extracted, concentrated, separated, and/or isolated oligosaccharides are quantified. In some embodiments, the separated and/or isolated oligosaccharides are quantified. In some embodiments, the isolated oligosaccharides are quantified.

[00284] In some embodiments, the oligosaccharides are quantified by weight, concentration, and/or relative abundance. In some embodiments, the oligosaccharides are quantified by weight. In some embodiments, at least a portion of the oligosaccharides in the DP2 fraction are isolated and then quantified by weight. In some embodiments, at least a portion of the anhydro-subunit containing oligosaccharides in the DP2 fraction are isolated and then quantified by weight. In some embodiments, the anhydro-subunit containing oligosaccharides within the isolated oligosaccharides are quantified, such as by relative abundance via mass spectrometry. For example, the anhydro-subunit containing DP2 oligosaccharides in the isolated DP2 fraction are quantified by relative abundance shown on a mass spectrometry.

[00285] In some embodiments, a majority of the quantified oligosaccharides are from the oligosaccharide preparation. In some embodiments, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, more than 95%, or more than 99 % by weight of the quantified oligosaccharides are from the oligosaccharide preparation. In some embodiments, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, more than 95%, or more than 99 % of the quantified oligosaccharides, by relative abundance, are from the oligosaccharide preparation.

Signal Analysis

[00286] In some embodiments, a method described herein comprises analyzing at least a portion of the extracted, digested, undigested, separated, or concentrated oligosaccharides and obtains a signal. In some embodiments, the method described herein comprises analyzing, through analytical instrumentation, the separated oligosaccharides. In some embodiments, the method described herein comprises analyzing, through analytical instrumentation, the isolated oligosaccharides. In some embodiments, the method described herein comprises analyzing,

through analytical instrumentation, the digested oligosaccharides. In some embodiments, the method described herein comprises analyzing, through analytical instrumentation, the quantified oligosaccharides.

[00287] In some cases, a derivatization step is performed for the oligosaccharides, either before or at the time their being analyzed through various instrumentations. A derivatization step can operate to alter the chemical and/or physical properties of the oligosaccharides and facilitate their quantification or separation. One or more functional or tagging groups can be attached to the oligosaccharides during derivatization. A derivatization step can involve chemical reactions that add polar or non-polar groups to the oligosaccharides; for example, chemical reactions such as silylation, acylation, alkylatioin, esterification, and transesterification can be performed. Methods of derivatization are described in the art, e.g., Ruiz-Matute et al. J. Chromatography B, vol. 879 (17-18), 1226-1240, and S. Ahuja, J. Pharmaceutical Sciences, vol 65 (2), Feb 1976, 163-182, which are incorporated by reference in their entirety. In some embodiments, a derivatization step is performed prior to the detection of the signals.

[00288] In some embodiments, a method described herein comprises analyzing glycosidic linkages of the oligosaccharides by NMR, thereby determining or detecting one or more described signals (such as the level of the signals, the first signal, and the second signal) associated with the corresponding glycosidic linkages. In some embodiments, the method comprises analyzing the glycosidic linkages by 1D ^{1}H NMR, 1D ^{13}C NMR, 2D NMR such as 2D JRES, HSQC, HMBC, COSY, ECOSY, TOCSY, NOESY, and ROESY, or any combination thereof. In some embodiments, the analyzed glycosidic linkages are α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

[00289] In some embodiments, the method comprises analyzing α-(1,2) glycosidic linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the α-(1,2) glycosidic linkages. In some embodiments, the method comprises analyzing α-(1,3) glycosidic linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the α-(1,3) glycosidic linkages. In some embodiments, the method comprises analyzing α-(1,6) glycosidic linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the α-(1,6) glycosidic linkages. In some embodiments, the method comprises analyzing β-(1,2) linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the β-(1,2)

glycosidic linkages. In some embodiments, the method comprises analyzing β-(1,3) linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the β-(1,3) glycosidic linkages. In some embodiments, the method comprises analyzing β-(1,4) linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the β-(1,4) glycosidic linkages. In some embodiments, the method comprises analyzing β-(1,6) linkages of the oligosaccharides by NMR, thereby determining or detecting a described signal that is associated with the β-(1,6) glycosidic linkages.

[00290] In some embodiments, a method described herein comprises analyzing the anhydro-subunit containing oligosaccharides, thereby determining or detecting a described signal (such as the level of the signals, the first signal, and the second signal) that is indicative of one or more anhydro-subunit containing oligosaccharides. In some embodiments, the anhydro-subunit containing oligosaccharides are analyzed by weight determination, HPLC, NMR, SEC, mass spectrometry such as LC-MS/MS, GC-MS, and MALDI-MS, or a combination thereof. In some embodiments, the anhydro-subunit containing oligosaccharides are analyzed by LC-MS/MS. In some embodiments, the anhydro-subunit containing oligosaccharides are analyzed by GC-MS. In some embodiments, the anhydro-subunit containing oligosaccharides are analyzed by MALDI-MS. In some embodiments, the anhydro-subunit containing oligosaccharides are analyzed by HPLC. In some embodiments, the anhydro-subunit containing oligosaccharides are analyzed by weight determination of fractions isolated and/or purified by liquid chromatography.

[00291] In some embodiments, a method described herein comprises analyzing the anhydro-subunit containing oligosaccharides in any one or more of the DP1 to DPn fractions. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides in any one of the DP1 to DP5 fraction. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides in the DP1 fraction. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides in the DP2 fraction. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides in the DP3 fraction. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides in the DP4 fraction. In some embodiments, the method comprises analyzing the anhydro-subunit containing oligosaccharides in the DP5 fraction.

[00292] In some embodiments, a method described herein comprises analyzing the DP distribution of the oligosaccharides thereby determining or detecting a described signal (such as the level of the signals, the first signal, and the second signal) that is associated with the DP distribution. In some embodiments, the described signal is provided by HPLC or SEC.

[00293] In some embodiments, analyzing the DP distribution of the oligosaccharides comprises determining, detecting, or quantifying the oligosaccharides in any one or more of the DP1 to DPn fractions. In some embodiments, analyzing the DP distribution of the oligosaccharides comprises determining, detecting, or quantifying the oligosaccharides in any one of the DP1 to DP5 fraction. In some embodiments, analyzing the DP distribution of the oligosaccharides comprises determining, detecting, or quantifying the oligosaccharides in the DP1 fraction. In some embodiments, analyzing the DP distribution of the oligosaccharides comprises determining, detecting, or quantifying the oligosaccharides in the DP2 fraction. In some embodiments, analyzing the DP distribution of the oligosaccharides comprises determining, detecting, or quantifying the oligosaccharides in the DP3 fraction.

[00294] In some embodiments, the method comprises quantifying at least a portion of the oligosaccharides in any one of the DP1 to DPn fraction. In some embodiments, the method comprises quantifying at least a portion of the oligosaccharides in any one of the DP1 to DP5 fraction. In some embodiments, the method comprises quantifying at least a portion of the oligosaccharides in the DP1 fraction. In some embodiments, the method comprises quantifying at least a portion of the oligosaccharides in the DP2 fraction. In some embodiments, the method comprises quantifying at least a portion of the oligosaccharides in the DP3 fraction.

[00295] Depending on the analytical instrumentation, a methods described herein can comprise different variations. In some variations, the method comprises detecting the presence or absence of a signal. In some embodiments, the detecting can be performed manually, automatically such as by a machine, or any combination thereof. In some variations, the method comprises determining the characteristics of a signal, including without limitation, the intensity, the strength, shape, and/or area of a signal. In some embodiments, determining the characteristics of a signal comprises determining the presence or absence of a signal. In some embodiments, determining the characteristics of a signal comprises determining the level of the signal.

[00296] For example, in some embodiments, the absence of DP2 anhydro-subunit containing oligosaccharides signal on mass spectrometry indicates that the sample of the nutritional composition does not contain an oligosaccharide preparation or does not contain a detectable level of the oligosaccharide preparation. For another example, in some embodiments, the presence and/or level of DP2 anhydro-subunit containing oligosaccharides signal on mass spectrometry indicates that the sample of the nutritional composition can contain a level of oligosaccharides preparation that corresponds to the level of the signal. For yet another example, in some embodiments, the presence and/or level of α-(1,6) glycosidic linkages signal on NMR spectra

indicates that the sample of the nutritional composition can contain a level of oligosaccharides preparation that corresponds to the level of the signal.

[00297] In some embodiments, the method comprises correlating (e.g., calculating or estimating) a concentration of oligosaccharide preparation in the nutritional composition based on a described signal (such as the level of a signal, the first signal, and the second signal). For example, the relative abundance of oligosaccharides can be correlated based on a mass spectrometry signal. In some embodiments, the concentration and/or relative abundance of oligosaccharides can be correlated based on an SEC, HLPC, and/or NMR signal.

[00298] In some embodiments, the oligosaccharide preparation is analyzed in order to calculate or estimate its concentration in the nutritional composition. For example, in some embodiments, the level of anhydro-subunit containing oligosaccharides in the oligosaccharide preparation is calculated when the quality control step determines or detects a signal that is indicative of anhydro-subunit containing oligosaccharides. For another example, in some embodiments, the DP distribution of the oligosaccharide preparation is determined, when the quality control step determines or detects a signal that is associated with a DP distribution of oligosaccharides. For yet another example, in some embodiments, the relative abundance of α-(1,6) glycosidic linkages is determined for the oligosaccharide preparation, when the quality control step determines or detects a signal that is associated with α-(1,6) glycosidic linkages.

[00299] In some embodiments, the method comprises comparing signals, wherein any characteristics of a signal can be compared. For example, in some embodiments, the method comprises comparing the first signal and the second signal. In some embodiments, the method comprises comparing the level of the first signal and the level of the second signal. In some embodiments, a difference between the first signal and the second signal indicates that the oligosaccharide preparation is inconsistently distributed in the nutritional composition. In other embodiments, the similarities between the first signal and the second signal indicate that the oligosaccharide preparation is consistently distributed in the nutritional composition.

[00300] It is to be understood that a quality control method as provided herein can comprise any combination of the steps and embodiments. Further, in some embodiments, the quality control method is repeated. In certain embodiment, the quality control method is performed for multiple samples taken from the same batch or different batches of nutritional composition.

[00301] In certain embodiments, the first sample and the second sample are taken from the same batch of the nutritional composition. In certain embodiments, the first sample and the second sample are taken from different batches of the nutritional composition. In some embodiments, the first sample and the second sample are taken from the same or different manufacturing facilities.

In some embodiments, the first sample and the second sample are taken at the same or different times. In certain embodiments, the second sample is taken more than 1 day, more than 1 week, more than 1 month, more than 6 months, or more than 1 year after the first sample is taken. In certain embodiments, the first sample and the second sample are taken within a period of 1 day, 1 week, 1 month, 6 months, or 1 year. In some embodiments, the first sample and the second sample are taken from different locations of the same mixer, where the oligosaccharide preparation and the base nutritional composition are combined. In some embodiments, the first sample is taken during the mixing of the oligosaccharide preparation and the nutritional composition, and the second sample is taken after the mixing.

[00302] In some embodiments, a detection limit exists for the method described herein, wherein a level of oligosaccharide preparation outside the limit may not be detected by the method. In some embodiments, the detection limit for oligosaccharide preparation is above 1 ppm, above 5 ppm, above 10 ppm, above 50 ppm, above 100 ppm, above 200 ppm, above 300 ppm, above 400 ppm, above 500 ppm, above 600 ppm, above 700 ppm, above 800 ppm, above 900 ppm, or above 1000 ppm relative to the nutritional composition. In some embodiments, the detection limit for oligosaccharide preparation is above 10 ppm, above 50 ppm, above 100 ppm, or above 500 ppm relative to the nutritional composition.

Additional Steps

[00303] In some embodiments, a method described herein comprises accepting and/or rejecting a batch of the nutritional composition. In some embodiments, a batch of the nutritional composition is accepted or rejected after the quality control step (or after performing a method described herein). In some embodiments, the accepting or rejecting is performed manually, automatically such as by a machine, or by a combination thereof.

[00304] In some embodiments, the rejecting or accepting is wholly or partially based on the presence or absence of a described signal. In other embodiments, the rejecting or accepting is wholly or partially based on the level of a described signal, upon which the concentration of the oligosaccharide preparation in the nutritional composition is calculated. In some embodiments, the rejecting or accepting is wholly or partially based on comparing the first signal and the second signal.

[00305] In further embodiments, the rejecting or accepting is wholly or partially based on a pre-determined range of concentration of oligosaccharide preparation in the nutritional composition. For example, the pre-determined range of concentration can vary according to the specific animal feed composition. As an example, in some embodiments, a batch of nutritional composition can

be rejected if the level of a signal indicates that the oligosaccharide preparation is not within 10 to 1000 ppm, 10 to 500 ppm, or 50 to 500 ppm relative to the nutritional composition.

[00306] In some embodiments, a method described herein comprises adjusting the level of the oligosaccharide preparation after the determining or detecting, e.g., after the quality control step. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adjusting the level of the base nutritional composition, adjusting the level of the oligosaccharide preparation, or a combination thereof. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adding additional oligosaccharide preparation into the nutritional composition or removing a portion of the oligosaccharide preparation from the nutritional composition. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adding additional base nutritional composition into the nutritional composition or removing a portion of the base nutritional composition from the nutritional composition. In some embodiments, adjusting the level of the oligosaccharide preparation comprises adding additional oligosaccharide preparation into the nutritional composition. In some embodiments, adjusting the level of the oligosaccharide preparation comprises mixing the nutritional composition to increase consistency. In certain embodiments, the method comprises adjusting the level of the oligosaccharide preparation to a pre-determined range, which can vary according to the specific animal feed composition.

[00307] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[00308] The present invention is further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

EXAMPLES

Example 1: Synthesis of a gluco-galacto-oligosaccharide preparation

[00309] Synthesis of a gluco-galacto-oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures that were selected to enable suitable production at the kg scale.

[00310] D-glucose monohydrate (825.16 g), D-lactose monohydrate (263.48 g) and 2-pyridinesulfonic acid (1.0079 g, Sigma-Aldrich, St. Louis, US) were added to a three-liter, three-neck round bottom flask with a center 29/42 ground glass joint and two 24/40 side ground glass joints. A 133 mm Teflon stirring blade was affixed to a glass stir shaft using PTFE tape. The stir rod was secured through the center point using a Teflon bearing adapter and attached to an

overhead high-torque mechanical mixer via flexible coupler. The flask was secured inside a hemispherical electric heating mantle operated by a temperature control unit via a J-type wand thermocouple inserted through a rubber septum in one of the side ports. The tip of the thermocouple was adjusted to reside within the reaction mixture with several mm clearance above the mixing element. A secondary temperature probe connected to an auxiliary temperature monitor was also inserted and secured by the same means. The second side port of the flask was equipped with a reflux condenser cooled by a water-glycol mixture maintained below 4 °C by a recirculating bath chiller.

[00311] The reaction mixture was gradually heated to 130 °C with continuous mixing with a stir rate of 80-100 rpm. When the reaction mixture reached 120 °C, the reflux condenser was re-positioned into a distillation configuration, with the distillated collected in a 250 mL round bottom flask placed in an ice bath. The mixture was maintained at 130 °C with continuous mixing for 6 hours, after which the thermocouple box was powered off. The distillation apparatus was removed and 390 g of 60 °C distilled water was gradually added into the three-neck flask. The resulting mixture was left to stir at 40 RPM for 10 hours. Approximately 1,250 g of a viscous, light-amber material was collected and measured by refractive index to have a concentration of 71.6 Brix.

Example 2: Synthesis of a gluco-oligosaccharide preparation

[00312] Synthesis of a gluco-oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures that were selected to enable suitable production at the kg scale.

[00313] D-glucose monohydrate (1,150g) was added to a three-liter, three-neck round bottom flask with one center 29/42 ground glass joint and two side 24/40 ground glass joints. A 133mm Teflon stirring blade was affixed to glass stir shaft using PTFE tape. The stir rod was secured through the center port of the flask using a Teflon bearing adapter and attached to an overhead high-torque mechanical mixer via flex coupling. The flask was secured inside a hemispherical electric heating mantle operated by a temperature control unit via a J-type wand thermocouple inserted through a rubber septum in one of the side ports. The tip of the thermocouple was adjusted to reside within the reaction mixture with several mm clearance above the mixing element. A secondary temperature probe connected to an auxiliary temperature monitor was also inserted and secured by the same means. The second side port of the flask was equipped with a reflux condenser cooled by a water-glycol mixture maintained below 4 °C by a recirculating bath chiller.

[00314] The reaction mixture was gradually heated to 130 °C with continuous mixing with a stir rate of 80-100 rpm. When the reaction temperature increased to between 120 °C and 130 °C, (+)-

Camphor-10-sulfonic acid (1.16 g, Sigma-Aldrich, St. Louis) was added to the three-neck flask and the apparatus was switched from a reflux condenser to a distillation configuration with a round bottom collection flask placed in an ice bath. This setup was maintained for 1 and a half hours, after which the thermocouple box was powered off, the distillation apparatus was removed, and 390 g of 23 C distilled water was gradually added into the three-neck flask. The resulting mixture was left to stir at 40 rpm for 10 hours until the moment of collection. Approximately 1300 g of a viscous, dark-amber material was collected and measured to have a concentration of 72.6 brix.

Example 3: Synthesis of a gluco-galacto-manno-oligosaccharide preparation

[00315] Synthesis of a gluco-galacto-manno-oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures that were selected to enable suitable production at the kg scale. MH47-32-A / MH46-35-B: 8/10/18

[00316] The gluco-galacto-manno-oligosaccharide preparation was prepared as two separate components synthesized in separate reaction vessels that were independently collected. Each synthesis used different starting reactants but followed the same procedure and methods to completion. The final gluco-galacto-manno-oligosaccharide preparation was a homogeneous syrup formed from the mixing of both synthesis products.

[00317] For the synthesis of the first component, 990.54 g of glucose monohydrate, 105.58 g of lactose monohydrate and 1.00 g of 2-pyridinesulfonic acid were added to a three-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. A 133mm Teflon stirring blade was affixed to a 440 mm glass stir shaft using PTFE tape. The stir rod was secured through the center point using a Teflon bearing adapter and attached to an overhead high-torque mechanical mixer via flexible coupler. The flask was placed inside a hemispherical electric heating mantle operated by a temperature control unit via a J-type wand thermocouple inserted through a rubber septum in one of the side ports. The tip of the thermocouple was adjusted to reside within the reaction mixture with several mm clearance above the mixing element. A secondary temperature probe connected to an auxiliary temperature monitor was also inserted and secured by the same means. The second side port of the flask was equipped with a reflux condenser cooled by a water-glycol mixture maintained below 4 °C by a recirculating bath chiller.

[00318] The reaction mixture was gradually heated to 130 °C with continuous mixing with a stir rate of 80-100 rpm. Once a temperature control box reading between 120 C and 130 C was observed, the apparatus was switched from a reflux condenser to a distillation configuration with a round bottom collection flask placed in an ice bath. This setup was maintained for approximately

6 hours and 10 minutes, after which the heating mantle was powered off, the distillation apparatus was removed, and 390 g of 60 °C distilled water was gradually added into the three-neck flask. The resulting mixture was left to stir at 40 rpm for 10 hours until the moment of collection. Approximately 1250 g of a viscous, light-amber material was collected and measured by refractive index to have a concentration of 73.1 Brix.

[00319] For the synthesis of the second component, 825.04 g of glucose monohydrate, 251.16 g of pure mannose from wood, 25.10 g distilled water, and 1.00 g of 2-pyridinesulfonic acid were added to a three-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. The remainder of the second component's synthesis followed the same procedure and methods as those of the first, until the moment of collection. Approximately 1250 g of a viscous, dark-amber material was collected and measured to have a concentration of 72.3 brix.

[00320] The entirety of the first and second components were transferred into a suitably sized HDPE container and mixed thoroughly by hand until homogenous. The final syrup mixture was approximately 2.5 kg, dark-amber in color, viscous and was measured to have a concentration of approximately 72 brix.

Example 4: Synthesis of a gluco-manno-oligosaccharide preparation

[00321] Synthesis of a gluco-oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures that were selected to enable suitable production at the kg scale.

[00322] A gluco-manno-oligosaccharide preparation was prepared as two separate components synthesized in separate reaction vessels that were independently collected. Each synthesis used different starting reactants but followed the same procedure and methods to completion. The final gluco-manno-oligosaccharide preparation was a homogeneous syrup formed from the mixing of both synthesis products.

[00323] For the synthesis of the first component, 1264.80 g of glucose monohydrate was added to a three-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. A 133mm Teflon stirring blade was affixed to a 440 mm glass stir shaft using PTFE tape. The stir rod was secured through the center point using a Teflon bearing adapter and attached to an overhead high-torque mechanical mixer via flexible coupler. The flask was placed inside a hemispherical electric heating mantle operated by a temperature control unit via a J-type wand thermocouple inserted through a rubber septum in one of the side ports. The tip of the thermocouple was adjusted to reside within the reaction mixture with several mm clearance above

the mixing element. A secondary temperature probe connected to an auxiliary temperature monitor was also inserted and secured by the same means. The second side port of the flask was equipped with a reflux condenser cooled by a water-glycol mixture maintained below 4 °C by a recirculating bath chiller.

[00324] The reaction mixture was gradually heated to 130 °C with continuous mixing with a stir rate of 80-100 rpm. Once a temperature control box reading between 120 C and 130 C was observed, 1.15 g of (+)-camphor-10-sulfonic acid was added to the three-neck flask and the apparatus was switched from a reflux condenser to a distillation configuration with a round bottom collection flask placed in an ice bath. This setup was maintained for approximately 1 hour, after which the thermocouple box was powered off, the distillation apparatus was removed, and 390 g of 23 °C distilled water was gradually added into the three-neck flask. The resulting mixture was left to stir at 40 rpm for 10 hours until the moment of collection. Approximately 1350 g of a viscous, light-amber material was collected and measured to have a concentration of 71.8 brix.

[00325] For the synthesis of the second component, 949.00 g of glucose monohydrate, 288.00 g of pure mannose from wood, 27.94 g distilled water, and 1.15 g of 2-pyridinesulfonic acid were added to a three-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. The remainder of the second component's synthesis followed the same procedure and methods as those of the first until the moment of collection, except (+)-camphor-10-sulfonic acid was not added as the reflux condenser was switched to a distillation configuration and the resulting setup was maintained for approximately 6 hours. Approximately 1350 g of a viscous, dark-amber material was collected and measured to have a concentration of 72.0 brix.

[00326] The entirety of the first and second components were transferred into a suitably sized HDPE container and mixed thoroughly by hand until homogenous. The final syrup mixture was approximately 2.7 kg, dark-amber in color, viscous and was measured by refractive index to have a concentration of approximately 72 Brix.

Example 5: Synthesis of a gluco-manno-oligosaccharide preparation

[00327] Kilogram scale production of the oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures found to be suitable for production at the 1kg scale.

[00328] A gluco-manno-oligosaccharide preparation was prepared as two separate components synthesized in separate reaction vessels that were independently collected. Each synthesis used different starting reactants but followed the same procedure and methods to completion. The final

gluco-manno-oligosaccharide preparation was a homogeneous syrup formed from the mixing of both synthesis products.

[00329] For the synthesis of the first component, 1261.00 g of glucose monohydrate and 1.15 g of 2-pyridinesulfonic acid were added to a three-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. A 133mm Teflon stirring blade was affixed to a 440 mm glass stir shaft using PTFE tape. The stir rod was secured through the center point using a Teflon bearing adapter and attached to an overhead high-torque mechanical mixer via flexible coupler. The flask was secured inside a hemispherical electric heating mantle operated by a temperature control unit via a J-type wand thermocouple inserted through a rubber septum in one of the side ports. The tip of the thermocouple was adjusted to reside within the reaction mixture with several mm clearance above the mixing element. A secondary temperature probe connected to an auxiliary temperature monitor was also inserted and secured by the same means. The second side port of the flask was equipped with a reflux condenser cooled by a water-glycol mixture maintained below 4 °C by a recirculating bath chiller.

[00330] The reaction mixture was gradually heated to 130 °C with continuous mixing with a stir rate of 80-100 rpm. Once a temperature control box reading between 120 °C and 130 °C was observed, the apparatus was switched from a reflux condenser to a distillation configuration with a round bottom collection flask placed in an ice bath. This setup was maintained for approximately 6 hours, after which the thermocouple box was powered off, the distillation apparatus was removed, and 390 g of 23 °C distilled water was gradually added into the three-neck flask. The resulting mixture was left to stir at 40 rpm for 10 hours until the moment of collection. Approximately 1250 g of a viscous, light-amber material was collected and measured to have a concentration of 73.5 brix.

[00331] For the synthesis of the second component, 949.00 g of glucose monohydrate, 288.00 g of pure mannose from wood, 28.94 g distilled water, and 1.15 g of 2-pyridinesulfonic acid were added to a three-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. The remainder of the second component's synthesis followed the same procedure and methods as those of the first until the moment of collection. Approximately 1250 g of a viscous, dark-amber material was collected and measured to have a concentration of 73.3 brix.

[00332] The entirety of the first and second components were transferred into a suitably sized HDPE container and mixed thoroughly by hand until homogenous. The final syrup mixture was approximately 2.5 kg, dark-amber in color, viscous and was measured to have a concentration of approximately 73 brix.

Example 6: Synthesis of a gluco-galacto-oligosaccharide preparation

[00333] Kilogram scale production of the oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures found to be suitable for production at the 1kg scale.

[00334] A 3L three-neck flask was equipped with an overhead mixer connected via a 10mm diameter glass stir-shaft to a 14 cm crescent-shaped mixing element. The mixing element was positioned with approximately 5 mm clearance from the walls of the flask. The flask was heated via a hemispherical electric heating mantle powered by a temperature control unit connected to a wand-type thermocouple probe inserted into the reaction flask. The thermocouple probe was placed to provide 5 – 10 mm clearance above the mixing element. The flask was charged with 576 grams of food-grade dextrose monohydrate and 577 grams of food-grade D-galactose monohydrate and heated to approximately 115 °C to obtain a molten sugar syrup. Once the syrup was obtained, the flask was fitted with a jacketed reflux condenser cooled to 4 °C by circulating chilled glycol/water and the temperature. 31 grams of Dowex Marathon C (moisture content 0.48 g H_2O / g resin) were added to the mixture to form a stirred suspension. The condenser was repositioned into distillation configuration and the suspension was heated to 145 °C.

[00335] A mixing rate of approximately 80 RPM and a temperature of 145 °C was maintained for 3.8 hours, after which the set point on the temperature control unit was reduced to 80 °C and 119 mL of 60 °C deionized water was gradually added to the flask to obtain a dark amber syrup containing residual Dowex resin. The resulting suspension was further diluted to 60 Brix, cooled to room temperature and vacuum filtered through a 0.45 micron filter to remove the resin. 1,200 grams of light-amber syrup at 60 Brix concentration was obtained.

Example 7: Synthesis of a gluco-oligosaccharide preparation

[00336] Kilogram scale production of the oligosaccharide preparation was performed in a three-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures found to be suitable for production at the 1kg scale.

[00337] A 3L three-neck flask was equipped with an overhead mixer connected via a 10mm diameter glass stir-shaft to a 14 cm crescent-shaped mixing element. The mixing element was positioned with approximately 5 mm clearance from the walls of the flask. The flask was heated via a hemispherical electric heating mantle powered by a temperature control unit connected to a wand-type thermocouple probe inserted into the reaction flask. The thermocouple probe was placed to provide 5 – 10 mm clearance above the mixing element. The flask was gradually

charged with 1,148 grams of food-grade dextrose monohydrate and heated to approximately 115 °C to obtain a molten sugar syrup. Once the syrup was obtained, the flask was fitted with a jacketed distillation condenser cooled to 4 °C by circulating chilled glycol/water. The reaction temperature was gradually increased to 145 °C. Once the temperature was obtained and stable, 31 grams of Dowex Marathon C (moisture content 0.48 g H2O / g resin) was added to the mixture and a mixing rate of approximately 80 RPM and a temperature of 145 °C was maintained for 3.8 hours.

[00338] After 3.8 hours, the set point on the temperature control unit was reduced to 80 °C and 119 mL of 60 °C deionized water was gradually added to the flask to obtain a dark amber syrup containing residual Dowex resin. The resulting suspension was further diluted to 60 Brix, cooled to room temperature and vacuum filtered through a 0.45 micron filter to remove the resin. 1,113 grams of dark-amber gluco-oligosaccharide syrup at 60 Brix concentration was obtained.

Example 8: Single-Pot Syntheses of Oligosaccharide Preparations

[00339] A single pot (single component) synthesis of the oligosaccharide from Example 3 was demonstrated at 300 gram scale in a one-liter reaction vessel using catalyst loadings, reaction times, and reaction temperatures found to be suitable for the single pot reaction.

[00340] 272.30 g of food-grade D-glucose monohydrate from corn, 37.50 g of fodo grade D-mannose from wood, 15.60 g of food-grade D-lactose monohydrate, 3.96 g of distilled water and 0.270 g of 2-pyridinesulfonic acid (Sigma-Aldrich, St. Louis) were added to a one-liter, three-neck round bottom flask with one center 29/42 ground joint flanked by two 24/40 ground joints. A Teflon stirring blade was affixed to a 220 mm glass stir shaft using PTFE tape. The stir rod was secured through the center point using a Teflon bearing adapter and attached to an overhead high-torque mechanical mixer via flexible coupler. The flask was secured inside a hemispherical electric heating mantle operated by a temperature control unit via a J-type wand thermocouple inserted through a rubber septum in one of the side ports. The tip of the thermocouple was adjusted to reside within the reaction mixture with several mm clearance above the mixing element. A secondary temperature probe connected to an auxiliary temperature monitor was also inserted and secured by the same means. The second side port of the flask was equipped with a reflux condenser cooled by a water-glycol mixture maintained below 4 °C by a recirculating bath chiller.

[00341] The reaction mixture was gradually heated to 130 °C with continuous mixing with a stir rate of 80-100 rpm. Once a temperature control box reading between 120 C and 130 C was observed, the apparatus was switched from a reflux condenser to a distillation configuration with a round bottom collection flask placed in an ice bath. The mixture was maintained at 130 °C with

continuous stirring for approximately 5 hours and 40 minutes, after which the heating mantle and distillation apparatus was removed. Approximately 40 g of 23 C distilled water was gradually added into the three-neck flask. The resulting mixture was left to stir at 40 rpm for 10 hours until the moment of collection. Approximately 389 g of a viscous, dark-amber material was collected and measured to have a concentration of 67.0 brix. Consistency with the oligosaccharide preparation from Example 3 was confirmed by SEC chromatography and 2D ^{1}H, ^{13}C- HSQC NMR spectroscopy.

Example 9: Characterization of Oligosaccharide Preparations

[00342] The methods and procedures from Examples 1 – 8 were used to prepare replicate batches and blends of the oligosaccharides of Examples 1 – 7. The resulting materials were analyzed by HPLC Size Exclusion Chromatography (SEC) to characterize the molecular weight distribution, LC-MS/MS analysis to quantify the DP2 anhydro-sugar content, and 2D ^{1}H, ^{13}C- HSQC NMR to fingerprint the molecular structure of the corresponding oligosaccharide preparations.

[00343] Example 9.1: eleven batches of the oligosaccharide preparation from Example 1 were prepared and blended into four separate lots.

[00344] Example 9.2: seven batches of the oligosaccharide preparation from Example 2 were prepared and blended into two separate lots.

[00345] Example 9.3: twelve batches of the oligosaccharide preparation from Example 3 were prepared and blended into five separate lots.

[00346] Example 9.4: four batches of the oligosaccharide preparation from Example 4 were prepared and blended into a single lot.

[00347] Example 9.5: four batches of the oligosaccharide preparation from Example 5 were prepared and blended into a single lot.

[00348] Example 9.6: two batches of the oligosaccharide preparation from Example 6 were prepared and blended into a single lot.

[00349] Example 9.7: two batches of the oligosaccharide preparation from Example 7 were prepared and blended into a single lot.

[00350] In the batches of Examples 9.1 – 9.7, both multi-pot (multi-component) and single-pot variants of the respective synthetic schemes were employed.

[00351] Further structural variants of oligosaccharide preparations of Examples 1 – 7 were synthesized at 300 gram scale using the methods of Examples 1 – 7 but varying the starting sugar compositions, acid, acid loading, time, and reaction temperature. Oligosaccharide preparations were synthesized as follows:

[00352] Example 9.8: 300 grams of sucrose, 3 grams of phosphoric acid, and 27 grams of water were reacted at 125 °C for about one hour to obtain a dark brown oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00353] Example 9.9: 270 grams of glucose, 30 grams of sucrose, 0.3 grams of phenylphosphonic acid, and 27 grams of water were reacted at 130 °C for between one to four hours to obtain a dark brown oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00354] Example 9.10: 225 grams of glucose, 75 grams of lactose, 3 grams of butylphosphonic acid and 27 grams of water were reacted at 130 °C for between one to four hours to obtain a dark amber oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00355] Example 9.11: 225 grams of glucose, 75 grams of lactose, 3 grams of phenylphosphonic acid and 27 grams of water were reacted at 130 °C for between one to five hours to obtain a dark amber oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00356] Example 9.12: 270 grams of glucose, 30 grams of lactose, 3 grams of phenylphosphinic acid and 27 grams of water were reacted at 130 °C for between three to five hours to obtain a dark brown oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00357] Example 9.13: 300 grams of glucose, 3 grams of phenylphosphinic acid, and 27 grams of water were reacted at 130 °C for one to three hours to obtain a dark amber oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00358] Example 9.14: 300 grams of glucose, 2 grams of propionic acid, and 27 grams of water were reacted at 130 °C for one to four hours to obtain an amber oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00359] Example 9.15: 300 grams of glucose, 0.15 grams of 8-hydroxy-5-quinolinesulfonic acid hydrate, and 27 grams of water were reacted at 130 °C for two to four hours to obtain an amber oligosaccharide syrup that was then diluted to 60 Brix with distilled water.

[00360] In the above reactions, all masses refer to the pure component masses, and the total mass of reactant water was inclusive of any carry-along water provided by the moisture content and/or water of hydration of the reactant sugars.

Characterization of Oligosaccharide Preparations:

[00361] The resulting materials were analyzed by HPLC Size Exclusion Chromatography (SEC) to characterize the molecular weight distribution, LC-MS/MS analysis to quantify the DP2 anhydrosugar content, and 2D ^{1}H, ^{13}C- HSQC NMR to fingerprint the molecular structure of the corresponding oligosaccharide preparations.

Polymer MW Analysis by HPLC:

[00362] The number average molecular weight (MWn) and weight-average molecular weight (MWw) of the oligosaccharide preparations of Examples 9.1 – 9.7 were determined by HPLC. SEC analysis was performed on an Agilent 1100 series HPLC with refractive index detection using an Agilent PL aquagel-OH 20 column at 40 °C with distilled water at 0.45 mL/min as the mobile phase. Retention-time to MW calibration was performed using standard solutions with known molecular weight and standard methods from the art were used to determine the various distribution properties from the SEC chromatogram.

Table 1. Polymer molecular weight (MW) for oligosaccharide preparations with multiple lots

Example	MWn (g/mol)	MWw (g/mol)
Ex. 9.1	719 ± 11	1,063 ± 23
Ex. 9.2	808 ± 30	1,336 ± 122
Ex. 9.3	757 ± 15	1,186 ± 49
Ex. 9.4	761	1,196
Ex. 9.5	755	1,177
Ex. 9.6	505	709
Ex. 9.7	762 ± 12	1,154 ± 14

Anhydro-DP2 Content Analysis by LC-MS/MS:

[00363] The anhydro DP2 oligosaccharides content of oligosaccharide preparations was determined by LC-MS/MS using a Capcell Pak NH2 (Shiseido; 250x4. 6mm, 5 µm) column at a flowrate of 1 mL/min under isocratic conditions of water/acetonitrile 35/65. Prior to MS the flow was split 1:4 and a makeup flow of 50 µL 0.05 % NH4OH was added to enhance ionization. For MS detection ESI probe was used in negative mode and multiple reaction monitoring (MRM) method allowed targeted analysis.

[00364] The anhydro DP2 contents of the oligosaccharide preparations was first determined relative to that of the oligosaccharide preparation of Example 9.7 as a reference composition. The absolute anhydro DP2 content of the reference oligosaccharide preparation of Example 9.7 was then determined by HPLC-MS/MS to be about 10% and the anhydro DP2 contents of the oligosaccharide preparations of Examples 9.1 to 9.6 were then obtained by calculation. The relative and absolute DP2 contents were determined as follows:

Table 2. Anhydro DP2 content for oligosaccharide preparations with multiple lots

Example	Relative Anhydro DP2 Content % Relative to Ex 9.7	Anhydro DP2 Content (g Anhydro DP2 / g total DP2)
Ex. 9.1	53%	5.3%
Ex. 9.2	14%	1.4%
Ex. 9.3	57%	5.7%

Ex. 9.4	53%	5.3%
Ex. 9.5	33%	3.3%
Ex. 9.6	50%	5.0%
Ex. 9.7	100%	10.0%

Molecular Fingerprint by 2D ^{1}H, ^{13}C- HSQC *NMR:*

[00365] The molecular structures of the oligosaccharide preparations of Example 9 were characterized by 2D ^{1}H, ^{13}C- HSQC NMR spectroscopy. Samples were prepared by dissolving 50 mg of dry oligosaccharide preparation in D_2O. NMR spectra were acquired at 300K on either a Bruker Avance NMR spectrometer operating at a proton frequency of 400 MHz or on a Bruker Avance III NMR spectrometer operating at a proton frequency of 600 MHz equipped with a cryogenically cooled 5 mm TCI probe. FIG. 1 provides an illustrative 2D ^{1}H,^{13}C HSQC NMR spectrum of the oligosaccharide preparation of Example 9.7.

[00366] The anomeric region of the ^{1}H, ^{13}C- HSQC spectrum, F2 ($\delta^{1}H$) = 4.2 – 6.0 ppm and F1($\delta^{13}C$) = 90 – 120 ppm, was used to fingerprint the linkage distribution of the oligosaccharide preparations. Each peak in the anomeric region was integrated and its relative abundance was determined relative to that of the total anomeric region. 2D ^{1}H, ^{13}C HSQC fingerprinting was performed on the four lots of the oligosaccharide preparation of Example 9.1, resulting in the following relative abundances provided in Table 3.

Table 3. Relative abundance of 2D ^{1}H,^{13}C HSQC NMR peaks of oligosaccharide preparation of Example 9.1

F2 (ppm)	**F1 (ppm)**	**AUC (Average ± SEM)**
5.43	92.42	0.4% ± 0.3%
5.44	102.07	0.4% ± 0.1%
5.43	90.05	0.5% ± 0.2%
5.40	100.22	1.6% ± 0.4%
5.37	98.33	0.7% ± 0.4%
5.35	99.70	2.7% ± 0.6%
5.33	96.53	0.3% ± 0.2%
5.24	100.86	0.5% ± 0.2%
5.22	92.71	20.2% ± 3.9%
5.21	102.45	0.5% ± 0.4%
5.18	93.86	0.9% ± 0.4%
5.17	96.01	0.4% ± 0.1%
5.09	96.88	0.6% ± 0.3%
5.03	108.49	0.4% ± 0.2%
5.02	109.16	0.4% ± 0.4%
4.98	99.19	0.6% ± 0.3%

4.95	98.51	30.6% ± 4.1%
4.86	98.53	0.7% ± 0.5%
4.79	96.84	0.6% ± 0.3%
4.71	103.48	2.5% ± 0.7%
4.64	103.56	0.8% ± 0.4%
4.63	102.49	0.7% ± 0.5%
4.62	104.56	1.4% ± 0.4%
4.57	97.07	1.6% ± 0.3%
4.50	103.30	25.9% ± 2.2%
4.45	103.56	2.4% ± 1.3%

Example 10: Determination of the Anhydro Sugar Subunits of an Oligosaccharide Preparation

[00367] The relative abundance of anhydro sugar subunits in the oligosaccharide preparations of Example 9 was determined by MALDI-MS on a Bruker Ultraflex instrument. Samples were dissolved in water to a concentration of 10 mg/ml, from which 5 µl were mixed with matrix solution (30 mg/ml DHB in 80 % ethanol and water in a ratio 1:10). Plates were prepared by applying 1 µl of the analyte solution to the target plate and dried at ambient air. In some cases, samples were re-crystalized by applying 1 µl ethanol prior to MS analysis.

[00368] FIG. 2 provides an illustrative MALDI spectrum of an oligosaccharide preparation from Example 9. Anhydro-sugar subunits are clearly observed as offset peaks shifted by -18 g/mol relative to its respective principal DP parent. Offset peaks are observed at all values of DP, indicating that anhydrosugar subunits are detected at all oligosaccharide sizes. The relative intensity of the anhydro subunit peak was determined to be about 10% of the total peak intensity for each DP.

[00369] FIG. 20A and FIG. 20B illustrate MALDI spectra of an oligosaccharide preparation from Example 2. Anhydro-sugar subunits are observed at every DP level with an relative intensity in the range of 5-10%.

Example 11: Characterization of the Anhydro Sub-Units of an Oligosaccharide Preparation

[00370] The anhydrosugar subunits of the oligosaccharide preparations of Example 9 were characterized using a combination of LC-MS, GC-MS, LC-MS/MS, and NMR methods.

Characterization of Anhydro-DP1 Components:

[00371] The anhydro DP1 oligosaccharide component of an oligosaccharide preparation from Example 9 was isolated by preparative liquid chromatography. The isolated anhydro-DP1 component was prepared for NMR by dissolving it in 0.75 mL of D2O. FIG. 3 provides an

illustrative 1D ^{1}H-NMR spectrum of an anhydro DP1 fraction isolated from an oligosaccharide of Example 9 and FIG. 4 provides an illustrative APT ^{13}C-NMR spectrum of the same isolated anhydro DP1 fraction. The NMR peak assignments are provided in Table 4 and FIG. 5.

Table 4. NMR peak assignments

	1,6-anhydro-beta-D-glucofuranose		1,6-Anhydro-beta-D-glucopyranose	
#	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)
1	5.33	101.9	5.01	104.4
2	3.40	70.6	4.37	79.8
3	3.56 (ov)[a]	73.0	4.27	78.3
4	3.56 (ov)[a]	71.3	4.38	80.6
5	4.50	76.7	3.74	64.1
6	3.97, 3.64	65.7	4.14, 3.72	66.7

[a] *Ov stands for overlapped signal*

[00372] The ratio of 1,6-anhydro-beta-D-glucofuranose to 1,6-Anhydro-beta-D-glucopyranose was determined by NMR to be 2:1.

Example 12: Characterization of the Anhydro Sub-Units of an Oligosaccharide Preparation

[00373] The anhydrosugar subunits of the oligosaccharide preparations of Example 9 were characterized using a combination of LC-MS, GC-MS, LC-MS/MS, and NMR methods.

Characterization of the Anhydro-DP2 Components

[00374] The anhydro DP2 oligosaccharides content of the oligosaccharide preparations of Example 9 were determined by GC-MS and LC-MS/MS analysis. Gas chromatography was performed using a 30m x 0.25 mm fused silica column containing HP-5MS stationary phase, with 21.57 psi constant pressure Helium as the carrier gas. Aliquots were pre-derivatized by acetylation by dissolving 20 mg of sample in 0.5 mL pyridine with 0.5 mL acetic anhydride for 30 minutes at 60 °C. 1 uL samples were injected at 300 °C with an oven temperature program starting at 70 °C and ramping by 10 °C per minute to 315 °C. Detection was performed on an Agilent 5975C MSD with an electron energy of 70 eV.

[00375] FIG. 6 illustrates an enlargement of the GC-MS chromatogram for the oligosaccharide preparation of Example 9.7. The TIC and XIC (m/z 229) plots demonstrate that the anhydro-DP2 components elute before the DP2 components.

[00376] FIGs. 25A-25B, 26A-26B, 27A-27B, and 28A-28B illustrate the presence of the DP1, anhydro DP1, DP2 and anhydro DP2 fractions as detected by GC-MS in an oligosaccharide

preparation of Example 1, Example 3, Example 4, and Example 7, respectively. As shown in FIGs. 25A-25B, 26A-26B, 27A-27B, and 28A-28B, anhydro DP1 and DP1 fractions have a retention time of from about 12-17 minutes, and anhydro DP2 and DP2 fractions have a retention time of about from 22-25 minutes.

[00377] FIG. 30 illustrates MALDI-MS spectra comparing the oligosaccharide preparation from Example 9 at different laser energies. Relative abundancy of signals were nearly unchanged, demonstrating that no loss of water is introduced by the laser ionisation. Hence, proving the presence of anhydro-sugar subunits in the oligosaccharide preparation.

Example 13: Comparative Example

[00378] A commercial 5 kDa dextran was analyzed by MALDI-MS for the presence of anhydrosugar subunits. FIG. 7 illustrates the clear presence of the offset peak shifted –18 g/mol from the principal DP peak (Na+ adduct at 851.268 g/mol). By contrast the dextran sample was found to be essentially free of anhydro sugar subunits.

Example 14: Quantification of the Anhydro DP Component by LC-MS/MS

[00379] Samples were dissolved in water and separated using a Capcell Pak NH2 (Shiseido; 250x4. 6mm, 5 μm) column at a flowrate of 1 mL/min under isocratic conditions of water/acetonitrile 35/65. In some cases, following chromatographic separation, 50 μL 0.05 % NH_4OH was added to enhance ionization. The anhydro DP2 oligosaccharides content was determined by MS/MS detection. For MS detection ESI probe was used in negative mode and MRM method allowed targeted analysis. FIG. 8 illustrates detection of an oligosaccharide preparation from Example 9 over a concentration range of 1 – 80 μg / mL in water, with a linear calibration curve (shown in FIG. 9) from the area under the LC-MS/MS chromatogram to concentration.

[00380] FIGs. 21A-21C, 22A-22C, 23A-23C, and 24A-24C illustrate the presence of anhydro DP2, anhydro DP1, and DP2 species detected by LC-MS/MS in an oligosaccharide preparation of Example 1, Example 3, Example 4, and Example 7, respectively.

Example 15: Preparation of Feed Comprising Oligosaccharide Preparations

[00381] Poultry and swine diets were prepared to demonstrate the incorporation of oligosaccharide preparations into the diet. Control feeds exhibiting a variety of ingredient compositions and corresponding treated feeds obtained by augmenting the respective control feeds with the oligosaccharide preparations of Example 9 were prepared as follows:

[00382] Example Feed 15.1: Control Feed 15.1 (CTR) was prepared using 62% corn meal and 32% soybean meal. Treated Feed 15.1 (TRT) was prepared by augmenting the control feed 15.1 (CTR) with 500 mg/kg of an oligosaccharide preparation from Example 9. For the treated diet, the oligosaccharide preparation was provided in a powder form by drying the oligosaccharide onto ground rice hulls as a carrier and adding the powder to the mixer using a micro-ingredient balance prior to pelleting.

[00383] Example Feed 15.2: Control Feed 15.2 (CTR) was prepared using 62% corn meal, 3% soybean concentrate, and 26% soybean meal. Treated Feed 15.2 (TRT) was prepared by augmenting the control feed 15.2 (CTR) with 500 mg/kg of an oligosaccharide preparation from Example 9. For the treated diet, the oligosaccharide preparation was provided in a powder form by drying the oligosaccharide onto ground rice hulls as a carrier and adding the powder to the mixer using a micro-ingredient balance prior to pelleting.

[00384] Example Feed 15.3: Control Feed 15.3 (CTR) was prepared using 52% corn meal, 6% corn starch, 5% soybean concentrate, 26% soybean meal, and a titanium oxide micro-tracer. Treated Feed 15.3 (TRT) was prepared by augmenting the control feed 15.3 (CTR) with 500 mg/kg of an oligosaccharide preparation. For the treated diet, the oligosaccharide preparation was provided in a powder form by drying the oligosaccharide onto ground rice hulls as a carrier and adding the powder to the mixer using a micro-ingredient balance prior to pelleting.

[00385] Example Feed 15.4: Control Feed 15.4 (CTR) was prepared using 55% corn meal and 39% soybean meal. Treated Feed 15.4 (TRT) was prepared by augmenting the control feed 15.4 (CTR) with 1,000 mg/kg of an oligosaccharide preparation. For the treated diet, the oligosaccharide preparation was provided in a powder form by drying the oligosaccharide onto ground rice hulls as a carrier and adding the powder to the mixer using a micro-ingredient balance prior to pelleting.

[00386] Example Feed 15.5: Control Feed 15.5 (CTR) was prepared using 62% corn meal, 3% soybean concentrate, and 26% soybean meal. Treated Feed 15.5 (TRT) was prepared by augmenting the control feed 15.5 (CTR) with 500 mg/kg of an oligosaccharide preparation from Example 9. For the treated diet, the oligosaccharide preparation was provided in a powder form and adding the powder to the mixer using a micro-ingredient balance prior to pelleting.

[00387] Example Feed 15.6: Control Feed 15.6 (CTR) was a commercial U.S. corn-soy starter poultry feed. Treated Feed 15.6 (TRT) was a commercial U.S. corn-soy starter poultry feed containing 500 ppm of an oligosaccharide preparation. For the treated diet, the oligosaccharide preparation was provided in a powder form and adding the powder to the mixer using a micro-ingredient balance prior to pelleting.

[00388] Example Feed 15.7: Control Feed 15.7 (CTR) was a research corn-soy poultry feed with the following diet composition: corn meal 62.39%, soybean meal 31.80%, calcium carbonate 0.15%, bicalcic-phosphate 2.2%, sodium chloride 0.15%, DL-Methionine 0.15%, L-Lysine 0.10%, Soya Oil 2.00%, vitamin-mineral premix 1.00%, and coccidiostat 0.06%. Treated Feed 15.7 (TRT) was obtained by supplementing the control feed 15.7 (CTR) with 1000 ppm of the oligosaccharide preparation of Example 9.1. For the treated diet, the oligosaccharide preparation was provided as 60% syrup in water and was applied by spraying the syrup onto the feed post pelleting.

[00389] Example Feed 15.8: Control Feed 15.8 (CTR) was a research corn-soy poultry feed with the following composition: wheat meal 48.45%, soybean meal 32.00%, rye 12%, calcium carbonate 0.20%, bicalcic phosphate 2.00%, sodium chloride 0.20%, DL-methionine 0.15%, soya oil 4.00%, vitamin-mineral premix 1.00%.Treated Feed 15.7 (TRT) was obtained by supplementing the control feed 15.7 (CTR) with 1000 ppm of the oligosaccharide preparation of Example 9.3. For the treated diet, the oligosaccharide preparation was provided as 60% syrup in water and was applied by spraying the syrup onto the feed post pelleting.

[00390] As would be understood by one skilled in the art, the 15.1 – 15.6 also contained industry standard levels of fat, vitamins, minerals, amino acids, other micronutrients and feed enzymes. In some cases the feeds contained a cocciodiostat, but were free in all cases of antibiotic growth promoters. The feeds were provided as either mash, pelletized, or crumbled diets, according to standard practices.

Example 16: Extraction of Feed Samples

[00391] Diets prepared according to the procedures of Example 15 were processed by extraction. Feed samples were ground with a mill. Five grams of the resulting milled feed were weighted into a 50 mL volumetric flask and hot water (approx. 80 °C) was added. After shaking, the mixture was incubated in an ultrasonic water bath at 80°C for 30 minutes. After cooling, the solution was centrifuged for 20 min at 3000xg and the supernatant was filtered through a 1.2 μm filter followed by a 0.45 μm filter (and in some cases by a 0.22 μm filter). The resulting filtered solutions were evaporated to dryness with a rotary evaporator.

[00392] In some cases, the extraction was performed using 50 wt% ethanol in water as an alternative extraction solvent. In some cases, the filtration step was performed using a 5,000 Dalton molecular-weight cutoff membrane filter. A list of feed samples for extraction and digestion is provided in Table 5.

Example 17: Enzymatic Processing of Feed Extracts

[00393] The feed extracts of Example 16 were subjected to one or more enzymatic hydrolysis steps to digest oligosaccharides naturally present in the feed. A mixture of α-Amylase and amyloglycosidases were used to digest α-(1,4) linkages of gluco-oligosaccharides and starch. Invertase and α-galactosidase were used to remove sucrose, raffinose, and other common fiber saccharides.

[00394] Enzyme solutions were prepared as follows: Amyloglucosidase (*A. niger*) 36000 U/g solution at 800 U/mL in ammonium acetate buffer (ammonium acetate 0.2 M pH 5 containing 0.5 mM MgCl2 and 200 mM CaCl2), α-Amylase (Porcine Pancreas) 100000 U/g Megazyme: solution at 800 U/mL in ammonium acetate, Invertase from Backer's yeast (*S. cerevisiae*) 300U/mg Sigma: solution at 600 U/mL in ammonium acetate buffer, α-Galactosidase from *A. niger* Megazyme 1000 U/mL.

[00395] The dry feed extracts of Example 16 were re-suspended in 10 mL ammonium acetate buffer. 50 µl of α-amylase (4 U/mL final), 50 µl of amyloglucosidase (4 U/mL final), 50 µl of invertase (3 U/mL final) were added. 20 µl α-galactosidase (2U /mL final) was added optionally. The solution was incubated for 4 hours at 60°C. The digested extract was then filtered through an ultrafiltration filter (Vivaspin Turbo 4, 5000 MWCO, Sartorius) before being evaporated to dryness on a nitrogen evaporation system. In variations of the enzymatic digestion, one or more of the above enzymes were used in combinations and the digestion period was varied between 4 hours to overnight digestion. The enzyme concentrations were varied up to twice the above loadings, and the full enzymatic digestion procedure was repeated multiple times in sequence on the same feed.

Table 5. List of Feed Samples for Extraction and Digestion

	Matrix	Extraction solvent	Filtration	Enzyme digestion
1	Blank feed	Water	0.22 µM	_
2	Anhydro-Oligomers feed 1000 mg/kg	Water	0.22 µM	_
3	Blank Feed	ethanol/water 50/50	0.22 µM	_
4	Anhydro-Oligomers feed 1000 mg/kg	ethanol/water 50/50	0.22 µM	_
5	Anhydro-Oligomers	Water	0.22 µM	a + b (4h 60 °C)
6	Blank feed	Water	0.22 µM	a + b (4h 60 °C)
7	Anhydro-Oligomers feed 1000 mg/kg	Water	0.22 µM	a + b (4h 60 °C)
10	Blank feed	ethanol/water 50/50	0.22 µM	_

11	Anhydro-Oligomers feed 1000 mg/kg	ethanol/water 50/50	0.22 µM	_
12	Anhydro-Oligomers feed 1000 mg/kg	Water	0.45 µM	a + b (4h 60 °C)
13	Anhydro-Oligomers feed 1000 mg/kg	Water	0.22 µM	a + b (4h 60 °C)
14	Blank starter feed A	Water	0.45µM	a + b (4h 60 °C)
15	Anhydro-Oligomers starter feed B 2000 mg/kg	Water	0.45 µM	a + b (4h 60 °C)
16	Blank feed	Water	0.45 µM	a + b + c (4h 60 °C)
17	Anhydro-Oligomers feed 1000 mg/kg	Water	0.45 µM	a + b + c (4h 60 °C)
18	Rice spelt/ Anhydro-Oligomers	Water	0.45 µM	_
19	Rice spelt/ Anhydro-Oligomers	ethanol/water 50/50	0.45 µM	_
20	Blank feed	Water	0.45 µM	a + b + c (4h 60 °C)
21	Anhydro-Oligomers feed 1000 mg/kg	Water	0.45 µM	a + b + c (4h 60 °C)
22	Grower feed C (blank)	Water	0.45 µM	
23	Grower feed D (Anhydro-Oligomers 2000mg/kg)	Water	0.45 µM	
24	Pre starter feed A (blank)	Water	0.45 µM	
25	Pre starter feed D (Anhydro-Oligomers 1000mg/kg)	Water	0.45 µM	
26	Grower feed C (blank)	Water	0.45 µM	a + b + c + d (4h 60°C)
27	Grower feed D (Anhydro-Oligomers 2000mg/kg)	Water	0.45 µM	a + b + c + d (4h 60°C)
28	Pre starter feed A (blank)	Water	0.45 µM	a + b + c + d (4h 60°C)
29	Pre starter feed D (Anhydro-Oligomers 1000mg/kg)	Water	0.45 µM	a + b + c + d (4h 60°C)
30	Maize	Water	0.45 µM	_
31	Wheat	Water	0.45 µM	_
32	Soy	Water	0.45 µM	_
33	Maize	Water	0.45 µM	a + b + c + d (4h 60°C)
34	Wheat	Water	0.45 µM	a + b + c + d (4h 60°C)
35	Soy	Water	0.45 µM	a + b + c + d (4h 60°C)
41	Blank Feed	Water	0.45 µM	
42	Anhydro-Oligomers feed 1000 mg/kg	Water	0.45 µM	

43	Blank feed	Water	ultra 5000 MWCO	a + b + c X2 (overnight 60°C)
44	Anhydro-Oligomers feed 1000 mg/kg	Water	ultra 5000 MWCO	a + b + c X2 (overnight 60°C)

[00396] Anhydro-Oligomers refer to anhydro-subunit containing oligosaccharides.

[00397] Blank feeds refer to nutritional compositions without added anhydro-oligomers.

[00398] Enzyme a = Amyloglucosidase (A. niger) 36000 U/g Megazyme

[00399] Enzyme b = α-Amylase (Porcine Pancreas) 100000 U/g Megazyme

[00400] Enzyme c = Invertase from Baker's yeast (S. cerevisiae) 300 U/mg Sigma

[00401] Enzyme d = α-Galactosidase from A. niger 620 U/mg Megazyme

Example 18: Detection of Oligosaccharide Preparations in Feed

[00402] The Control and Treated diets according to Example 15 were assayed to detect the absence or presence of oligosaccharide preparations. After diet manufacture, 1 kg samples were drawn from the final feed. The extractable solids content of the feed was obtained by water extraction using the procedure of Example 16. The resulting extracts were analyzed for the presence of anhydro-DP species by LC-MS/MS according to the procedure of Example 14.

[00403] FIG. 10 clearly illustrates the absence of anhydro-DP2 species in the control feeds of Examples 15.1(CTR) – 15.6(CTR) versus the presence of anhydro-DP2 species in the treated feeds of Examples 15.1(TRT) – 15.6(TRT). Integration of the resulting LC-MS/MS chromatograms was used to determine the presence of the oligosaccharide compositions of Example 9 in the final feed. Specifically, feeds were determined to contain the respective oligosaccharide preparation if the area under the anhydro-DP2 peak exceeded the limit of detection (or any other suitable threshold established in the method).

Example 19: Quantification of Oligosaccharide Preparations in Feed

[00404] The Control and Treated diets according to Example 15 were assayed to determine the concentration of oligosaccharide preparations in the final feed. After diet manufacture, 1 kg samples were drawn from the final feed. The extractable solids content of the feed was obtained by water extraction using the procedure of Example 16. The resulting extracts were analyzed for the presence of anhydro-DP species by LC-MS/MS according to the procedure of Example 14 and the area of the anhydro-DP2 peak was compared against a standard calibration curve to determine the concentration of the oligosaccharide preparation in the feed.

Table 6. Quantification of oligosaccharides in feeds

Feed	Oligosaccharide Content (ppm) in Control Feed	Oligosaccharide Content (ppm) in Treated Feed
Example 15.1	Not detected	1642
Example 15.2	Not detected	953
Example 15.3	Not detected	1912
Example 15.4	Not detected	549
Example 15.5	Not detected	406
Example 15.6	Trace	401

Example 20: NMR Characterization of Anhydro-subunit Containing Gluco-oligosaccharides

[00405] Gluco-oligosaccharide preparations comprising anhydro-subunits were characterized by i) the degree of polymerization and ii) the glycosidic linkage distribution of the glucose units.

[00406] The relative molar abundances of α–(1,1)-α, α–(1,1)-β, β–(1,1)-β, α–(1,2), β–(1,2), α–(1,3), β–(1,3), α–(1,4), β–(1,4), α–(1,6), and β–(1,6) linkages were identified by NMR spectroscopy. For the determination of the linkage distribution, J-RES and ^{1}H, ^{13}C- HSQC were used. For some samples, the HSQC method showed a superior performance. For each analysis, approximately 50 mg of freeze-dried product were dissolved in D2O and transferred to a 5 mm NMR tube. Any residual catalyst or solids were removed by filtration. NMR experiments were performed on a Bruker Avance III NMR spectrometer operating at 600 MHz proton corresponding to 150 MHz carbon Larmor frequency. The instrument was equipped with a cryogenically cooled 5mm TCI probe. All experiments are carried out at 298 K. ^{1}H NMR spectra were recorded and calibrated in deuterated water (4.75 ppm). ^{13}C NMR spectra are calibrated with acetone (30.9 ppm). Data were acquired using TopSpin 3.5 and processed with ACD/Labs running on a personal computer.

[00407] FIG. 11 provides a representative 2D-1H JRES NMR spectrum of an anhydro-subunit containing gluco-oligosaccharide sample with solvent pre-saturation.

[00408] FIG. 12 provides a representative 1H, 13C- HSQC NMR spectrum of an anhydro-subunit containing gluco-oligosaccharides sample

[00409] FIG. 13 illustrates an overlay of ^{1}H DOSY spectra of three anhydro-subunit containing oligosaccharides. Diffusion-Ordered NMR Spectroscopy (DOSY) was performed to separate the NMR signals of different species according to diffusion coefficient and thus MW homologues. Signals at the upper part of the DOSY spectra in FIG. 13 correspond to slow diffusing species, while faster diffusing species appear below.

Example 21: Semi-preparative Isolation of DP1 & DP2 Fractions

[00410] Preparative isolation of the DP1 fraction was performed by preparative HPLC using a Waters BEH Amide 19x150mm column. As mobile phase water was used as solvent A and Acetonitrile as solvent B, each with 0.1% ammonia. The applied gradient is shown in table 7. The collected DP1 fraction of 8 separations were pooled, dried and resolubilized in 0.75ml D_2O for NMR analysis as described before.

[00411] For the characterization of the DP2 fraction a 2-step purification was done. The first step was performed on a flash chromatography system, using an ELSD (Evaporative light scattering detector). 2ml (2.65g) of an oligosaccharide preparation were diluted with 1ml DMSO, 0.5ml water and 0.5ml Acetonitrile. The solution was mixed and for 15min sonicated. 1ml of the solution was injected on a YMC DispoPackAT, NH2, spherical, 25 µm, 120 g column was used. The oligosaccharide preparation was separated running an isocratic gradient method with 75% acetonitrile in water at 40ml/min. The DP2 containing fraction was dried with nitrogen and resolubilized in DMSO/water (80:20, v/v).

[00412] For the 2nd purification step an analytical UPLC system with a YMC NH_2 4.6x250mm (5µm) column at 40° was used . The DP2 fraction was purified with an isocratic gradient (Table 8) and a flow rate of 1ml/min. A 1:5 post-column spilt was used in order to trigger the collection by ELSD. The DP2 fraction from 12 chromatographic runs were pooled, the acetonitrile removed by heated nitrogen and the residual water by freeze-drying. The dry fraction was resolubilized for subsequent LC-MS/MS & NMR analysis.

Table 7. Gradient Method

Time (min)	Flow rate (ml/min)	Solvent A	Solvent B
0	25	10	90
2.5	25	10	90
23	25	25	75
23.1	25	10	90
47	25	10	90

Table 8. Isocratic Method

Time (min)	Flow rate (ml/min)	Water (%)	ACN (%)
0	1	25	75
15	1	25	75

[00413] The second approach, an UPLC method, was set up based on a HILIC-mode separation with an Amide column.

[00414] Method details: system, Waters UPLC-SQ MS/ ELSD; solvent A, 30/70 acetonitrile/water: 0.1 % ammonia; solvent B, 80/20 acetonitrile/water: 0.1 % ammonia; weak

needle wash: 80/20 acetonitrile/water; strong needle wash: 20/80 acetonitrile/water; column: Waters BEH Amide 2.1x100 mm, 1.7 µm; injection: 0.5 µl in water.

Table 9. Gradient

Time (min)	Flow (ml/min)	A (%)	B (%)	Slope
Initial	0.17	0	100	Initial
13	0.17	60	40	6
14.5	0.17	100	0	6
16.5	0.17	100	0	6
16.6	0.17	0	100	6
30	0.17	0	100	6

[00415] This method provided good separation of a series of sugar reference compounds. Only glucose and galactose are co-eluting, however, with different peak shape.

[00416] The analysis of 1,6-Anhydro-ß-D-glucose (DP1-18) and the 1,6-Anhydro-ß-D-celobiose (DP2-18) is shown in FIG. 14, compared to an anhydro-subunit containing oligosaccharides sample.

[00417] Preparative isolation by Preparative HPLC-ELSD/MS: the optimization of a preparative HPLC separation is usually done by starting with an analytical column. Once the separation is optimal a column loading experiment is done. During the following upscale all column parameters like particle size, length, pore size, phase, linear flow rate (cm/min) are kept the same, only changing the diameter of the column.

Example 22: Analysis of Digested Feed samples

[00418] Interference by naturally occurring oligosaccharides in the feed extract with the analysis of anhydro-subunit containing oligosaccharides was reduced by selective digestion of background feed components without degrading the anhydro-subunit containing oligosaccharides. FIG. 15 illustrates mass chromatograms of anhydro-subunit containing oligosaccharides (top) and digested anhydro-subunit containing oligosaccharides (bottom) at selected MRM. FIG. 16 illustrates mass chromatograms of (1) feed that contains anhydro-subunit containing oligosaccharides, (2) digested feed that contains anhydro-subunit containing oligosaccharides, and (3) blank digested feed at selected MRM. Higher DPs species in the feed were eliminated or reduced. For example, as shown in FIG. 16, the second peak at DP3 level was essentially removed, and at DP2 level, both peaks were significantly reduced.

Example 23: Workflow for Quantifying Oligosaccharide Preparation

[00419] FIG. 17 illustrates a workflow used for quantifying oligosaccharide preparations. It consists of an extraction step of a water-soluble oligosaccharide preparation out of the feed, followed by removal of the water by evaporation or freeze-drying. The resulting extract will be investigated if direct analysis of the oligosaccharide preparations by either LC-MS or NMR is possible. If this is not the case, interfering oligosaccharides from the feed matrix will need to be removed by an enzymatic digest. This can exploit the fact that most glyosidic bonds in the oligosaccharide preparation have a connectivity and stereochemistry that do not occur naturally in base nutritional composition. It is thus expected that the oligosaccharide preparation is stable during the digest. In case of the glycosidic bonds present in the oligosaccharide preparation that can in principle be cleaved by the enzymes [mainly α-(1,4), α-(1,6), β-(1,6)], steric hindering due to the branching can prevent this for the majority of molecules. Following enzymatic digest, a concentration step will be needed prior to quantification.

[00420] The low DP oligosaccharides, in particular anhydro-DP1, anhydro-DP2, and DP2 oligosaccharides, generally consist of relatively a small number of chemical entities and have relatively high concentration. Those fractions can be quantified. In addition, small oligomers can show a much higher sensitivity in LC-MS detection than larger oligomers.

[00421] For quantification by MS, typically isotopically labelled material can be used as internal standard. If required, 13C-labeled synthesized oligosaccharide preparation can be used. After quantification, NMR and/or MS will be performed to analyze the quantified oligosaccharides.

Example 24: Additional Representative Workflows

[00422] In some embodiments, the method comprises the following workflow:

[00423] (a) obtaining or producing a sample of a nutritional composition; (b) extracting from the sample the soluble species using a solvent (water, a water alcohol mixture); (c) (optionally) introducing an internal standard to the extract; (d) (optionally) filtering or clarifying the extract (e.g., to remove fine particulates); (e) (optionally) concentrating the extract; (f) (optionally) digesting a portion of the background carbohydrates using a cocktail of hydrolytic enzymes (carbohydratases, proteases, lipases, etc.); (g) (optionally) performing a crude separation of the components of the (digested, concentrated) extract by e.g., precipitation, nano-filtration, etc.; (h) chromatographically separating some or all of the species contained in the extract, in particular the DP2 carbohydrate species; (i) (optionally) quantifying the species comprising primarily glycosidic linkages not commonly abundant in the nutrition composition itself; (j) (optionally) detecting for and quantifying the presence of particular anhydro DP1 and DP2 species; and (k) calculating the concentration of the carbohydrate preparation in the feed, e.g., by reference to a

standard curve prepared by performing the assay on a set of primary standards comprising nutritional compositions to which a known quantity of carbohydrate preparation was added.

[00424] The nutritional compositions can be a poultry feed, pig feed, fish feed, or ruminant feed.

Example 25: Growth Performance of Commercial Broiler Chickens fed an Oligosaccharide Composition

[00425] Broiler chickens were grown on the diets of Example 15.6 to determine the effect of the oligosaccharide preparation on the growth performance of the animals. Specifically, commercial corn-soymeal poultry feeds containing dried distillers grains with solubles (DDGS), a coccidiostat, and a standard micronutrient blend, were manufactured according to industry practices and a three phase feeding program. By proximate analysis, the feed compositions were determined as follows (Table 10).

Table 10. Determination of feed composition

Component	Starter	Grower	Withdrawal	Method
Moisture	13.0%	13.0%	12.9%	AOAC 930.15 (drafted oven)
Crude Protein (CP)	24.1%	21.5%	19.6%	AOAC 992.15; AOAC 990.03
Fat (EE)	3.2%	3.8%	3.9%	AOAC 920.39 (ether extraction)
Crude Fiber (CF)	2.7%	2.4%	2.4%	AOAC 962.09 (hydrolysis)
Ash (AR)	5.2%	4.3%	4.3%	AOAC 942.05 (muffle furnace)
NFE, by difference	51.9%	55.1%	56.9%	Calculated: 1-CP-EE-CF-AR
Total	100.0%	100.0%	100.0%	

[00426] Control (CTR) and treated (TRT) diets were prepared for each phase as described in Example 15.6, where the treat diets were prepared by augmenting the control diet with one pound per treated short ton using the oligosaccharide preparation of Example 9.7. In total, about 50 short tons of each diet were manufactured.

[00427] Day-of-hatch Hubbard M99 × Cobb 500 straight run chicks were obtained from a commercial poultry hatchery and placed randomly into 36 ft × 40 ft pens constructed into a tunnel-ventilated, dirt-floor poultry house. Approximately 30,000 birds were placed in total, with an equal number of birds in each pen. The house bedding consisted of built-up litter top-dressed with fresh wood shavings. A standard commercial environmental and lighting program were employed. Animals and housing facilities were inspected daily, including recording the general health status,

feed consumption, water supply and temperature of the facility. Any mortalities were recorded daily.

[00428] Birds in odd numbered pens were fed the treated diet (i.e., containing the feed additive at 2 lbs/ton inclusion), and birds in the even numbered pens received the control diet. All diets were provided *ad libitum* via automatic feeders in each pen, and on feeder trays from day one until day 7. Water was provided *ad libitum* from a nipple drinking line.

[00429] The starter phase took place from day 0 to day 13, the grower phase from day 14 to day 27, and the withdrawal phase from day 28 through the end of the study, day 31. Bird weights by pen were recorded on days 0 and 31. The total mass of consumed feed was recorded for each pen. Weight gain and FCR were then determined for each pen according to standard industry practices.

[00430] On day 31, six male birds were randomly selected from each pen for blood and cecal sampling. The live weight of each sampled bird was recorded. A blood sample was collected via wing puncture into vacutainer tubes and frozen following coagulation and serum separation. Each sampled bird was then euthanized via cervical dislocation followed by extraction of the ceca using standard veterinary methods. Following dissection, cecal contents were transferred to 15 mL conical tubes, the weight of the cecal contents was recorded, and the contents were flash frozen to -80 degrees Celsius. From the weights of the sampled birds, the treatment group exhibited an 11 point increase in body weight, significant at $P < 0.05$ (by ANOVA).

Example 26: Formulation of a Syrup Product

[00431] The oligosaccharide preparation of Example 9.7 was pH adjusted to a pH of 4.2 with food grade sodium hydroxide according to the procedure of Example 21. The resulting syrup was packaged into a 20 liter carboy with a tamper-resistant cap. Immediately prior to sealing the container, a 500 gram sample was taken and subjected to quality testing. The total solids content of the syrup was confirmed to be greater than 70 wt%, per the methods of FCC APPENDIX X: Carbohydrates (Starches, Sugars, and Related Substances): TOTAL SOLIDS. The reducing sugar content was confirmed to be less than 50% as D-glucose on a dry weight basis according to the method of FCC APPENDIX X: Carbohydrates (Starches, Sugars, and Related Substances): REDUCING SUGARS ASSAY. Sulfated ash was confirmed to be less than 1% on a dry weight basis using the method of FCC APPENDIX II: Physical Tests and Determinations: C. OTHERS: RESIDUE ON IGNITION (Sulfated Ash) Method II (for Liquids). The sulfur dioxide content was confirmed below 40 mg/kg using an optimized Monier Williams method. The lead content was confirmed to be below 1 mg/kg using the method of AOAC International Official Method 2013.06. The total aerobic plate count was confirmed to be below 1000 cfu/g using the methods

of CMMEF Chapter 7. Total yeast and mold was confirmed below 100 cfu/g using the method of AACC International Approved Method 42-50. Coliforms were confirmed below 10 MPN/g using the method of the FDA BAM Chapter 4. E. coli was confirmed below 3 MPN/g using the method of FDA BAM Chapter 4. Salmonella was confirmed to be not detected per a 25 gram sample according to the method of FDA BAM Chapter 5. Staphylococcus aureus was confirmed to be below 10 cfu/g using the method of FDA BAM Chapter 12. Color was confirmed by visual inspection to be caramel.

[00432] The container was sealed, the remaining retention sample was frozen and stored for future reference, and a certificate of analysis was issued for the resulting lot.

Example 27: Preparation of Treated Drinking Water

Drinking water containing 250 ppm of the oligosaccharide preparation of Example 9.7 was prepared as follows. 37 mL of the oligosaccharide syrup of Example 33 and 40 grams of potassium sorbate were added gradually to 50 gallons of potable tap water in a 55 gallon blue-poly drum. The solution was mixed manually using a paddle for 10 minutes at room temperature.

[00433] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein can be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents.

CLAIMS

What is claimed is:

1. A method of correlating a synthetic oligosaccharide preparation in a nutritional composition,

wherein the nutritional composition comprises the synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition,

the method comprising:

a. providing a sample of the nutritional composition,

b. detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition, and

c. correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition,

wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

2. A method of performing quality control of a nutritional composition comprising:

a. providing a batch of a nutritional composition, wherein the nutritional composition comprises a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition,

b. obtaining a sample of the nutritional composition from the batch,

c. detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, and

d. accepting or rejecting the batch of the nutritional composition,

wherein the signal is (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

3. The method of claim 1 or 2, wherein the signal is indicative of one or more anhydro-subunit containing oligosaccharides.

4. The method of claim 3, wherein the one or more anhydro-subunit containing oligosaccharides have a degree of polymerization of 2 (DP2).
5. A method of performing quality control of a nutritional composition comprising:
    a. providing a sample of a nutritional composition, wherein the nutritional composition comprises a naturally occurring oligosaccharide composition, and
    b. detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, wherein the signal is indicative of one or more anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2).
6. The method of claim 5, wherein the nutritional composition comprises a synthetic oligosaccharide preparation.
7. The method of claim 2 or 6, comprising correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition.
8. The method of any one of claims 1 to 7, wherein the signal is detected by high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, size exclusion chromatography (SEC), field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), weight determination of fractions by preparative chromatography, or any combination thereof.
9. The method of any one of claims 1 to 8, wherein the nutritional composition comprises a base nutritional composition.
10. The method of claim 9, wherein the base nutritional composition comprises the naturally occurring oligosaccharide composition.
11. The method of claim 9 or 10, wherein the base nutritional composition lacks a detectable level of anhydro-subunit containing oligosaccharides.
12. The method of any one of claims 9 to 11, wherein the base nutritional composition is essentially free of anhydro-subunit containing oligosaccharides.
13. The method of any one of claims 1 to 4 or 6 to 12, wherein the one or more anhydro-subunit containing oligosaccharides originate from the synthetic oligosaccharide preparation.
14. The method of any one of claims 1 to 3 or 8 to 13, wherein the signal is indicative of anhydro-subunit containing oligosaccharides having a degree of polymerization of 1 (DP1).

15. The method of claim 14, wherein the signal is attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof.
16. The method of any one of claims 1 to 3 or 8 to 13, wherein the signal is indicative of anhydro-subunit containing oligosaccharides having a degree of polymerization of 3 (DP3).
17. The method of any one of claims 1 to 3 or 8 to 13, wherein the signal is indicative of DP1, DP2, or DP3 anhydro-subunit containing oligosaccharides, or a combination thereof.
18. The method of any one of claims 1 to 13, wherein the signal is attributed to anhydro-cellobiose.
19. The method of any one of claims 1 to 18, wherein the detecting comprises a weight determination of one or more degree of polymerization (DP) fractions of oligosaccharides.
20. The method of any one of claims 1 to 19, wherein the detecting comprises a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the sample.
21. The method of claim 20, wherein the at least a portion of anhydro-subunit containing oligosaccharides have a degree of polymerization of 1, 2, or 3.
22. The method of any one of claims 19 to 21, wherein the one or more DP fractions of oligosaccharides or the at least a portion of anhydro-subunit containing oligosaccharides are isolated by preparative chromatography.
23. The method of any one of claims 1 to 22, wherein the signal is detected, at least in part, by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS).
24. The method of any one of claims 1 to 22, wherein the signal is detected, at least in part, by liquid chromatography–mass spectrometry (LC-MS)/MS.
25. The method of any one of claims 1 to 22, wherein the signal is detected, at least in part, by GC-flame ionization detector (GC-FID) or GC-MS.
26. The method of any one of claims 1 to 22, wherein the signal is detected, at least in part, by NMR spectroscopy.
27. The method of any one of claims 1 to 2 or 8 to 12, wherein the signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages of oligosaccharides.
28. The method of claim 27, wherein the detecting comprises obtaining an NMR spectrum.

29. The method of claim 27 or 28, wherein the signal is associated with α-(1,2) glycosidic linkages.
30. The method of claim 27 or 28, wherein the signal is associated with α-(1,3) glycosidic linkages.
31. The method of claim 27 or 28, wherein the signal is associated with α-(1,6) glycosidic linkages.
32. The method of claim 27 or 28, wherein the signal is associated with β-(1,2) glycosidic linkages.
33. The method of claim 27 or 28, wherein the signal is associated with β-(1,3) glycosidic linkages.
34. The method of claim 27 or 28, wherein the signal is associated with β-(1,4) glycosidic linkages.
35. The method of claim 27 or 28, wherein the signal is associated with β-(1,6) glycosidic linkages.
36. The method of any one of claims 1 to 35, wherein the detecting comprises determining the presence or absence of the signal.
37. The method of claim 36, wherein the detecting comprises determining the presence or absence of DP1 or DP2 anhydro-subunit containing oligosaccharides, or both.
38. The method of any one of claims 1 to 37, wherein the detecting comprises determining or correlating a level of the signal.
39. The method of claim 38, wherein the detecting comprises correlating a level of DP1 anhydro-subunit containing oligosaccharides in the nutritional composition.
40. The method of claim 38 or 39, wherein the detecting comprises correlating a level of DP2 anhydro-subunit containing oligosaccharides in the nutritional composition.
41. A method of performing quality control of a nutritional composition comprising a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition,

the method comprising:

a. providing a first sample of the nutritional composition,

b. providing a second sample of the nutritional composition,

c. detecting a first signal of at least a portion of oligosaccharides in the first sample,

d. detecting a second signal of at least a portion of oligosaccharides in the second sample, and

e. comparing the first signal and the second signal,

wherein the first signal and the second signal are independently (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

42. The method of claim 41, comprising correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition of the first sample, a concentration of the synthetic oligosaccharide preparation in the nutritional composition of the second sample, or both.
43. The method of claim 41 or 42, wherein the first signal and the second signal are each independently indicative of one or more anhydro-subunit containing oligosaccharides.
44. The method of claim 43, wherein the first signal and the second signal are attributed to the same species of an anhydro-subunit containing oligosaccharide.
45. The method of claim 43, wherein the first signal and the second signal are attributed to different species of anhydro-subunit containing oligosaccharides
46. The method of any one of claims 41 to 45, wherein the first sample and the second sample are taken from different batches of the nutritional composition.
47. The method of any one of claims 41 to 46, wherein the first signal and the second signal are each independently detected by high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, size exclusion chromatography (SEC), field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), weight determination of fractions by preparative chromatography, or any combination thereof.
48. The method of any one of claims 41 to 47, wherein the nutritional composition comprises a base nutritional composition.
49. The method of claim 48, wherein the base nutritional composition comprises the naturally occurring oligosaccharide composition.
50. The method of claim 48 or 49, wherein the base nutritional composition lacks a detectable level of anhydro-subunit containing oligosaccharides.
51. The method of any one of claims 48 to 50, wherein the base nutritional composition is essentially free of anhydro-subunit containing oligosaccharides.

52. The method of any one of claims 41 to 51, wherein the one or more anhydro-subunit containing oligosaccharides originate from the synthetic oligosaccharide preparation.
53. The method of any one of claims 41 to 52, wherein the first signal, the second signal, or both are independently attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 1 (DP1).
54. The method of claim 53, wherein the first signal, the second signal, or both are independently attributed to levoglucosan, 1,6-anhydro-β-D-glucofuranose, or a combination thereof.
55. The method of any one of claims 41 to 52, wherein the first signal, the second signal, or both are independently attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2).
56. The method of claim 55, wherein the signal is attributed to anhydro-cellobiose.
57. The method of any one of claims 41 to 52, wherein the first signal, the second signal, or both are independently attributed to anhydro-subunit containing oligosaccharides having a degree of polymerization of 3 (DP3).
58. The method of any one of claims 41 to 52, wherein the first signal, the second signal, or both are independently attributed to DP1, DP2, or DP3 anhydro-subunit containing oligosaccharides, or a combination thereof.
59. The method of any one of claims 41 to 58, wherein the detecting comprises a weight determination of one or more degree of polymerization (DP) fractions from the sample or the second sample.
60. The method of any one of claims 41 to 59, wherein the detecting comprises a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the first sample or the second sample.
61. The method of claim 60, wherein the at least a portion of anhydro-subunit containing oligosaccharides have a degree of polymerization of 1, 2, or 3.
62. The method of any one of claims 59 to 61, wherein the one or more DP fractions of oligosaccharides or the at least a portion of anhydro-subunit containing oligosaccharides are isolated by preparative chromatography.
63. The method of any one of claims 41 to 62, wherein the first signal, the second signal, or both are independently detected, at least in part, by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS).

64. The method of any one of claims 41 to 62, wherein the first signal, the second signal, or both are independently detected, at least in part, by liquid chromatography–mass spectrometry (LC-MS)/MS.
65. The method of any one of claims 41 to 62, wherein the first signal, the second signal, or both are independently detected, at least in part, by GC-flame ionization detector (GC-FID) or GC-MS.
66. The method of any one of claims 41 to 62, wherein the first signal, the second signal, or both are independently detected, at least in part, by NMR spectroscopy.
67. The method of any one of claims 41 or 47 to 66, wherein the first signal, the second signal, or both are independently associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, or β-(1,6) glycosidic linkages of oligosaccharides.
68. The method of claim 67, wherein the detecting comprises obtaining an NMR spectroscopy.
69. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with α-(1,2) glycosidic linkages.
70. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with α-(1,3) glycosidic linkages.
71. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with α-(1,6) glycosidic linkages.
72. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with β-(1,2) glycosidic linkages.
73. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with β-(1,3) glycosidic linkages.
74. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with β-(1,4) glycosidic linkages.
75. The method of claim 67 or 68, wherein the first signal, the second signal, or both are associated with β-(1,6) glycosidic linkages.
76. The method of any one of claims 41 or 47 to 66, wherein the first signal is indicative of one or more anhydro-subunit containing oligosaccharides and the second signal is associated with α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β

glycosidic linkages, β-(1,1)-α glycosidic linkages, or β-(1,1)-β glycosidic linkages of oligosaccharides.

77. The method of any one of claims 41 to 76, wherein the detecting comprises determining the presence or absence of the first signal and the second signal.
78. The method of claim 77, wherein the detecting comprises determining the presence or absence of DP1 or DP2 anhydro-subunit containing oligosaccharides, or both.
79. The method of any one of claims 41 to 76, wherein the detecting comprises determining or correlating a level of the first signal and the second signal.
80. The method of claim 79, wherein the detecting comprises correlating a level of DP1 anhydro-subunit containing oligosaccharides in the nutritional composition.
81. The method of claim 79 or 80, wherein the detecting comprises correlating a level of DP2 anhydro-subunit containing oligosaccharides in the nutritional composition.
82. The method of any one of claims 1 or 3 to 81, further comprising accepting or rejecting a batch of the nutritional composition.
83. The method of any one of claims 1 to 4 or 6 to 82, further comprising adjusting a level of the synthetic oligosaccharide preparation in the nutritional composition after the detecting.
84. The method of any one of claims 9 to 40 or 48 to 83, wherein the base nutritional composition comprises a plurality of oligosaccharides.
85. The method of claim 84, wherein the base nutritional composition comprises starch or plant fibers.
86. The method of any one of the claims 9 to 40 or 48 to 85, wherein a level of α-(1,2) glycosidic linkage, α-(1,3) glycosidic linkage, β-(1,2) glycosidic linkage, β-(1,3) glycosidic linkage, or β-(1,4) glycosidic linkage in the base nutritional composition is at least 10% lower than a level of the same glycosidic linkage in the synthetic oligosaccharide preparation.
87. The method of any one of claims 1 to 86, comprising a derivatization step prior to the detecting.
88. The method of any one of claims 1 to 87, further comprising extracting oligosaccharides from the sample of the nutritional composition.
89. The method of claim 88, further comprising filtering or clarifying the extracted oligosaccharides.
90. The method of claim 88 or 89, further comprising concentrating the extracted oligosaccharides.

91. The method of claim 90, wherein the concentrating comprises nano-filtration.
92. The method of claim 90, wherein the concentrating comprises freeze-drying.
93. The method of any one of claims 88 to 92, further comprising introducing an internal standard into the extracted or concentrated oligosaccharides.
94. The method of any one of claims 88 to 93, further comprising reducing the extracted or concentrated oligosaccharides.
95. The method of any one of the claims 88 to 93, further comprising digesting the extracted or concentrated oligosaccharides with one or more hydrolytic enzymes.
96. The method of claim 95, wherein the one or more hydrolytic enzymes comprise carbohydratase, protease, lipase, or any combination thereof.
97. The method of claim 95 or 96, wherein the one or more hydrolytic enzymes comprise α-amylase, amyloglycosidase, invertase, α-galactosidase, or any combination thereof.
98. The method of any one of claims 95 to 97, wherein the one or more hydrolytic enzymes cleave one or more naturally occurring glycosidic bonds.
99. The method of any one of claims 95 to 98, further comprising isolating the oligosaccharides that are not digested.
100. The method of any one of claims 88 to 98, further comprising separating the extracted, concentrated, digested, or reduced oligosaccharides.
101. The method of claim 100, wherein the oligosaccharides are separated chromatographically.
102. The method of claim 100, wherein the oligosaccharides are separated by nano-filtration.
103. The method of claim 101 or 102, further comprising isolating the separated oligosaccharides.
104. The method of any one of claims 100 to 103, wherein the oligosaccharides are separated or isolated by their degrees of polymerization.
105. The method of claim 104, the method comprises isolating or separating the oligosaccharides with a degree of polymerization of 1, 2, 3, 4, or 5.
106. The method of claim 105, wherein the DP1 oligosaccharides are isolated or separated.
107. The method of claim 105, wherein the DP2 oligosaccharides are isolated or separated.
108. The method of claim 105, wherein the DP3 oligosaccharides are isolated or separated.

109. The method of any one of claims 1 to 105, comprising isolating at least a portion of the DP1 anhydro-subunit containing oligosaccharides from the sample.

110. The method of any one of claims 1 to 105, comprising isolating at least a portion of the DP2 anhydro-subunit containing oligosaccharides from the sample.

111. The method of any one of claims 1 to 105, comprising isolating at least a portion of the DP3 anhydro-subunit containing oligosaccharides from the sample.

112. The method of any one of the claims 99 to 111, wherein the isolated or separated oligosaccharides are quantified.

113. The method of claim 112, wherein a majority of the quantified oligosaccharides originate from the synthetic oligosaccharide preparation.

114. The method of claim 112 or 113 , wherein more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, more than 95%, or more than 99% by weight of the quantified oligosaccharide originate from the synthetic oligosaccharide preparation.

115. A method of correlating a synthetic oligosaccharide preparation in a nutritional composition,

wherein the nutritional composition comprises (i) a synthetic oligosaccharide preparation that comprises anhydro-subunit containing oligosaccharides and (ii) a naturally occurring oligosaccharide composition,

the method comprising:

a. providing a sample of the nutritional composition,

b. isolating one or more anhydro-subunit containing oligosaccharides from the sample,

c. detecting a signal that is indicative of the one or more anhydro-subunit containing oligosaccharides, wherein the detecting comprises (i) a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the sample or (ii) analyzing at least a portion of anhydro-subunit containing oligosaccharides from the sample by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), liquid chromatography–mass spectrometry (LC-MS)/MS, or gas chromatography(GC)-MS, and

d. correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition.

116. The method of claim 115, wherein the detecting comprises a weight determination of at least a portion of the anhydro-subunit containing oligosaccharides having a degree of polymerization of 1 (DP1) from the sample.

117. The method of claim 115, wherein the detecting comprises a weight determination of at least a portion of the anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2) from the sample.

118. The method of claim 115, wherein the detecting comprises analyzing at least a portion of DP1 or DP2 anhydro-subunit containing oligosaccharides from the sample by MALDI-MS.

119. The method of claim 115, wherein the detecting comprises analyzing at least a portion of DP1 or DP2 anhydro-subunit containing oligosaccharides from the sample by LC-MS/MS.

120. The method of claim 115, wherein the detecting comprises analyzing at least a portion of DP1 or DP2 anhydro-subunit containing oligosaccharides from the sample by GC/MS.

121. The method of claim 115, wherein the detecting comprises analyzing at least a portion of DP3 anhydro-subunit containing oligosaccharides from the sample by weight determination, MALDI-MS, LC-MS/MS, or GC-MS.

122. The method of any one of claims 115 to 121, wherein the isolating comprises separating the one or more anhydro-subunit containing oligosaccharides by preparative chromatography.

123. The method of any one of claims 1 to 4 or 6 to 122, wherein the synthetic oligosaccharide preparation is present in the nutritional composition at a concentration of from about 1 to about 5000 ppm, from about 1 to about 1000 ppm, from about 1 to about 500 ppm, from about 10 to about 5000 ppm, from about 10 to about 2000 ppm, from about 10 to about 1000 ppm, from about 10 to about 500 ppm, from about 10 to about 250 ppm, from about 10 to about 100 ppm, from about 50 to about 5000 ppm, from about 50 to about 2000 ppm, from about 50 to about 1000 ppm, from about 50 to about 500 ppm, from about 50 to about 250 ppm, or from about 50 to about 100 ppm.

124. The method of any one of the claims 1 to 4 or 6 to 122, wherein the synthetic oligosaccharide preparation is present in the nutritional composition at a concentration of greater than 10 ppm, greater than 50 ppm, greater than 100 ppm, greater than 200 ppm, greater than 300 ppm, greater than 400 ppm, greater than 500 ppm, greater than 600 ppm, greater than 1000 ppm, or greater than 2000 ppm.

125. The method of any one of the claims 1 to 124, wherein the nutritional composition is an animal feed composition.

126. A method of manufacturing a nutritional composition comprising:

a. combining a base nutritional composition with a synthetic oligosaccharide preparation comprising anhydro-subunit containing oligosaccharides, and
b. performing a quality control method as provided in any one of claims 1 to 125.

127. The method of claim 126, wherein the synthetic oligosaccharide preparation comprises at least n fractions of oligosaccharides each having a distinct degree of polymerization selected from 1 to n (DP1 to DPn fractions), wherein n is an integer greater than or equal to 3; and wherein the DP1 and DP2 fractions each independently comprises from about 0.1% to about 15% of anhydro-subunit containing oligosaccharides by relative abundance as measured by mass spectrometry (MS).

128. The method of claim 127, wherein the relative abundance is determined by liquid chromatography–mass spectrometry (LC-MS)/MS.

129. The method of claim 127 or 128, wherein the relative abundance of oligosaccharides in each of the n fractions decreases monotonically with its degree of polymerization.

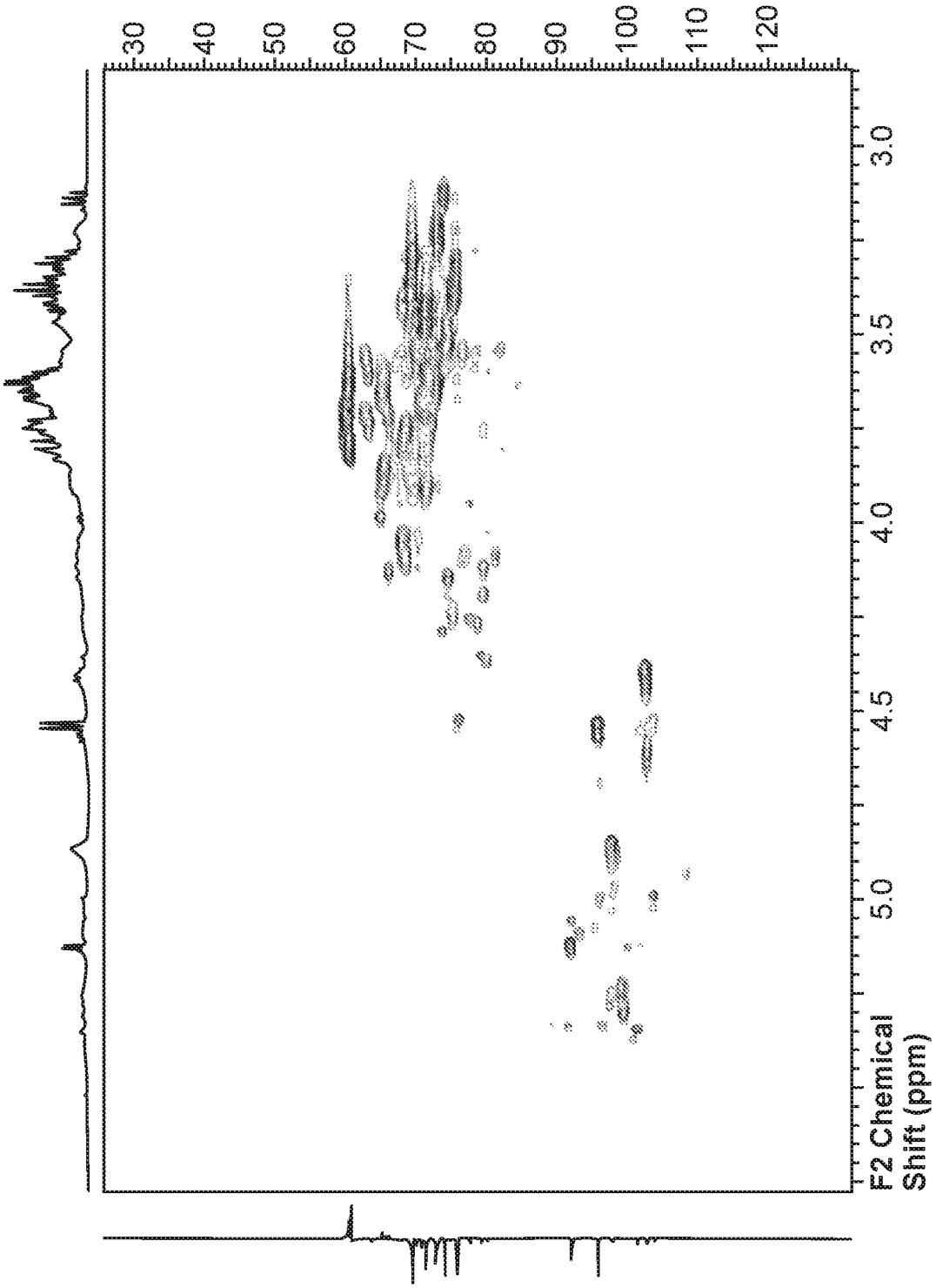

30
40
50
60
70
80
90
100
110
120
3.0
3.5
4.0
4.5
5.0
F2 Chemical Shift (ppm)

FIG. 1

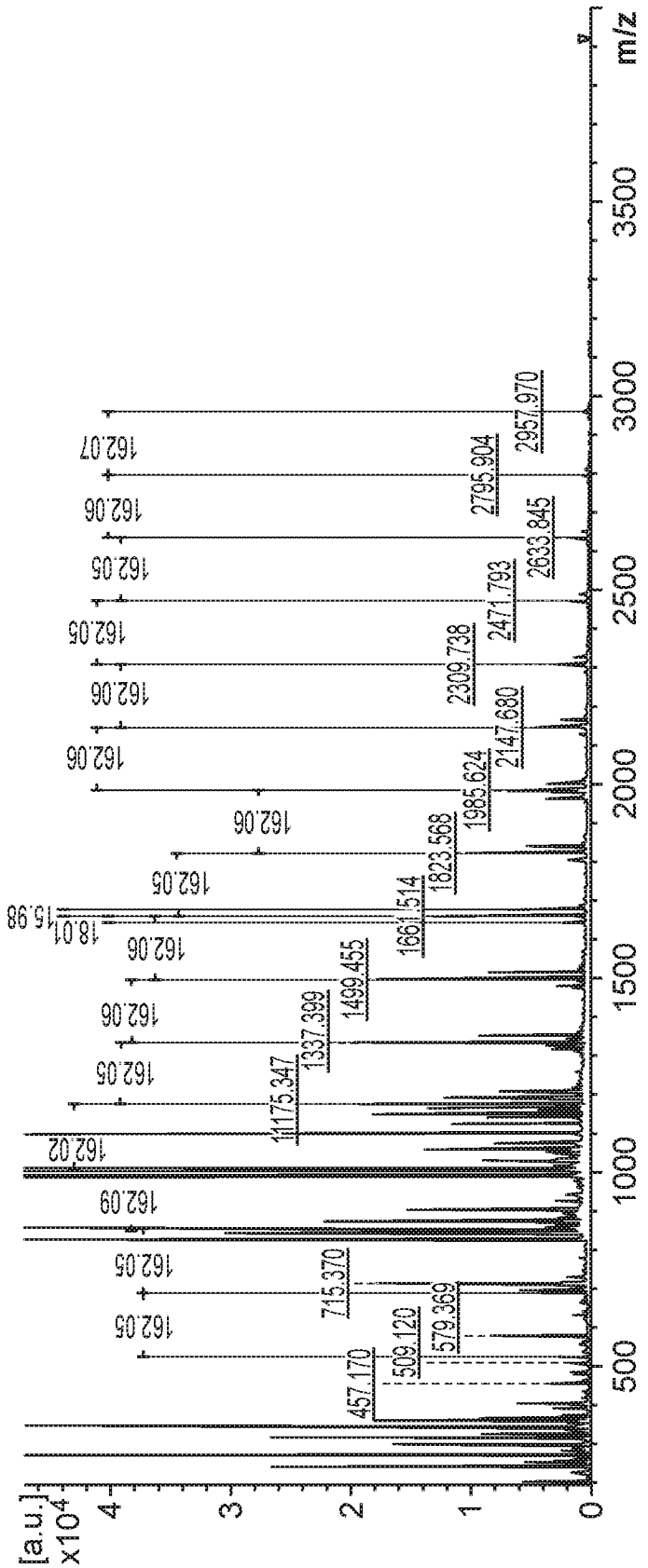

[a.u.]
x10^4
0
1
2
3
4
500
1000
1500
2000
2500
3000
3500
m/z
457.170
509.120
579.369
715.370
1175.347
1337.399
1499.455
1661.514
1823.568
1985.624
2147.680
2309.738
2471.793
2633.845
2795.904
2957.970
162.05
162.09
162.02
162.06
162.07
18.01
15.98

FIG. 2

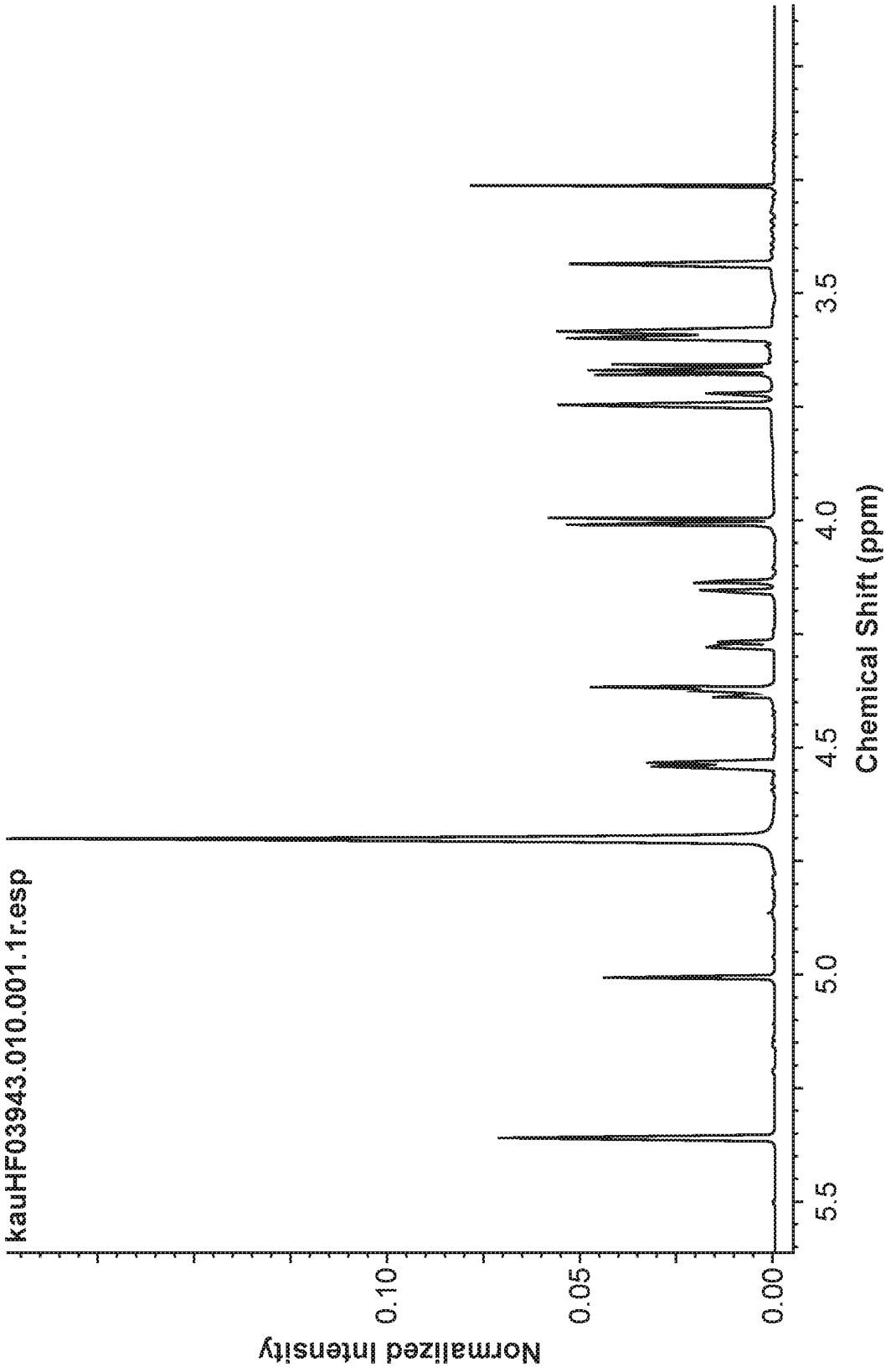

kauHF03943.010.001.1r.esp
Normalized Intensity
0.00
0.05
0.10
5.5
5.0
4.5
4.0
3.5
Chemical Shift (ppm)

FIG. 3

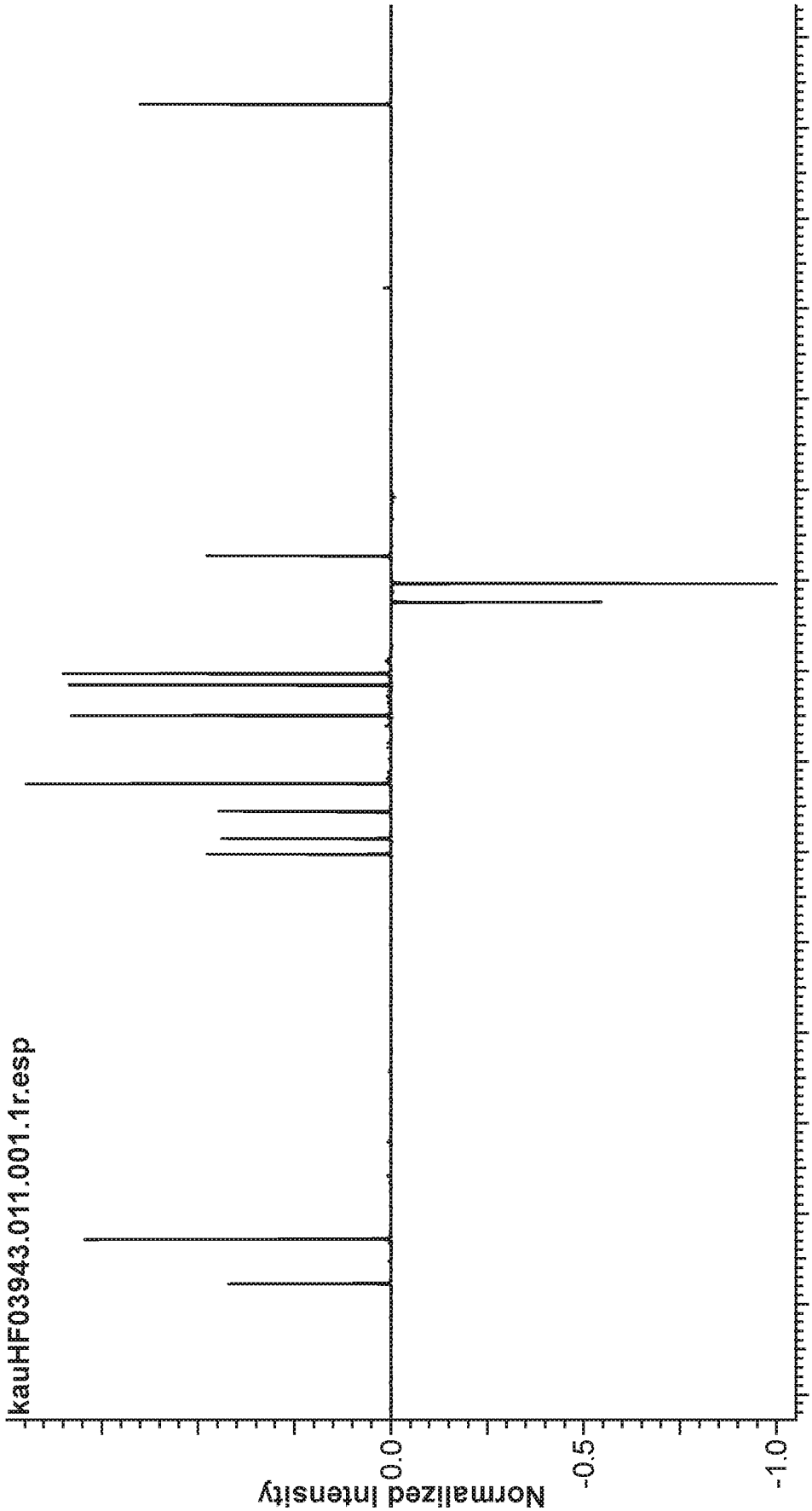
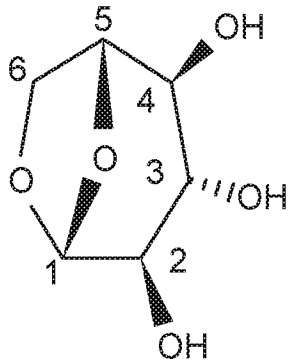
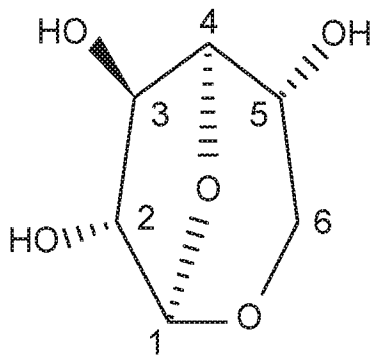

kauHF03943.011.001.1r.esp
Normalized Intensity
0.0
-0.5
-1.0
110
105
100
95
90
85
80
75
70
65
60
55
50
Chemical Shift (ppm)

FIG. 4



1,6-Anhydro-beta-
D-glucofuranose



1,6-Anhydro-beta-
D-glucopyranose

FIG. 5

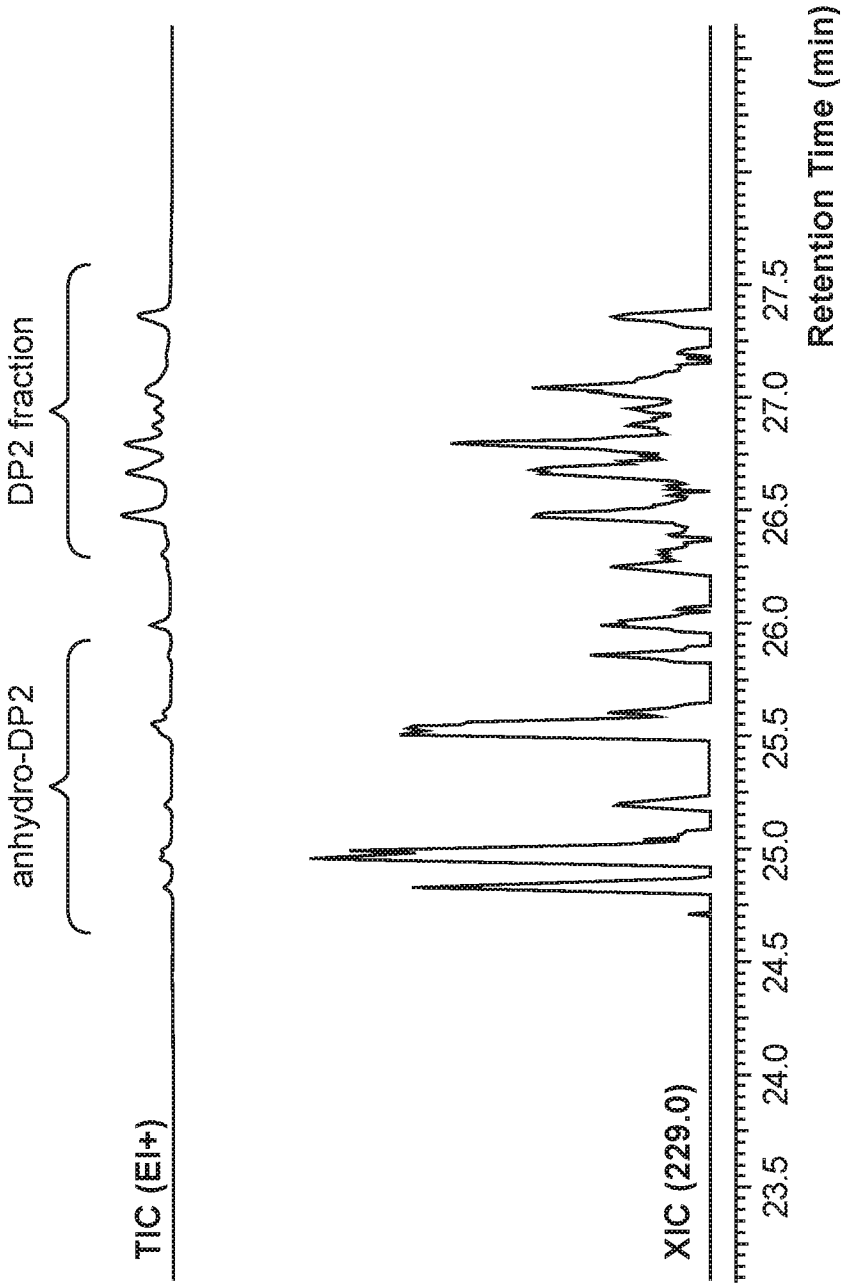

anhydro-DP2
DP2 fraction
TIC (EI+)
XIC (229.0)
23.5
24.0
24.5
25.0
25.5
26.0
26.5
27.0
27.5
Retention Time (min)

FIG. 6

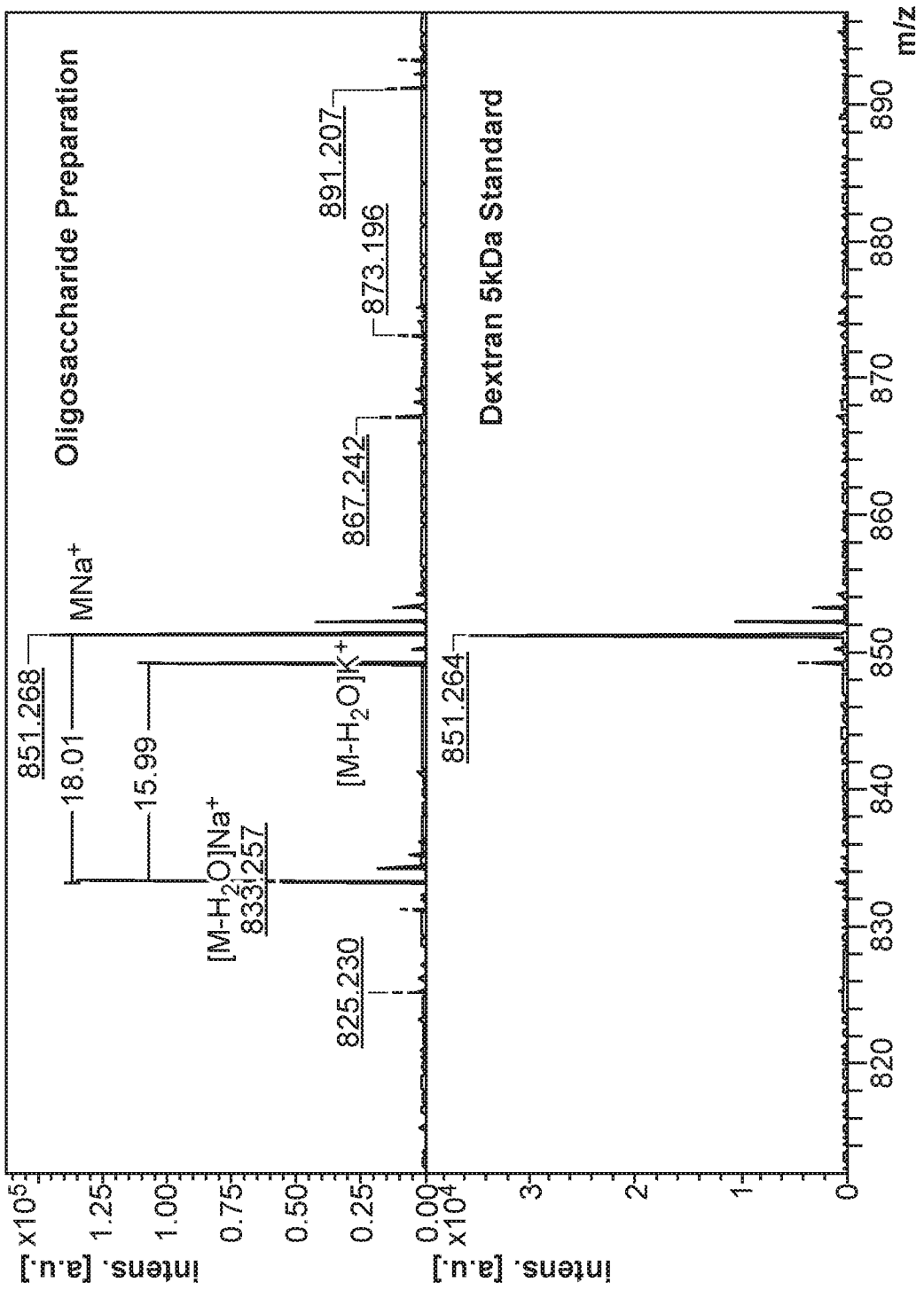

Oligosaccharide Preparation
Dextran 5kDa Standard
MNa+
[M-H2O]Na+
[M-H2O]K+
851.268
18.01
15.99
833.257
825.230
867.242
873.196
891.207
851.264
intens. [a.u.]
x10^5
x10^4
m/z

FIG. 7

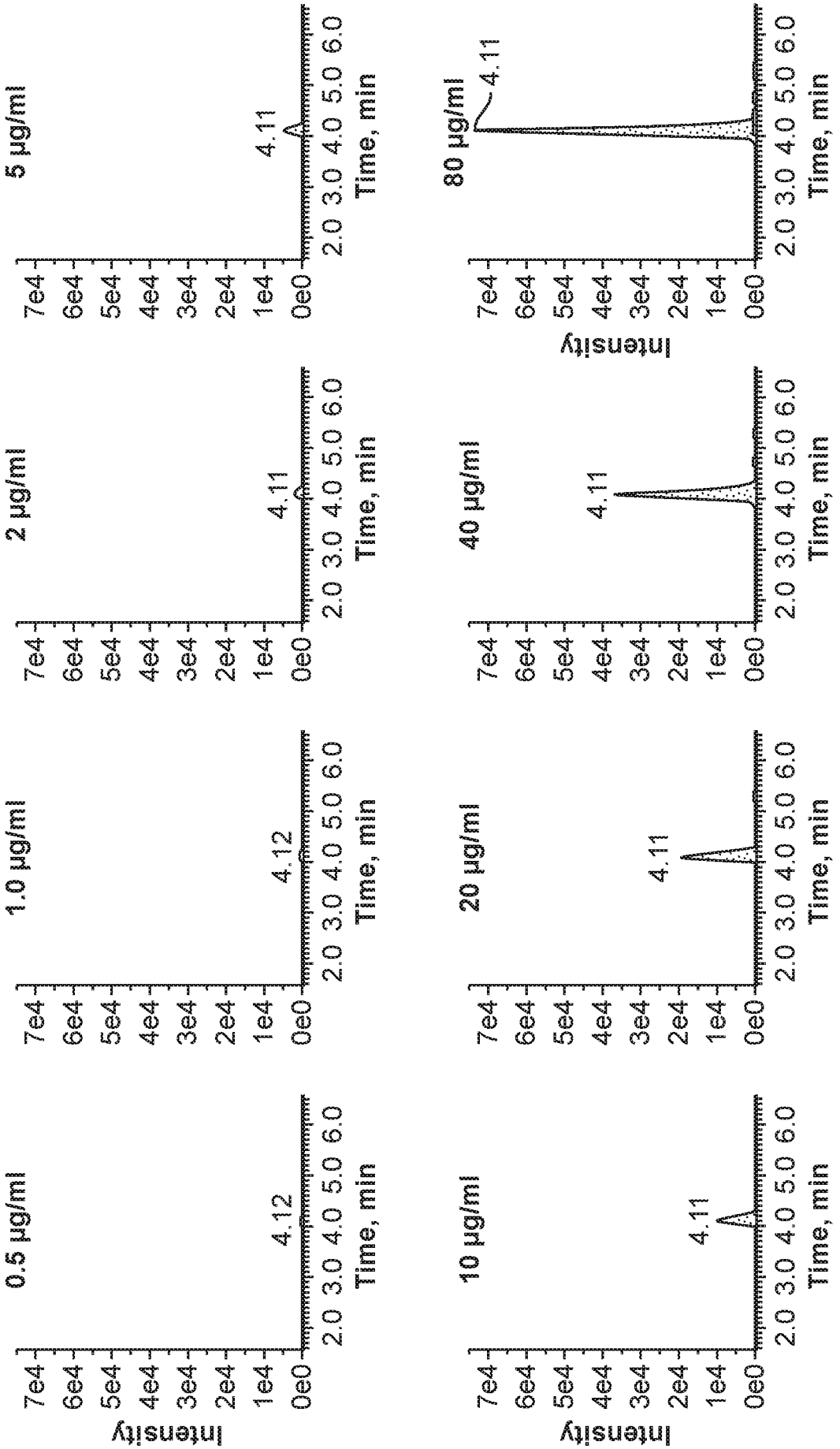

0.5 µg/ml
1.0 µg/ml
2 µg/ml
5 µg/ml
10 µg/ml
20 µg/ml
40 µg/ml
80 µg/ml
4.11
4.12
Intensity
Time, min
7e4
6e4
5e4
4e4
3e4
2e4
1e4
0e0
2.0 3.0 4.0 5.0 6.0

FIG. 8

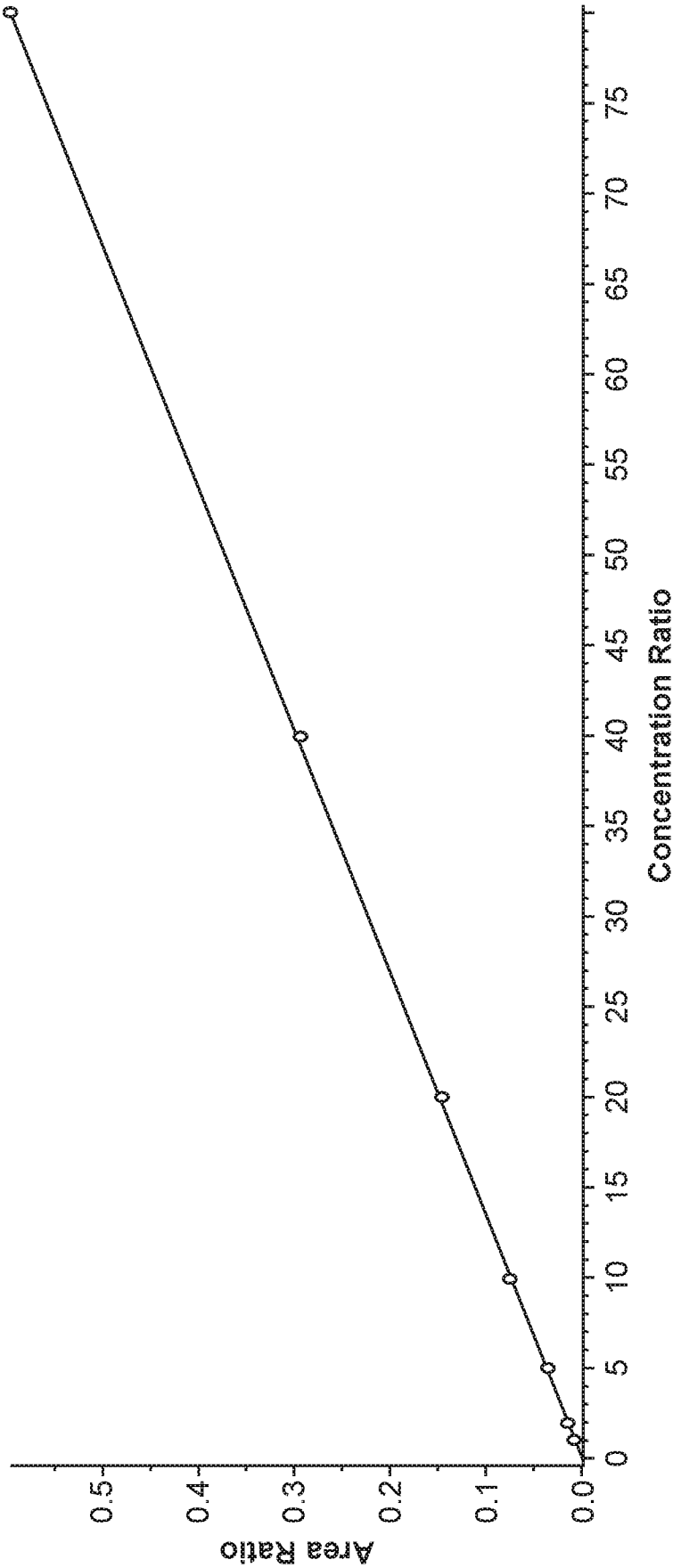

Area Ratio
0.0
0.1
0.2
0.3
0.4
0.5
Concentration Ratio
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75

FIG. 9

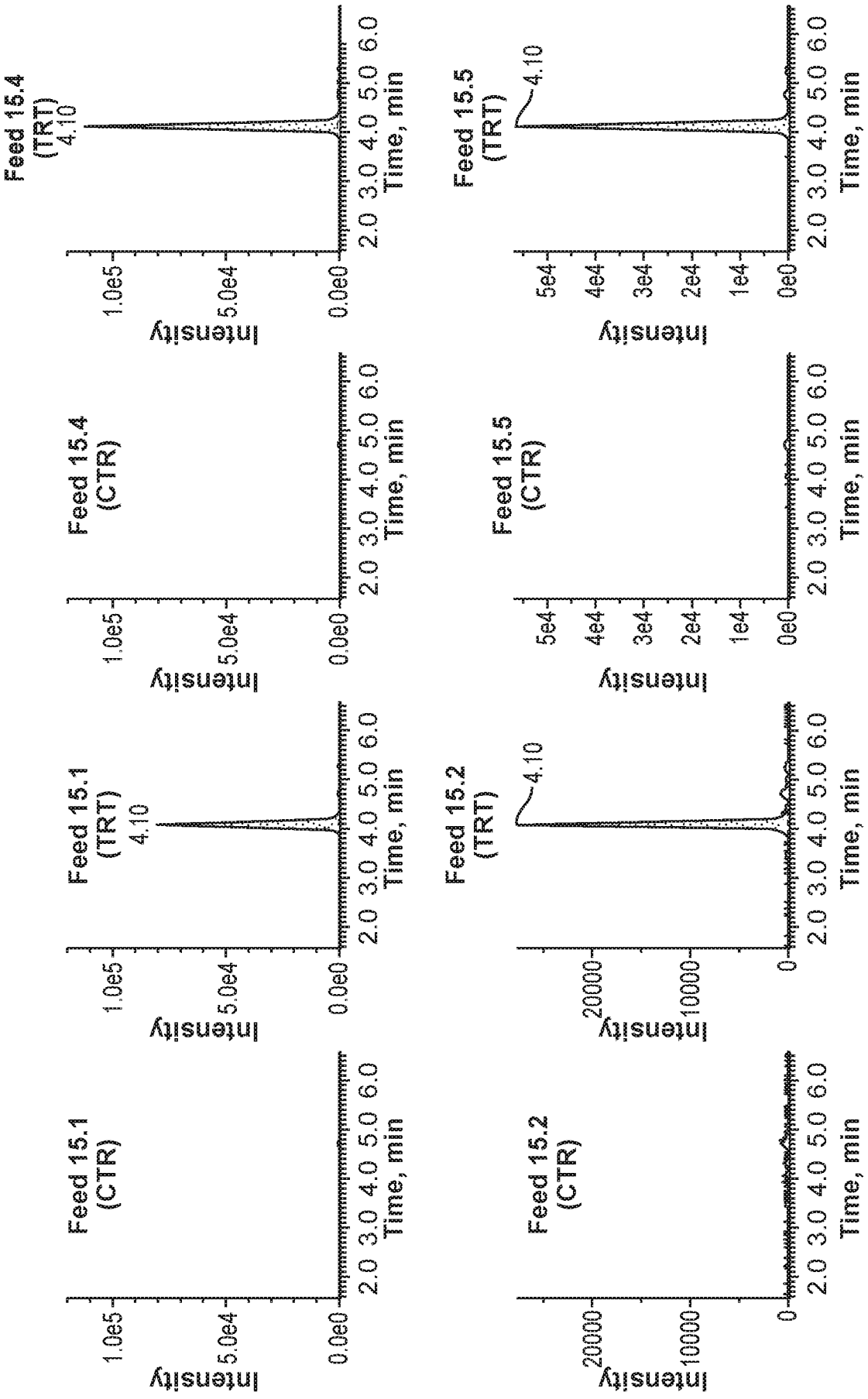

Feed 15.4 (TRT)
4.10
Feed 15.4 (CTR)
Feed 15.5 (TRT)
4.10
Feed 15.5 (CTR)
Feed 15.1 (TRT)
4.10
Feed 15.1 (CTR)
Feed 15.2 (TRT)
4.10
Feed 15.2 (CTR)
Intensity
Time, min
1.0e5
5.0e4
0.0e0
5e4
4e4
3e4
2e4
1e4
0e0
20000
10000
0
2.0 3.0 4.0 5.0 6.0

FIG. 10

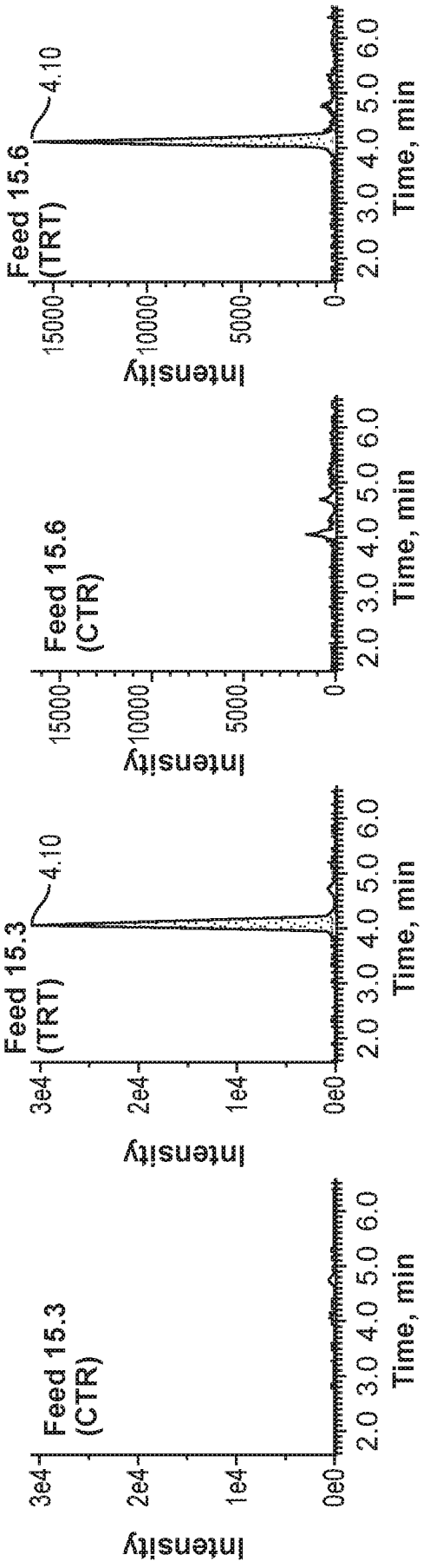

Feed 15.6
(TRT)
4.10
Intensity
15000
10000
5000
0
2.0 3.0 4.0 5.0 6.0
Time, min
Feed 15.6
(CTR)
Intensity
15000
10000
5000
0
2.0 3.0 4.0 5.0 6.0
Time, min
Feed 15.3
(TRT)
4.10
Intensity
3e4
2e4
1e4
0e0
2.0 3.0 4.0 5.0 6.0
Time, min
Feed 15.3
(CTR)
Intensity
3e4
2e4
1e4
0e0
2.0 3.0 4.0 5.0 6.0
Time, min

FIG. 10 (Cont.)

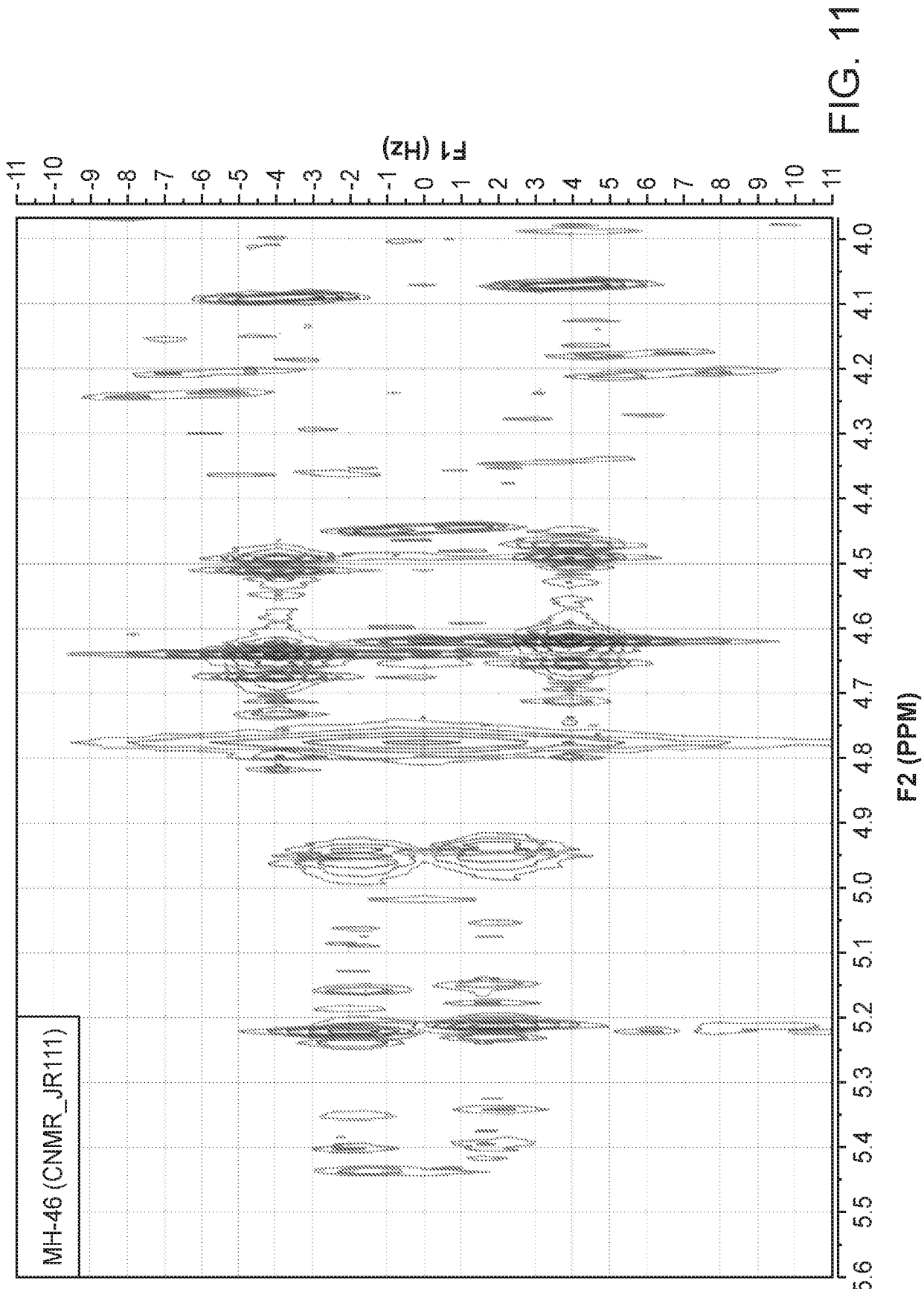

F1 (Hz)
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
F2 (PPM)
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
5.4
5.5
5.6
MH-46 (CNMR_JR111)

FIG. 11

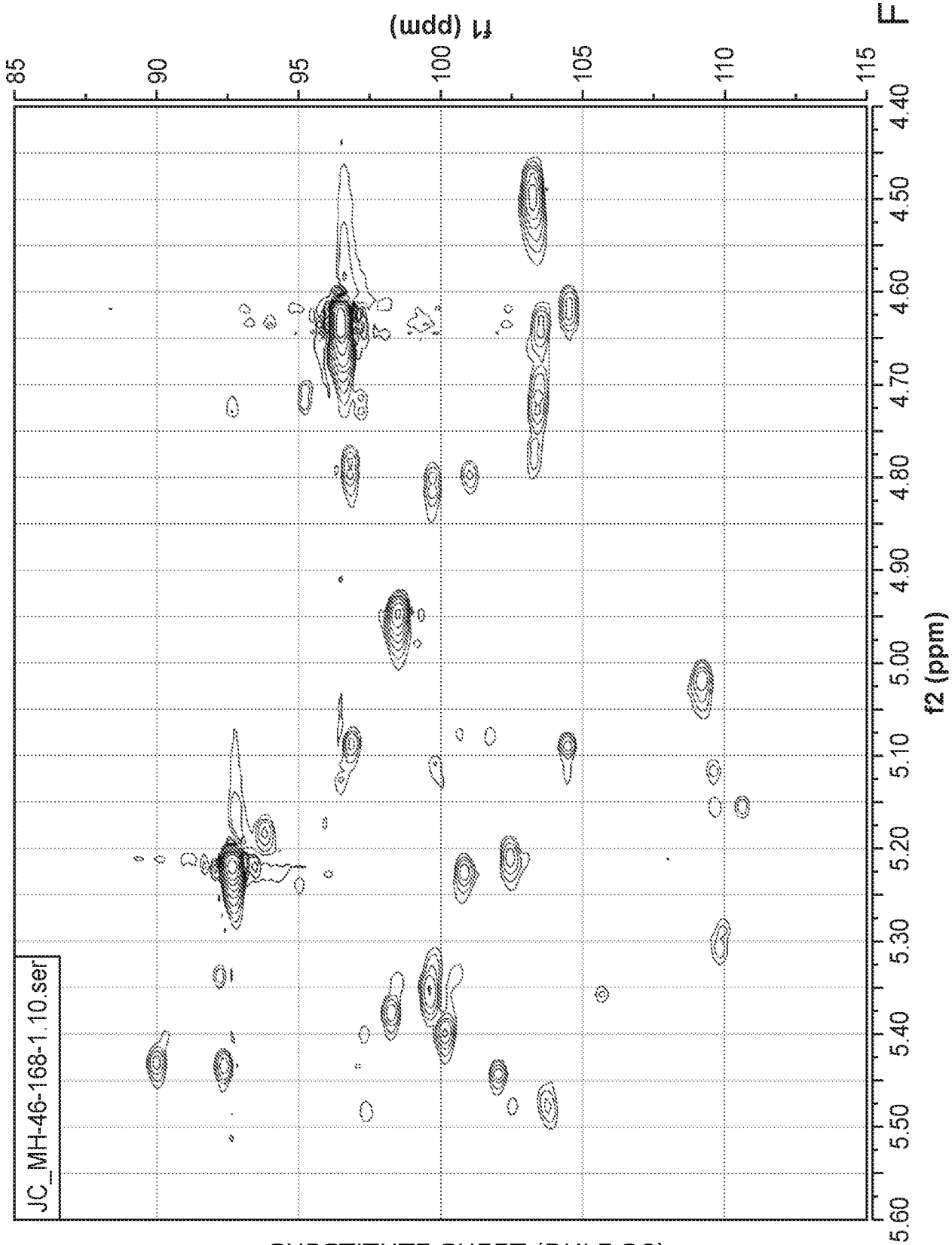

f1 (ppm)
85
90
95
100
105
110
115
f2 (ppm)
4.40
4.50
4.60
4.70
4.80
4.90
5.00
5.10
5.20
5.30
5.40
5.50
5.60
JC_MH-46-168-1.10.ser

FIG. 12

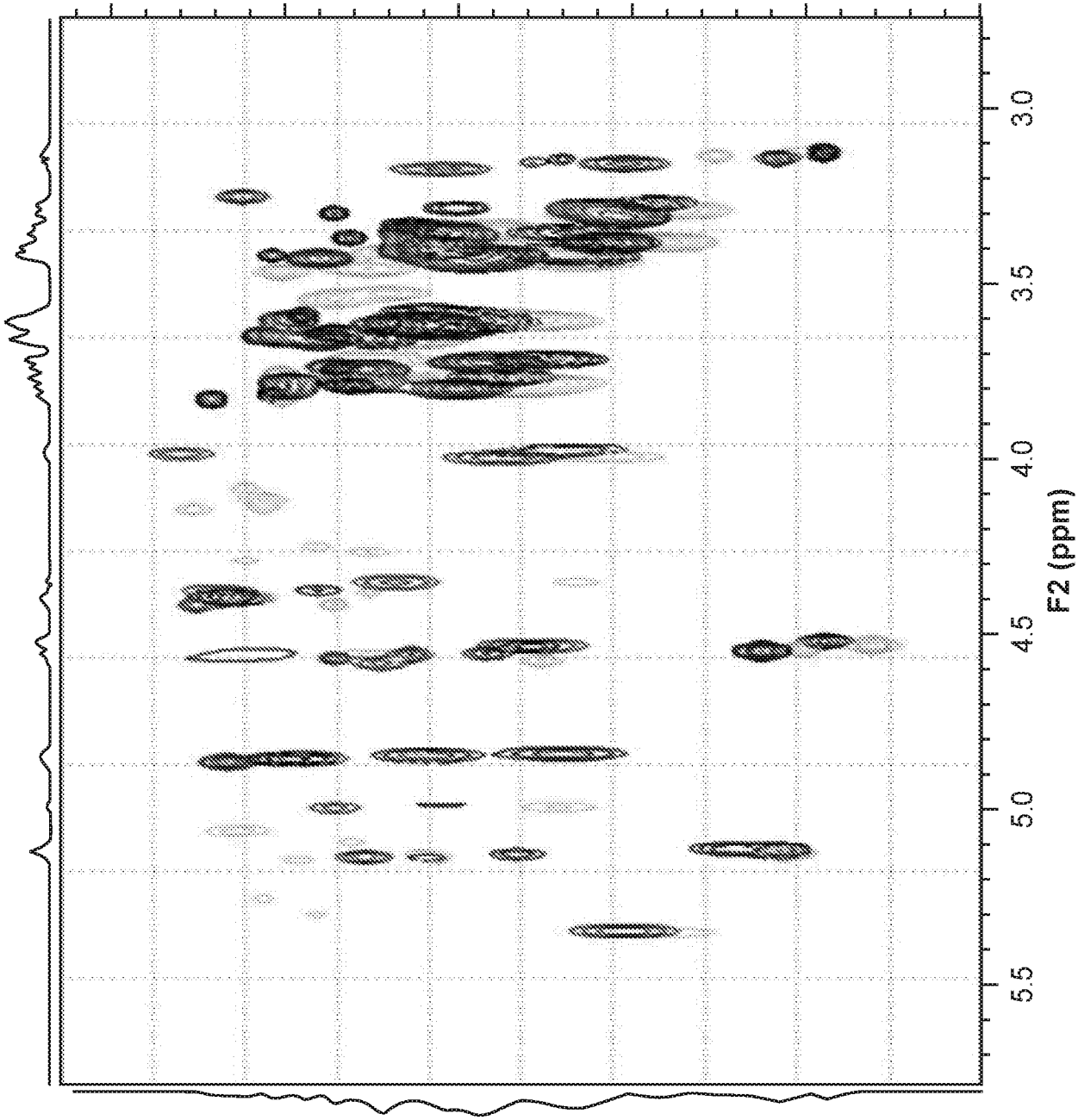


FIG. 13

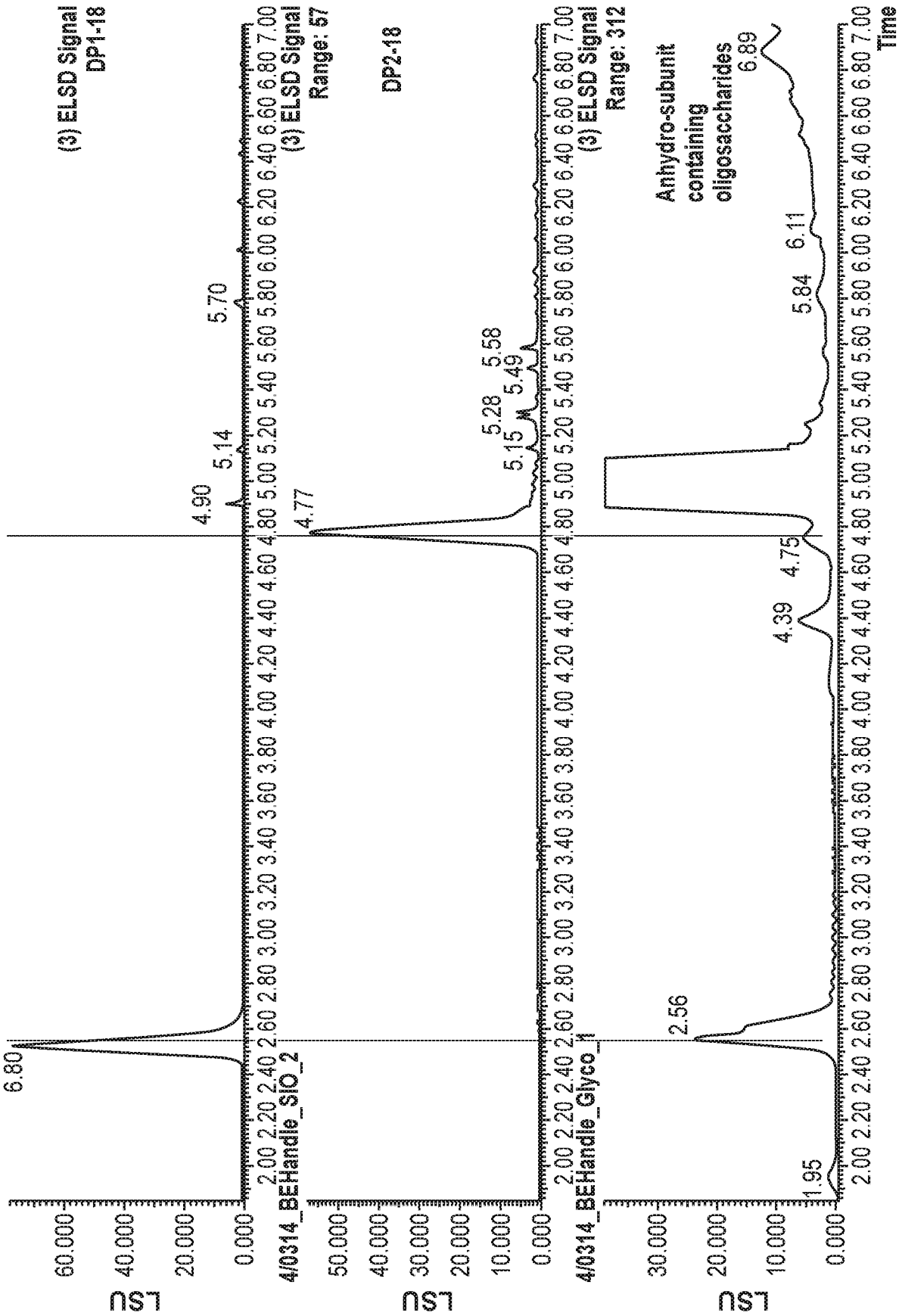

(3) ELSD Signal
DP1-18
Range: 57
DP2-18
(3) ELSD Signal
Range: 312
Anhydro-subunit containing oligosaccharides
(3) ELSD Signal
Time
4/0314_BEHandle_SIO_2
4/0314_BEHandle_Glyco_1
LSU
6.80
5.70
5.14
4.90
4.77
5.58
5.49
5.28
5.15
6.89
6.11
5.84
4.75
4.39
2.56
1.95

FIG. 14

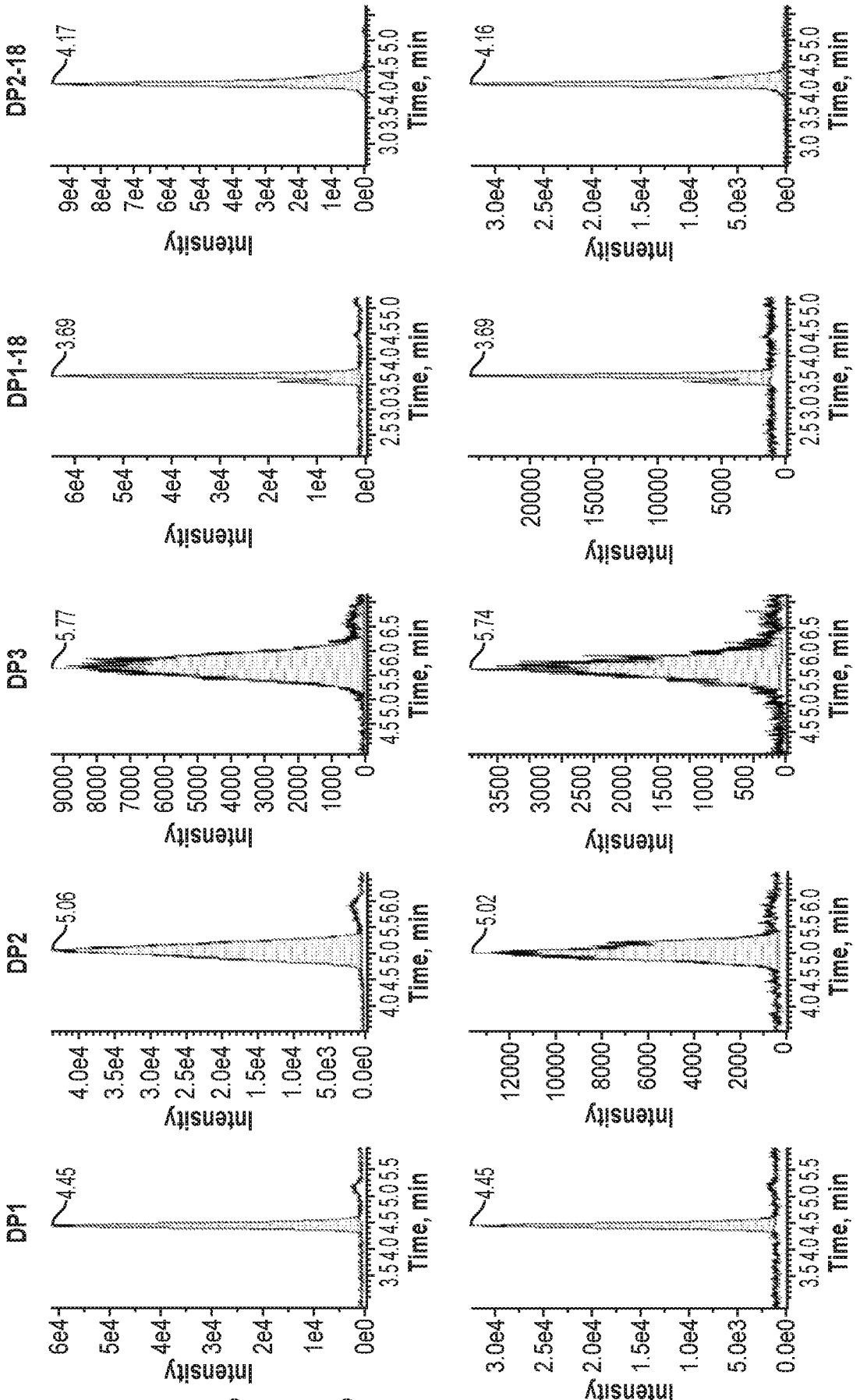

DP1
DP2
DP3
DP1-18
DP2-18
Intensity
Time, min
Anhydro-subunit containing gluco-oligosaccharides
Digested Anhydro-subunit containing gluco-oligosaccharides

FIG. 15

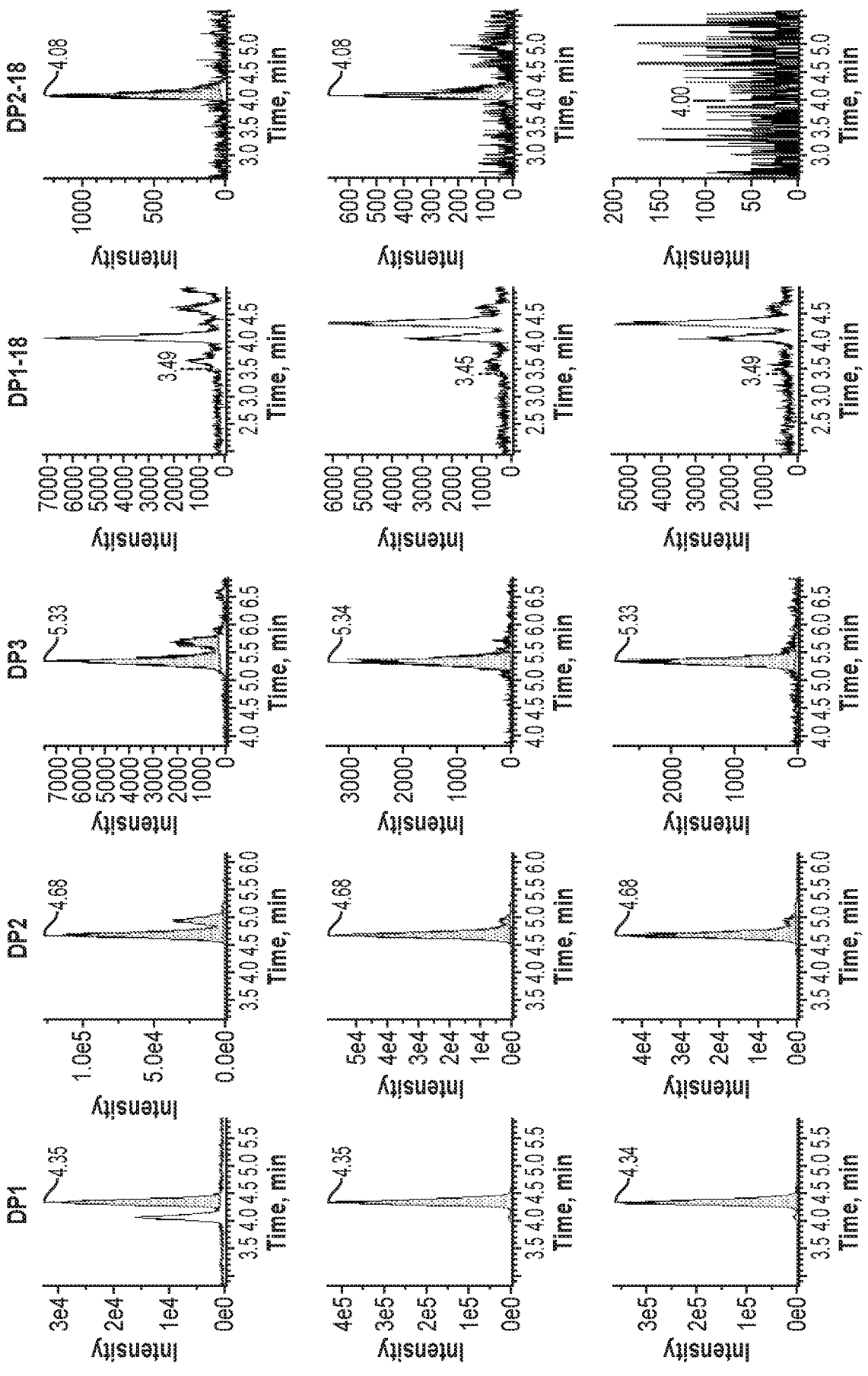


FIG. 16

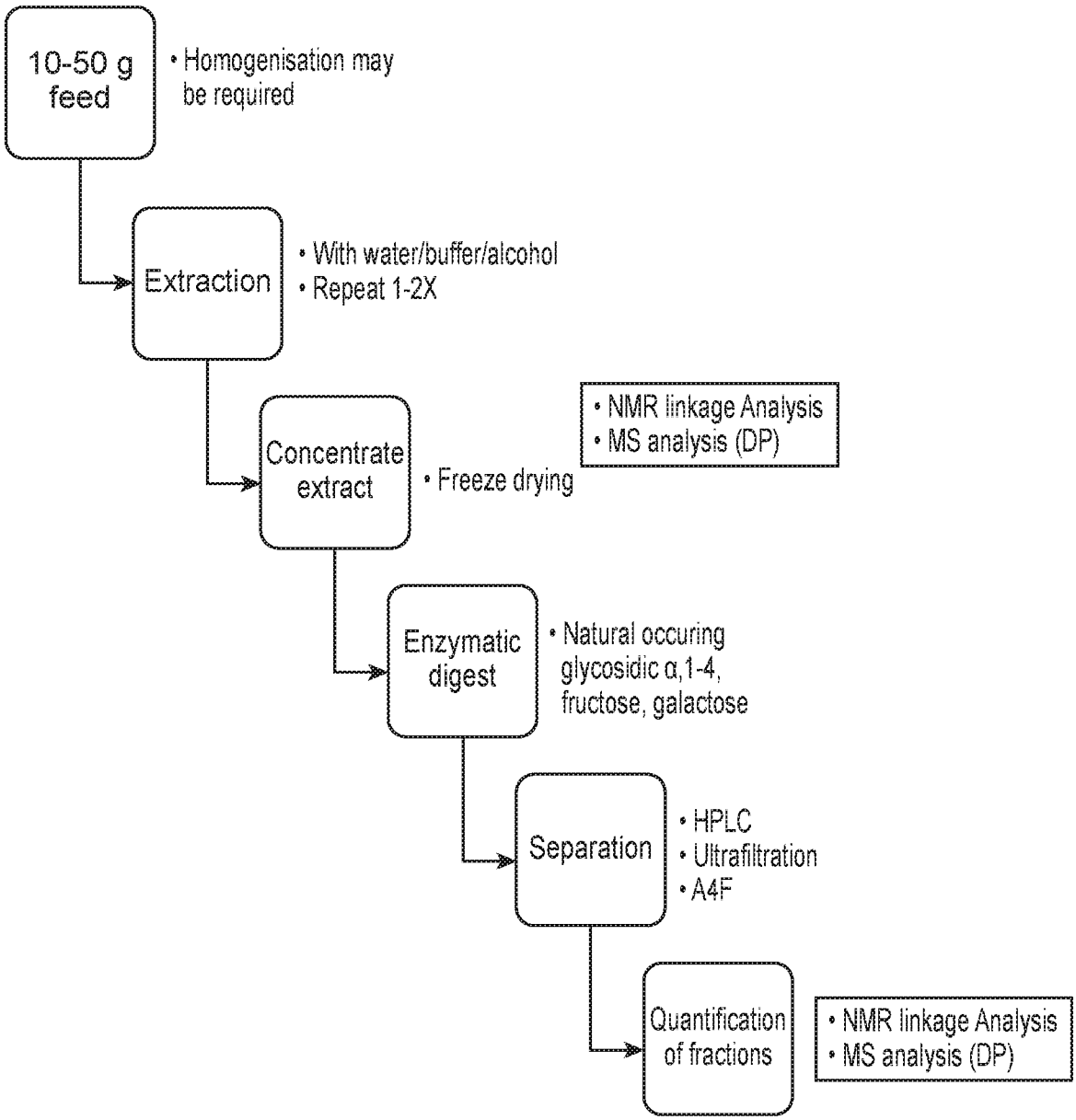
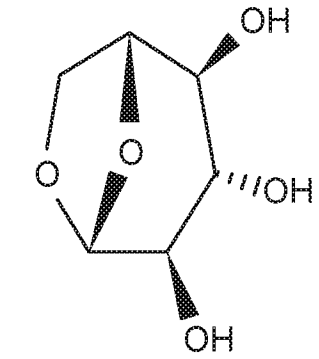
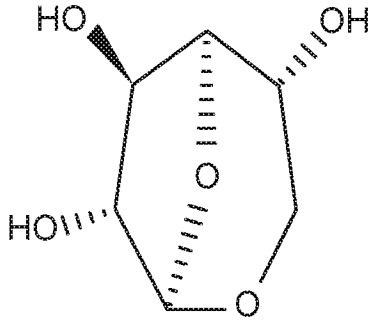

10-50 g feed
• Homogenisation may be required
Extraction
• With water/buffer/alcohol
• Repeat 1-2X
Concentrate extract
• Freeze drying
• NMR linkage Analysis
• MS analysis (DP)
Enzymatic digest
• Natural occuring glycosidic α,1-4, fructose, galactose
Separation
• HPLC
• Ultrafiltration
• A4F
Quantification of fractions
• NMR linkage Analysis
• MS analysis (DP)

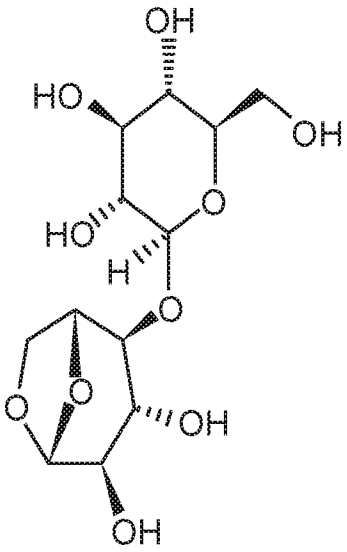
FIG. 17



Levoglucosane
CAS 498-07-7



1,6-Anhydro-β-D
Glucofuranose
CAS 7425-74-3,



Anhydro-cellobiose,

FIG. 18

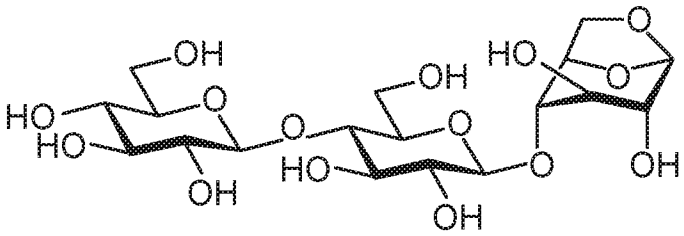


FIG. 19

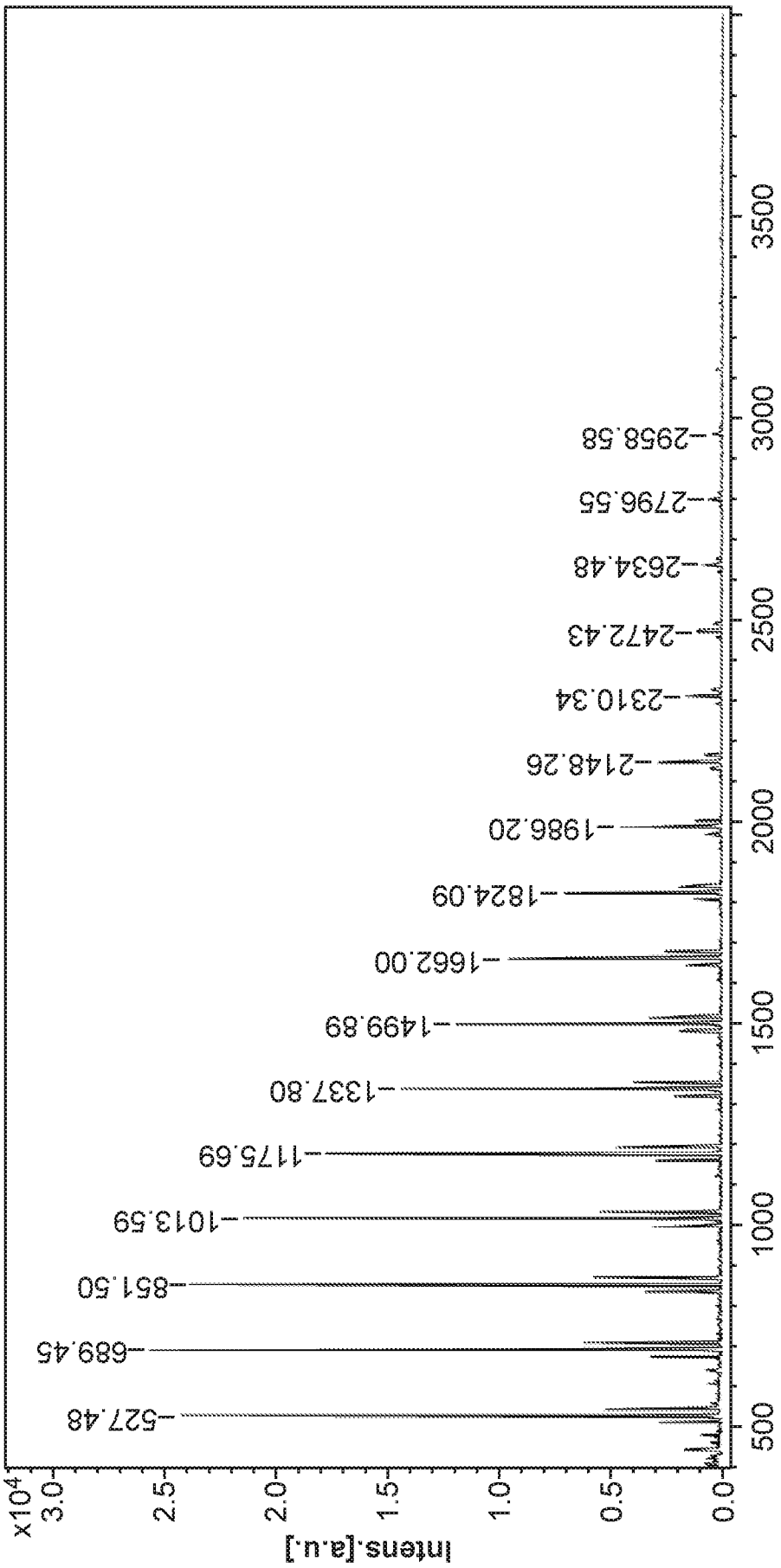

Intens.[a.u.]
x10^4
3.0
2.5
2.0
1.5
1.0
0.5
0.0
500
1000
1500
2000
2500
3000
3500
m/z
527.48
689.45
851.50
1013.59
1175.69
1337.80
1499.89
1662.00
1824.09
1986.20
2148.26
2310.34
2472.43
2634.48
2796.55
2958.58

FIG. 20A

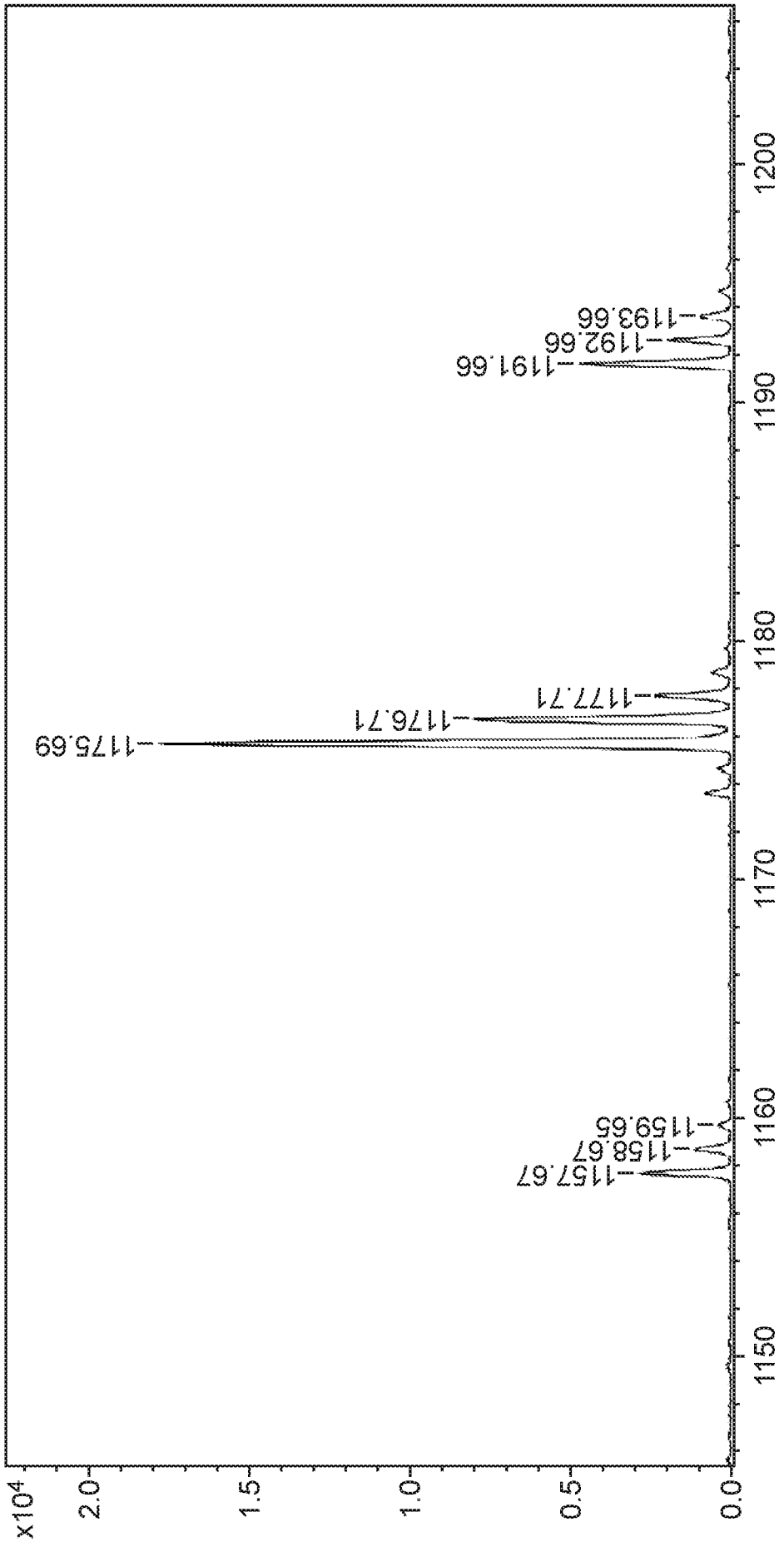

1193.66
1192.66
1191.66
1177.71
1176.71
1175.69
1159.65
1158.67
1157.67
1200
1190
1180
1170
1160
1150
m/z
x10^4
2.0
1.5
1.0
0.5
0.0

FIG. 20B

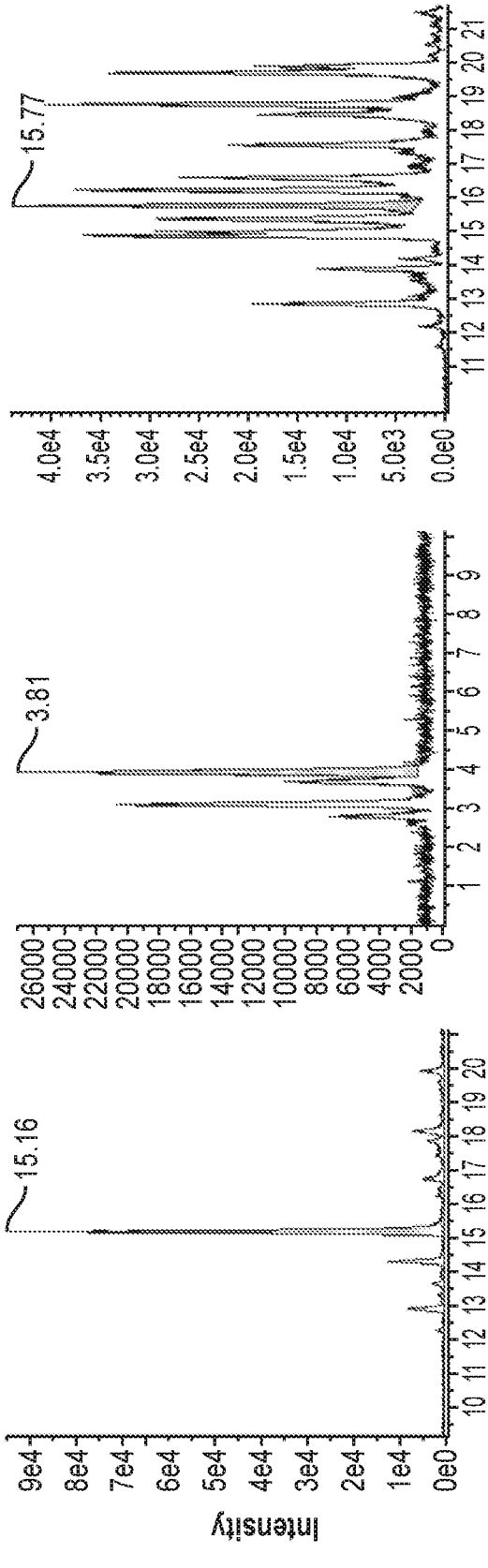


FIG. 21A

FIG. 21B

FIG. 21C

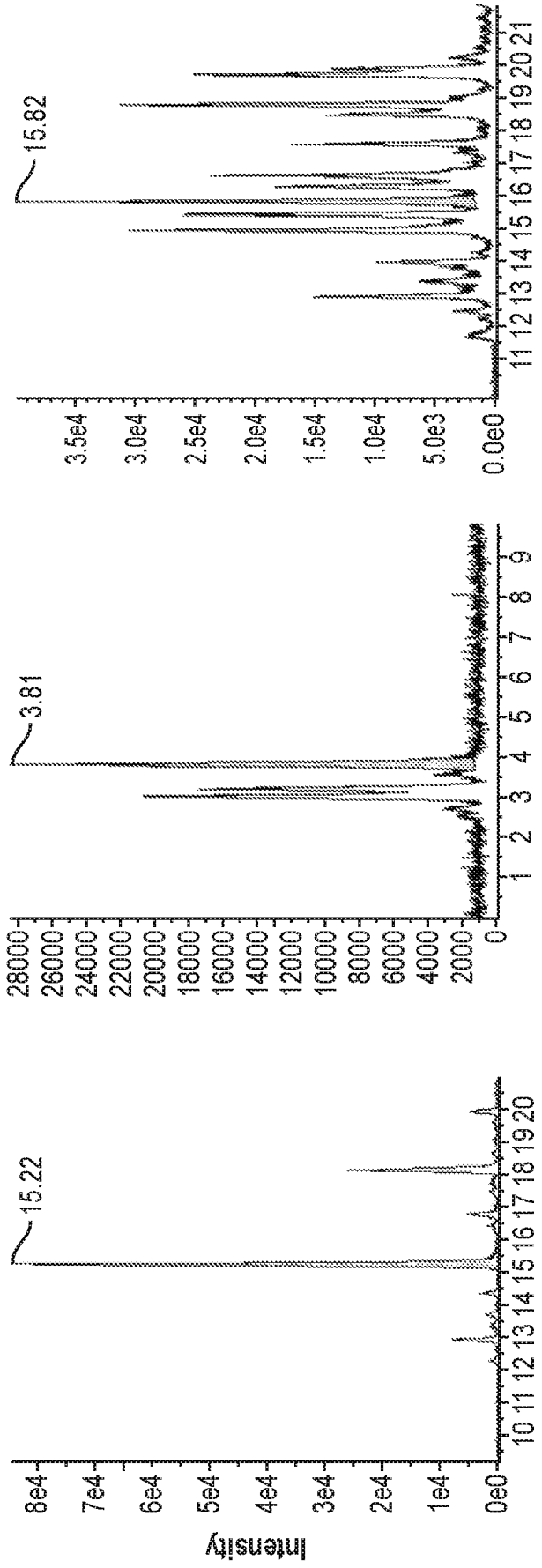


FIG. 22A

FIG. 22B

FIG. 22C

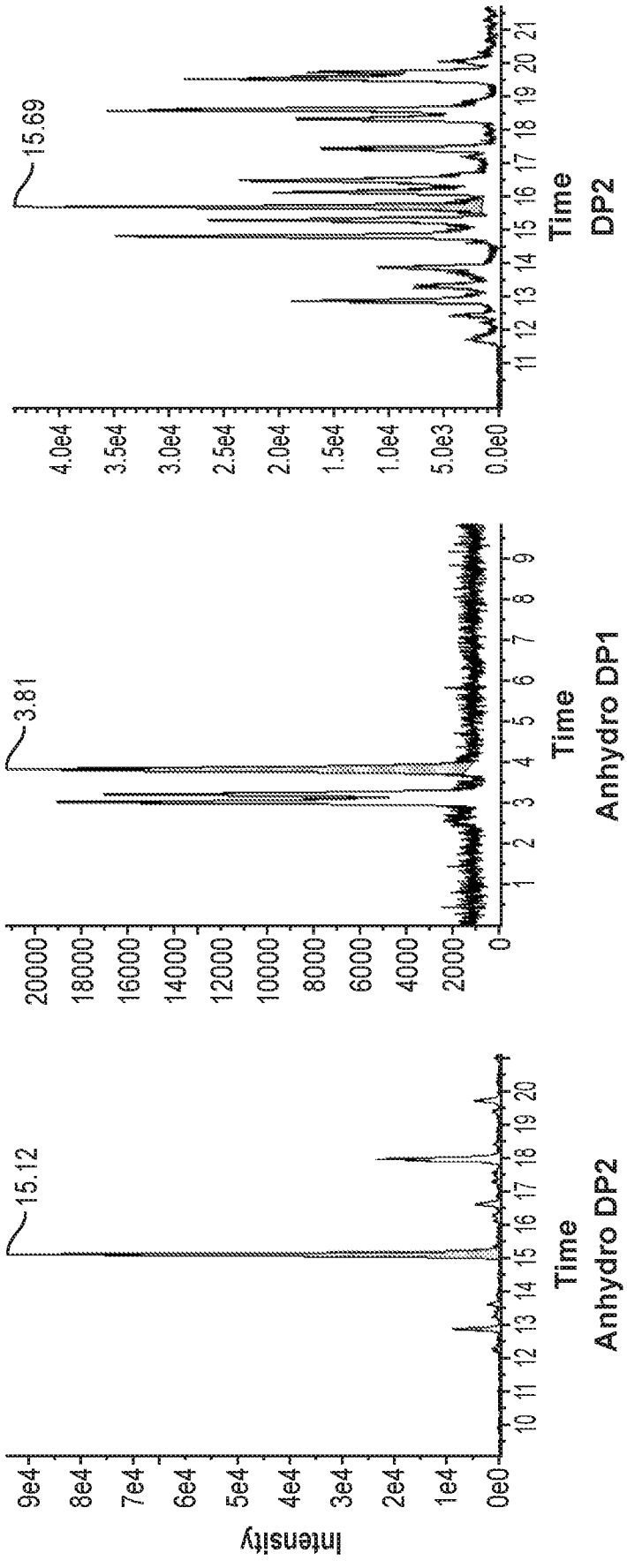


FIG. 23A

FIG. 23B

FIG. 23C

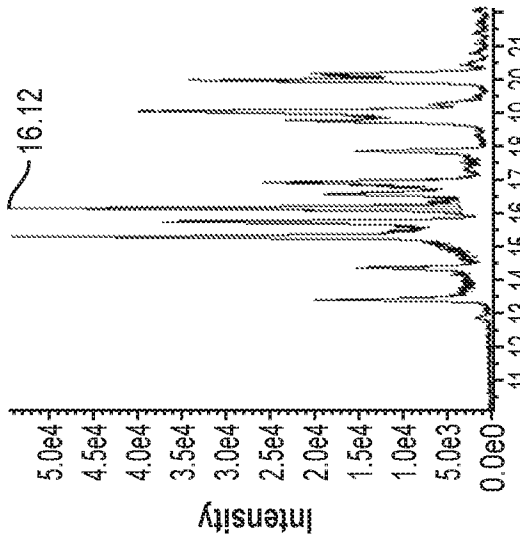


FIG. 24C

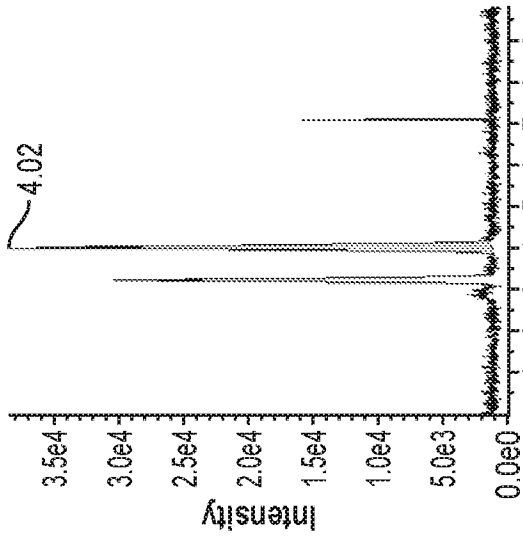


FIG. 24B

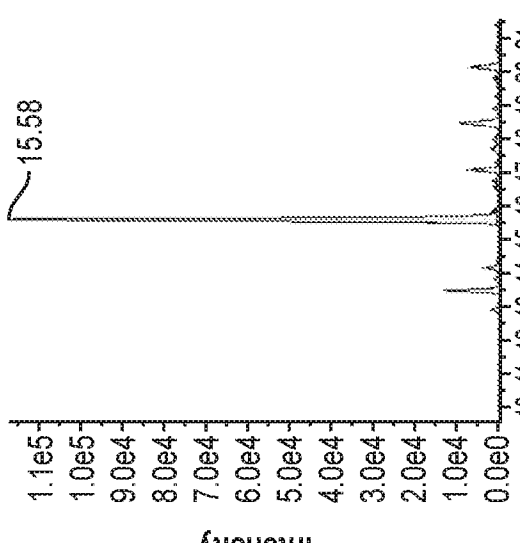


FIG. 24A

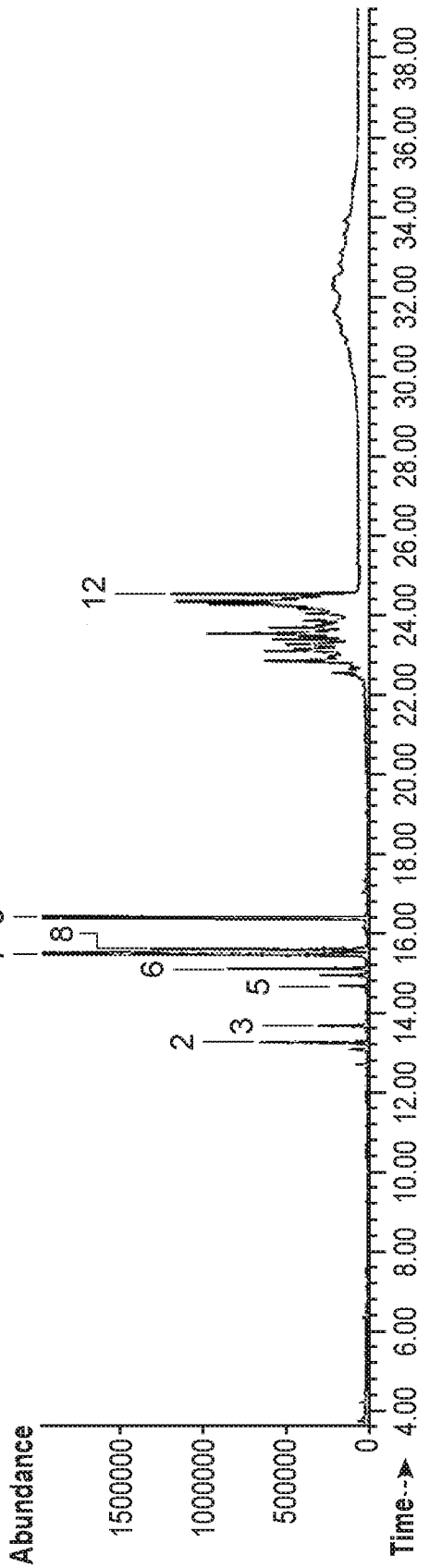


FIG. 25A

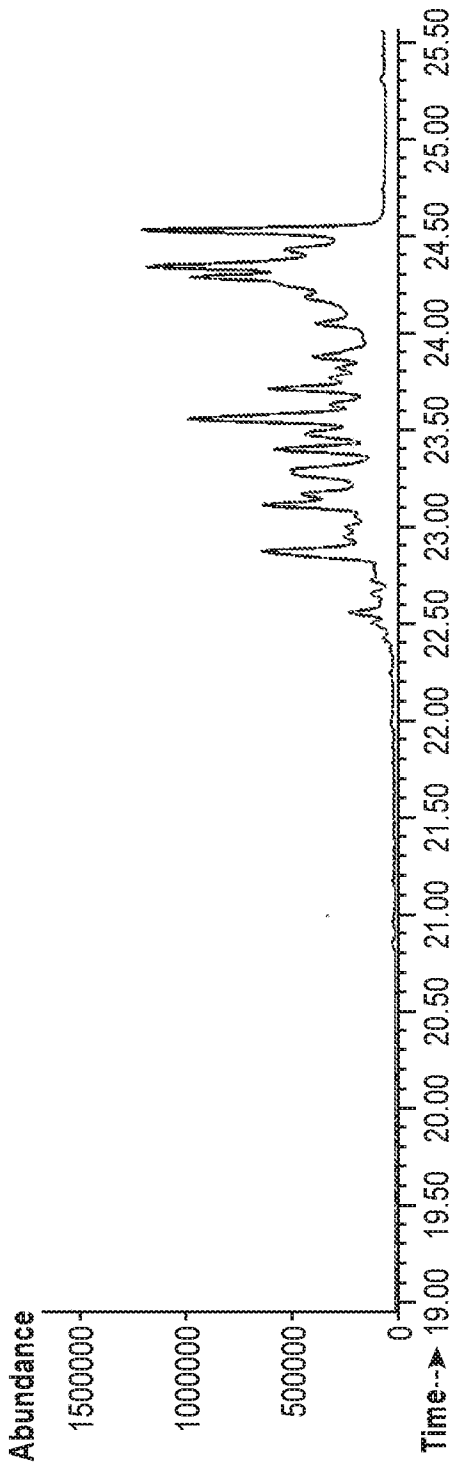


FIG. 25B

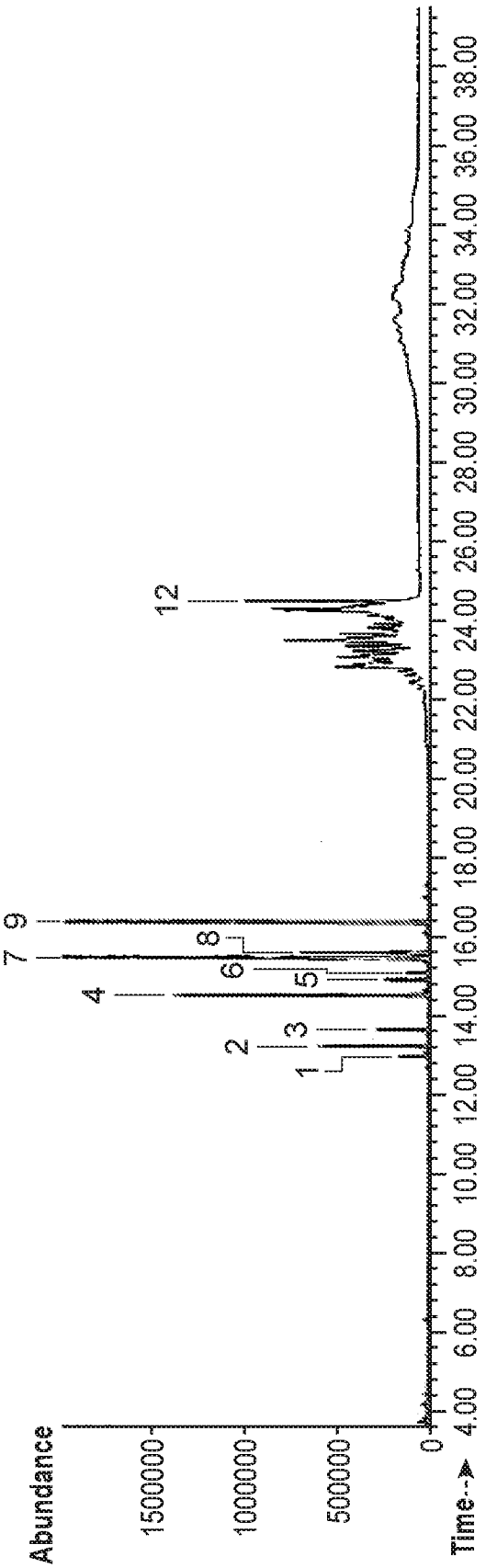


FIG. 26A

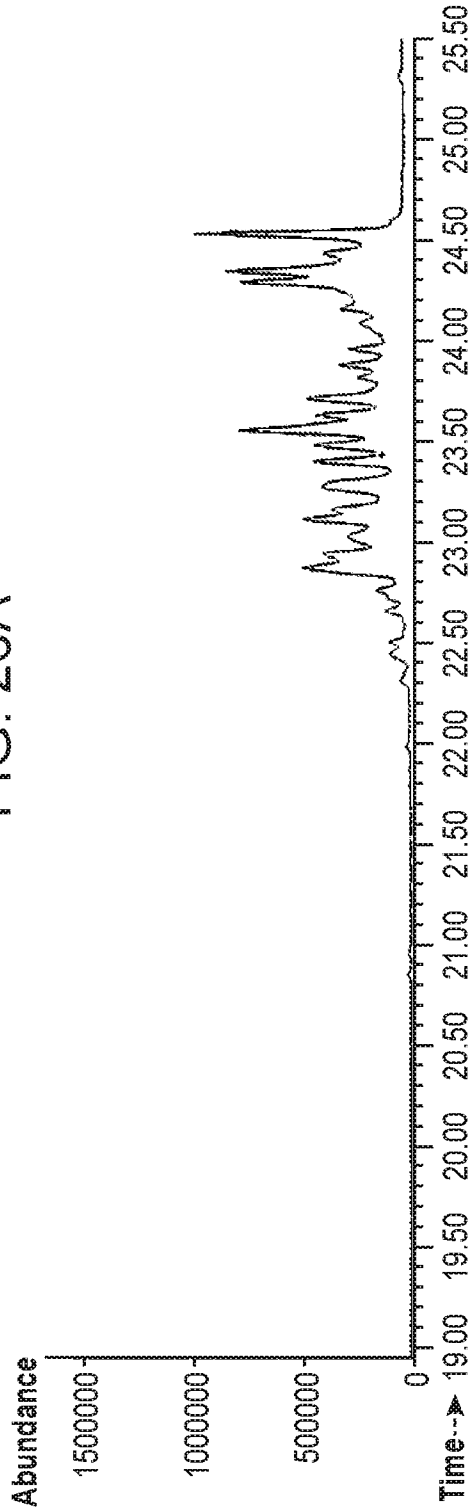


FIG. 26B

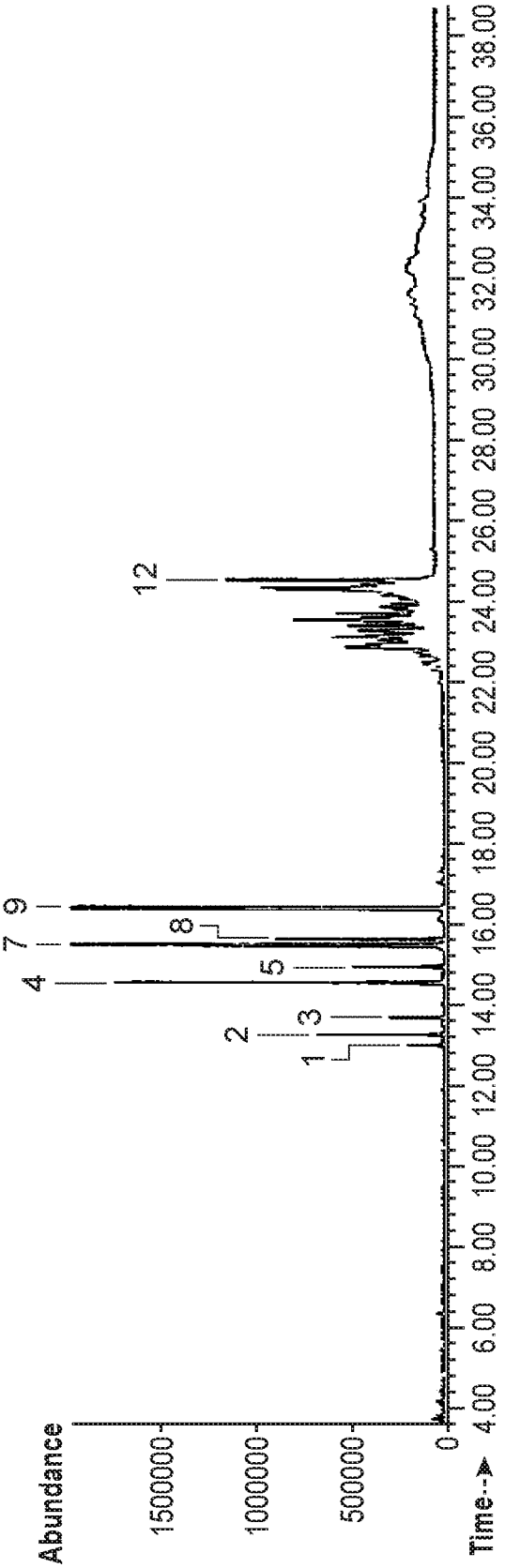


FIG. 27A

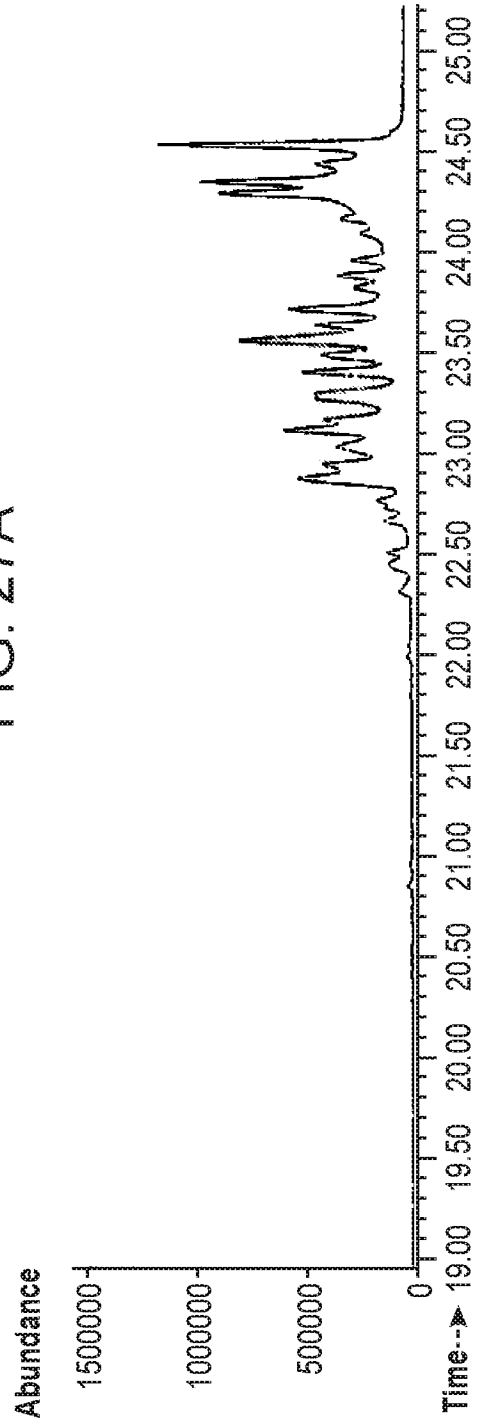


FIG. 27B

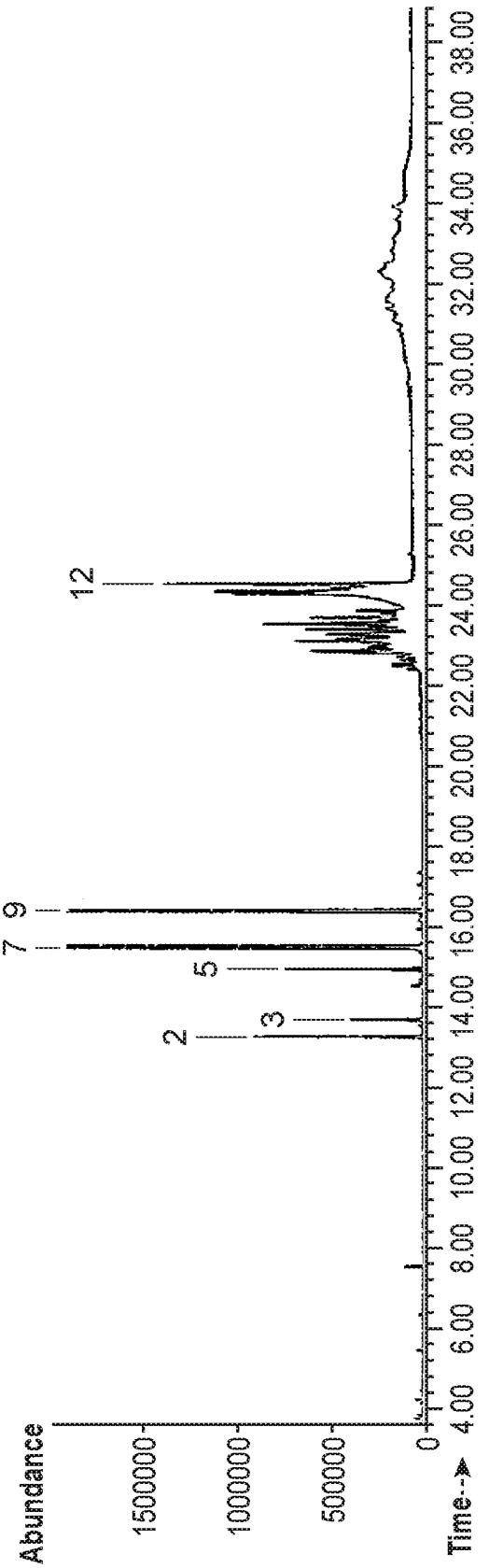


FIG. 28A

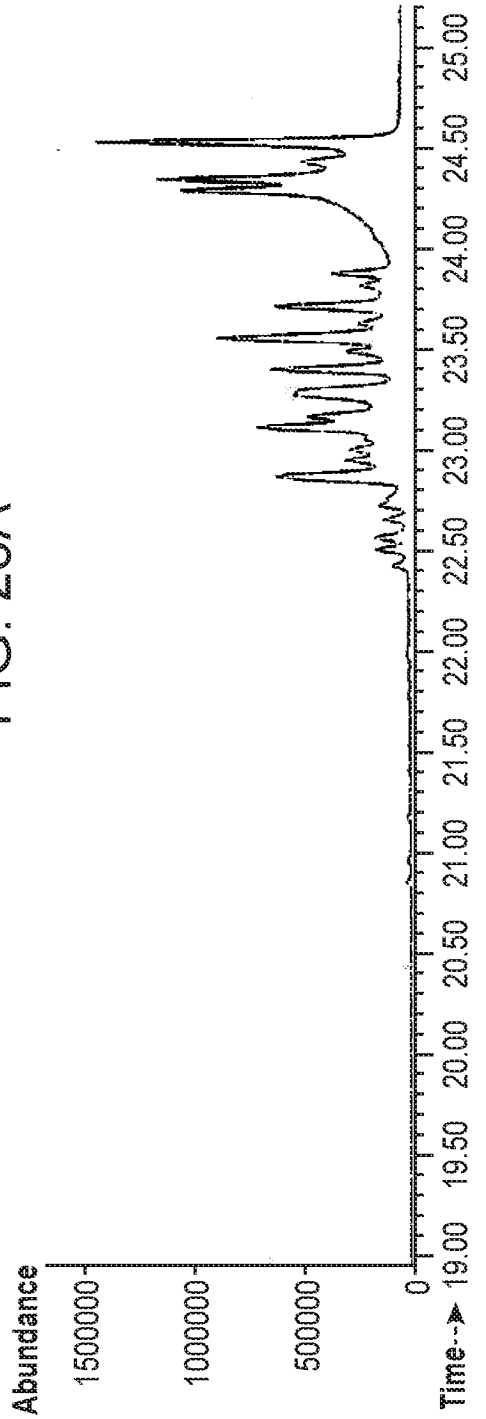


FIG. 28B

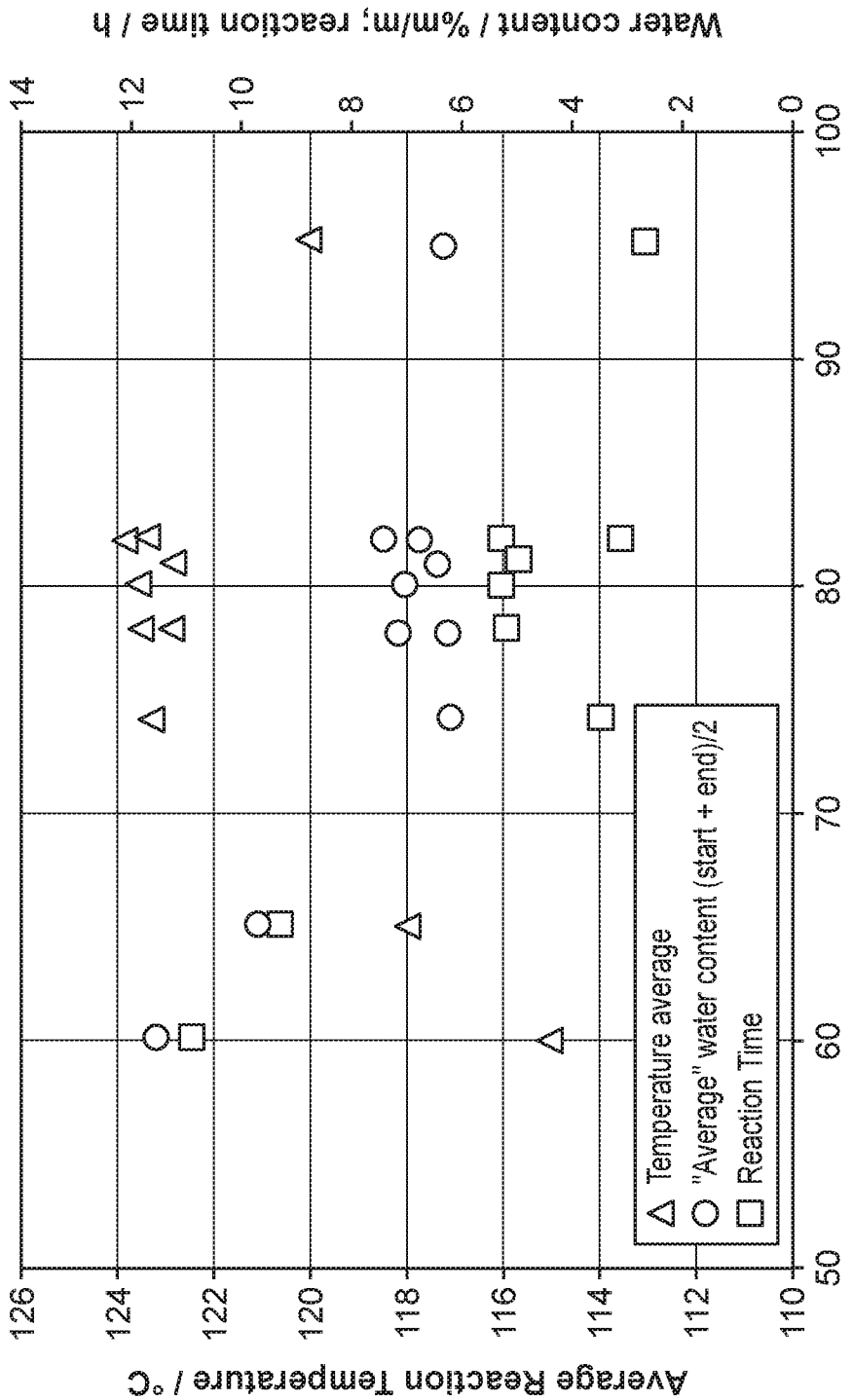

Water content / %m/m; reaction time / h
Average Reaction Temperature / °C
Temperature average
"Average" water content (start + end)/2
Reaction Time
DP2 Anhydro content relative to an oligosaccharide preparation of Example 2

FIG. 29

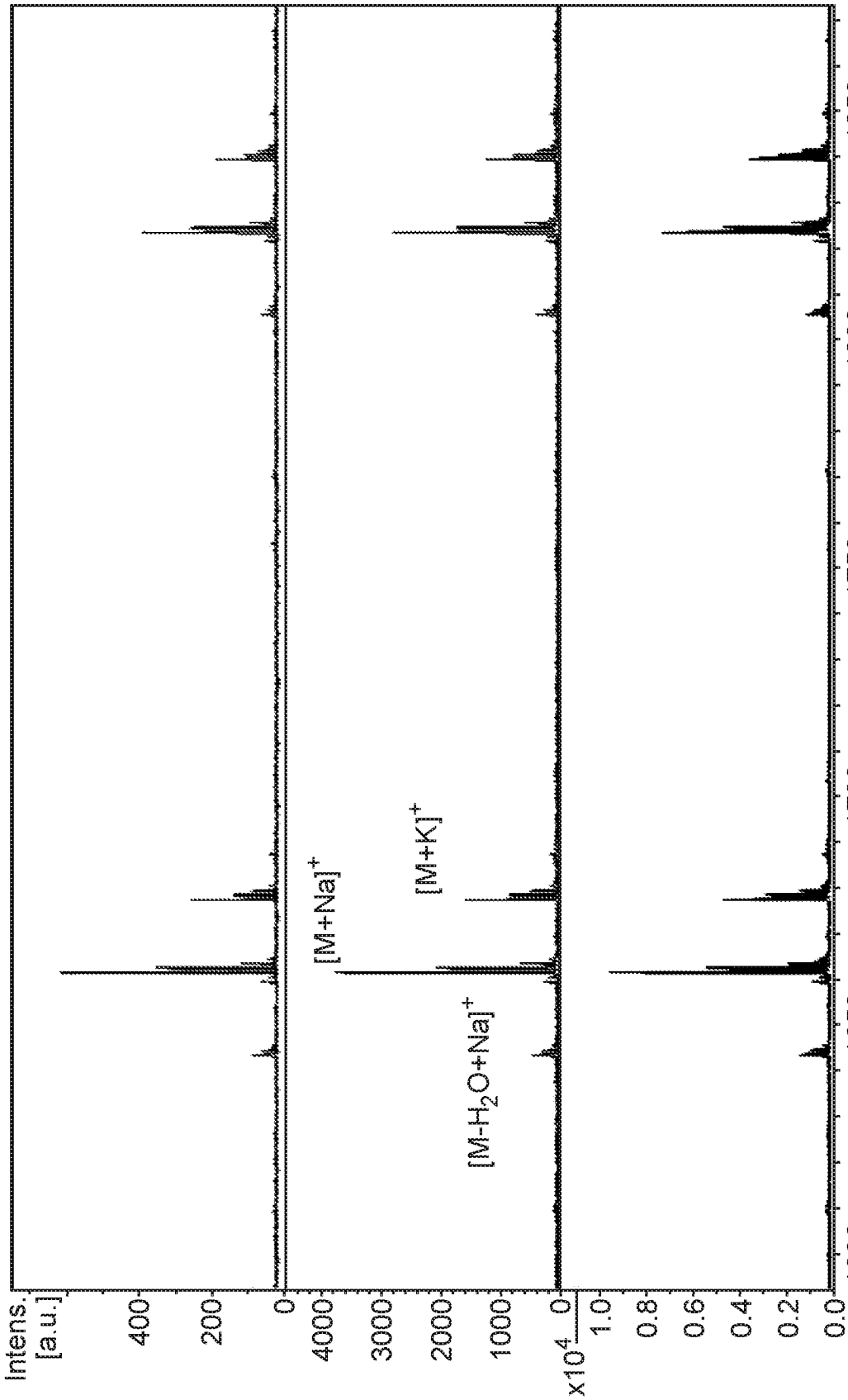

Intens. [a.u.]
400
200
0
4000
3000
2000
1000
0
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
[M+Na]+
[M+K]+
[M-H2O+Na]+
1600
1650
1700
1750
1800
1850

FIG. 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/60453

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C08B 30/20; A23G 3/00 (2020.01)

CPC - A21D 2/18; A21D 2/183; A23K 20/163; A23K 50/40

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)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/0366909 A1 (Midori USA, Inc.) 22 December 2016 (22.12.2016) para [0008], [0069], [0097], [0191], [0256], [1138]	1-4, 41-45
A	Wikipedia, 'High-performance liquid chromatography', 2004, para 5 [retrieved from the internet on 11 February 2020 (11.02.2020) at <https://en.wikipedia.org/wiki/High-performance_liquid_chromatography >]	1-4, 41-45
A	Wikipedia article 'Fructooligosaccharide', 1997, para 6 [retrieved from the internet on 11 February 2020 (11.02.2020) at <https://en.wikipedia.org/wiki/Fructooligosaccharide>]	1-2
A	US 8,101,733 B1 (Shriver et al.) 24 January 2012 (24.01.2012) entire document	1-4, 41-45
A	US 2017/0029458 A1 (Analyticon Discovery GMBH) 02 February 2017 (02.02.2017) entire document	1-4, 41-45

☐ Further documents are listed in the continuation of Box C. ☐ 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
"D" document cited by the applicant in the international application
"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
12 February 2020	19 MAR 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/60453

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.: 8-40, 46-114 and 123-129
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. For all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-4 and 41-45, directed to a method of correlating or undertaking quality control, the method comprising: (a) providing at least one sample of a nutritional composition, wherein the nutritional composition comprises a synthetic oligosaccharide preparation and a naturally occurring oligosaccharide composition; followed by (b) detecting at least one signal from the at least one sample of the nutritional composition; and lastly (c) comparing or correlating the at least one signal of the at least one sample of the nutritional composition, wherein the first signal and the second signal are independently (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with a-(1,2) glycosidic linkages, a-(1,3) glycosidic linkages, a-(1,6) glycosidic linkages, B-(1,2) glycosidic linkages, B-(1,3) glycosidic linkages, B-(1,4) glycosidic linkages, B-(1,6) glycosidic linkages, a-(1,1)-a glycosidic linkages, a-(1,1)-B glycosidic linkages, B-(1,1)-a glycosidic linkages, or B-(1,1)-B glycosidic linkages of oligosaccharides.

-*-Continued in Supplemental Box-*-

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 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.:
1-4, 41-45

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.

Continuation of

-*-Box III - Observations where unity of invention is lacking-*-

Group II: Claims 5-7, directed to a method of performing quality control of a nutritional composition comprising: (a) providing a sample of a nutritional composition, wherein the nutritional composition comprises a naturally occurring oligosaccharide composition, and followed by (b) detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, wherein the signal is indicative of one or more anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2).

Group III: Claims 115-122, directed a method of correlating a synthetic oligosaccharide preparation in a nutritional composition, wherein the nutritional composition comprises a synthetic oligosaccharide preparation that comprises anhydro-subunit containing oligosaccharides and a naturally occurring oligosaccharide composition, the method comprising: (a) providing a sample of the nutritional composition; followed by (b) isolating one or more anhydro-subunit containing oligosaccharides from the sample; then (c) detecting a signal that is indicative of the one or more anhydro-subunit containing oligosaccharides, wherein the detecting comprises (i) a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the sample or (ii) analyzing at least a portion of anhydro-subunit containing oligosaccharides from the sample by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), liquid chromatography-mass spectrometry (LC-MS)/MS, or gas chromatography(GC)MS; and lastly (d) correlating a concentration of the synthetic oligosaccharide preparation in the nutritional composition.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features required to unify the inventions described in each group:
Special Technical Features:

Group I requires the steps of: (c) comparing or correlating the at least one signal of the at least one sample of the nutritional composition, wherein the first signal and the second signal are independently (i) indicative of one or more anhydro-subunit containing oligosaccharides or (ii) associated with a-(1,2) glycosidic linkages, a-(1,3) glycosidic linkages, a-(1,6) glycosidic linkages, B-(1,2) glycosidic linkages, B-(1,3) glycosidic linkages, B-(1,4) glycosidic linkages, B-(1,6) glycosidic linkages, a-(1,1)-a glycosidic linkages, a-(1,1)-B glycosidic linkages, B-(1,1)-a glycosidic linkages, or B-(1,1)-B glycosidic linkages of oligosaccharides, which is not required by Groups II or III.

Group II requires the steps of: detecting a signal of at least a portion of oligosaccharides in the sample of the nutritional composition through analytical instrumentation, wherein the signal is indicative of one or more anhydro-subunit containing oligosaccharides having a degree of polymerization of 2 (DP2), which is not required by Group I or III.

Group III requires the steps of: (b) isolating one or more anhydro-subunit containing oligosaccharides from the sample; then (c) detecting a signal that is indicative of the one or more anhydro-subunit containing oligosaccharides, wherein the detecting comprises (i) a weight determination of at least a portion of anhydro-subunit containing oligosaccharides from the sample or (ii) analyzing at least a portion of anhydro-subunit containing oligosaccharides from the sample by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), liquid chromatography-mass spectrometry (LC-MS)/MS, or gas chromatography(GC)MS, neither or which is required by Groups I or II.

Common Technical Features:

Groups I-III share the common technical feature of being methods of correlating or undertaking quality control requiring providing a sample of a nutritional composition that at least comprises natural oligosaccharides. However, this shared technical feature does not represent a contribution over the prior art, because it is anticipated by US 6,045,854 A to Prieto et al. (hereinafter "Prieto") Prieto teaches a method of correlating or undertaking quality control (col. 6, ln. 15-21 "Oligosaccharide Analysis by High-Pressure Anion Exchange Chromatography: Samples from Example 1 were analyzed using a DioneX Bio-LC System, equipped with a Pulsed-electrochemical detector. Two CarboPac PA-1 analytical columns were connected in Serial by a very short tube, and were preceded by a guard column of the same material;" col. 6, ln. 52-56 " A series of 8 oligosaccharide standards from 50 to 200 ppm was analyzed with each instrument run. All Standards were purchased from V-Labs, Inc. (Covington, La.). Calculations of oligosaccharide content in each sample were determined from these standard curves.") and providing a sample of a nutritional composition that at least comprises natural oligosaccharides (col. 5, ln. 32-51 " Collection/Storage of Human Milk Samples: Milk Samples were collected through 5 different means: 1. The Center for Pediatric Research, Eastern Virginia Medical School, Norfolk, Va. 2. The University of Chile, Division of Medical Science Orient, Santiago, Chile. 3. Department of Infectious Diseases, National Institute of Nutrition, Mexico. 4. Clinical Study No. W93-180, Abbott International Division, North Chicago, Ill. 5. Children's Hospital, Columbus, Ohio 6. Dr. Milo Hilty, Ross Laboratories, Columbus, Ohio.").

As the shared technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups. Therefore, Groups I-III lack unity under PCT Rule 13.

Item 4 (contd.):
Claims 8-40, 46-114 and 123-129 are unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).