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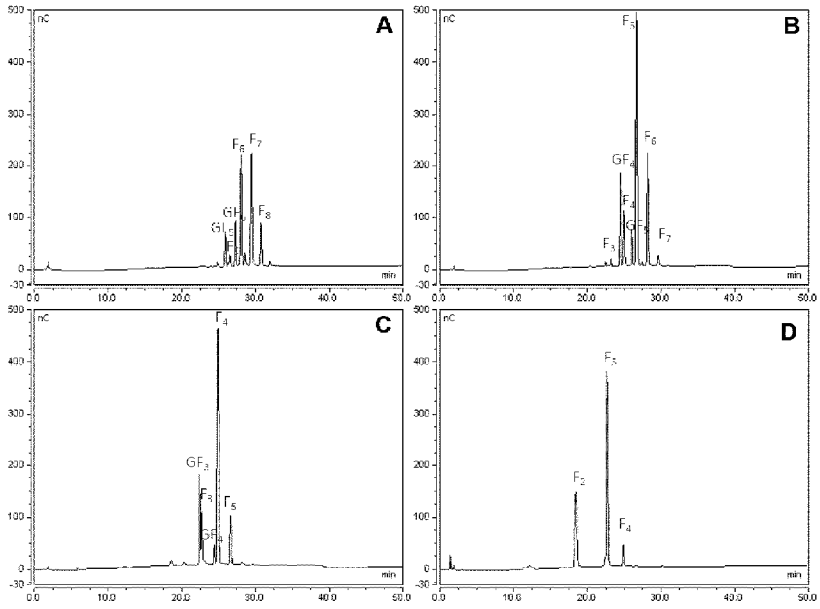
(54) Title: OLIGOSACCHARIDES AS OLIGOMOLECULAR TAGS

FIG. 1



(57) Abstract: The present disclosure is directed to oligomolecular tags (OMTs) comprising hydrolyzed inulin oligosaccharides useful for tracing a product, as well as methods of producing OMTs. Also provided are methods of tracing a product comprising an OMT, and systems of tracing a product, wherein the systems comprise an OMT and a detector.

[Continued on next page]

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TITLE: OLIGOSACCHARIDES AS OLIGOMOLECULAR TAGS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority from co-pending United States Provisional Patent Application No. 62/714,301 filed August 3, 2018, the contents of which are herein incorporated by reference in their entirety.

FIELD

[0002] The present disclosure is in the field of internal tracing systems, in particular internal tracing systems using hydrolyzed inulin oligosaccharides as oligomolecular tags (OMTs).

INTRODUCTION

[0003] The ability to monitor a food/ingredient throughout the entire supply chain, from harvest through transport, processing, storage and sale is referred to as food traceability. Currently, the global sourcing and manufacturing of foods and ingredients, as well as consumer demand for greater transparency in product labelling coupled with the proliferation of label claims, has placed increasing demands on traceability systems (Morrison C, 2003. Traceability in food processing: an introduction. In: *Food Authenticity and Traceability*. Lees, M. (Ed.), CRC Press, Boca Raton, FL. pp. 459-472). Traceability systems represent a proactive approach to improve food quality and safety and are designed to identify when and where potential hazards or adulterants enter the supply chain. In order for a traceability system to be useful it must be applicable to a wide variety of foods/ingredients, accurate, reproducible, cost effective, relatively easy to record and read/interpret, damage resistant and tamper-proof (Opara and Mazaud, 2001. Food traceability from field to plate. *Outlook on Agriculture*, 30: 239-247).

[0004] Currently, food traceability systems are based on external tags, such as barcodes and radio frequency identification (RFID) tags (Regattieri *et al*, 2007. Traceability of food products: general framework and experimental evidence. *Journal of Food Engineering*, 81: 347-356). While barcodes are easy to read, provide access to a wide variety of data (*e.g.* lot number, date and location of production) and are economical, products need to be positioned such that the barcodes can be detected and identified by readers, which requires the careful positioning of products during transport and storage

(Regattieri *et al*, 2007). Radio frequency identification (RFID) tags do not suffer from this issue as radio waves transmit information wirelessly enabling fully automated product monitoring (Regattieri *et al*, 2007). However, RFID tags are more expensive than barcodes, thereby limiting their application for lower-value products. The major limitations of external traceability systems are that they can be readily removed or manipulated, and that the food or ingredient within the container/package can be substituted in whole or in part without detection.

[0005] Methods to mitigate the issues associated with external tags have been developed where a tag is added directly to the product, that is, an internal traceability systems. In these cases, if the product was tampered with, the tag itself should also show tampering. In foods, an internal traceability system would not only allow for the detection of adulteration but could also provide a way to track it to its source. As such, a food/ingredient could be monitored from farm to fork. A number of different approaches to these internal traceability systems have been suggested. As an example, beads labelled with fluorescent dyes (*e.g.*, fluorescein, Texas red) could be added to products such as the plastics used in expensive consumer goods such as electronics. These beads could then be detected spectrophotometrically and the product could be monitored throughout its movement through the supply chain (Egner *et al*, 1997. Tagging in combinatorial chemistry: the use of coloured and fluorescent beads. *Chemical Communications*, 8: 735-736; Paunescu *et al*, 2016. Particles with an identity: tracking and tracing in commodity products. *Powder Technology,* 291: 344-350). To date, these fluorescently labelled beads are not approved for foods/ingredients and as such are not applicable to food traceability.

[0006] Another proposed internal traceability system is through the addition of oligonucleotides with defined sequences to a product. One of the major advantages of genetic (*i.e.* DNA) based molecular tags is that a nearly unlimited number of unique sequences can be produced, which would allow producers to change the tag used as required (Paunescu *et al*, 2016). Oligonucleotides have been added to anti-cancer drugs as a method for product traceability and have also been examined for use in food products, fuels and cosmetics (Puddu *et al*, 2014. Magnetically recoverable, thermostable, hydrophobic DNA/silica encapsulates and their application as invisible oil tags. *ACS Nano*, 8: 2677-2685; Paunescu *et al*, 2016). Limitations of this approach to

food traceability include: whether these oligonucleotides would be considered as food additives, and if so their approval; possible negative reactions from consumers to the addition of oligonucleotides (*i.e.* foreign DNA) to foods; food labeling issues; stability under food processing conditions; cost; and accuracy/reproducibility. The development of an alternate internal traceability system for foods/food ingredients is long overdue.

SUMMARY

[0007] The present disclosure relates to alternate internal traceability systems for foods/food ingredients. Inulin oligosaccharides are useful as internal traceability tags, for example, because: 1) these carbohydrates are not commonly found in the majority of foods; 2) production of a series of different oligosaccharide structures is possible by controlled hydrolysis and blending; 3) inulin is naturally present in some food products (*e.g.* agave syrup, chicory root); and 4) inulin oligosaccharides are food ingredients used in diabetic and low calorie foods and as such are considered to be safe. The present disclosure describes fractions of inulin oligosaccharides (*i.e.* hydrolyzed inulin oligosaccharides) as oligomolecular tags (OMTs) and uses thereof, methods of producing OMTs, methods of tracing a product containing an OMT, and a system of tracing a product containing an OMT. The present disclosure shows the efficacy of these OMTs as an internal traceability system in the production and storage of a product, for example a beverage such as apple juice.

[0008] Accordingly, the present disclosure describes an OMT for tracing a product, wherein the OMT comprises at least one combined fraction of hydrolyzed inulin, optionally chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin, suitably chicory inulin.

[0009] In some embodiments, the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides having the molecular weight between about 300 and about 1,800 Da.

[0010] In some embodiments, the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides having a degree of polymerization (DP) of (a) from 5 to 8; (b) from 3 to 7; (c) from 3 to 5; or (d) from 2 to 4.

[0011] In some embodiments, the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides:

(a) GF_5, GF_6, F_5, F_6, F_7, and F_8;

(b) GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7;

(c) GF_3, GF_4, F_3, F_4, and F_5; or

(d) F_2, F_3, and F_4.

[0012] In some embodiments, the oligosaccharides are GF_5, GF_6, F_5, F_6, F_7, and F_8.

[0013] In some embodiments, the oligosaccharides are GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7.

[0014] In some embodiments, the oligosaccharides are GF_3, GF_4, F_3, F_4, and F_5.

[0015] In some embodiments, the oligosaccharides are F_2, F_3, and F_4.

[0016] In some embodiments, the oligosaccharides are in solid, liquid solution or suspension form, optionally powder form.

[0017] In some embodiments, the product is a food product, a pharmaceutical product, a nutraceutical product, a textile product, a personal care product or an industrial product.

[0018] In some embodiments, the product is hydrophobic.

[0019] In some embodiments, the food product is a whole food or a food ingredient.

[0020] In some embodiments, the whole food is a beverage.

[0021] In some embodiments, the food ingredient is flour or a food additive.

[0022] In some embodiments, the beverage is water, milk, a juice, coffee, tea, or a soft drink.

[0023] In some embodiments, the water is coconut water.

[0024] In some embodiments, the juice is apple, pear, grape, cranberry, grapefruit, tomato, blackberry, raspberry, strawberry, orange, winter melon, pomegranate, aloe vera, clam, sugarcane, kiwifruit, lemon, lime, lychee, cantaloupe, honeydew, papaya, pineapple, guava, prune, beet, carrot, celery, cucumber, dandelion-green, parsley, spinach, tejuino, turnip, watercress or hemp juice.

[0025] In some embodiments, the pharmaceutical product is a biologic or a small molecule.

[0026] In some embodiments, the nutraceutical product is a dietary supplement or a functional food.

[0027] In some embodiments, the person care product is a personal hygiene or a cosmetic product.

[0028] In some embodiments, the industrial product is paint.

[0029] In some embodiments, the OMT is at a concentration of at least about 10 ppm.

[0030] In some embodiments, the OMT is stable for at least 12 months, optionally from about 4.0 °C to about 90 °C.

[0031] In some embodiments, the OMT comprises hydrophobic oligosaccharides.

[0032] In some embodiments, the hydrophobic oligosaccharides comprise acetylated oligosaccharides.

[0033] Also provided is a method for producing the OMT described herein, comprising hydrolysis of inulin, optionally chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin, suitably chicory inulin, by an endo-inulinase, wherein the endo-inulinase is from *Arthrobacter*, optionally *Arthrobacter* sp. S37, from *Aspergillus,* optionally *Aspergillus niger*, or from *Pseudomonas*, optionally *Pseudomonas mucidolens*.

[0034] In some embodiments, the hydrolyzed inulin is separated by size exclusion chromatography.

[0035] In some embodiments, the hydrolyzed inulin is identified by Capillary Gas Chromatography, High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), High Performance Anion Exchange Chromatography with Pulsed Electrochemical Detection (HPAE-PED), or liquid chromatography-mass spectrometry (LC-MS).

[0036] Also provided is a method of tracing a product, comprises introducing the OMT described herein into the product, optionally during or after production of the product, optionally before or during packaging of the product.

[0037] Also provided is a system of tracing a product, comprising the OMT described herein and a detector.

[0038] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating embodiments of the disclosure are given by way of illustration only, the scope of the claims should not be limited by the embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] The embodiments of the disclosure will now be described in greater detail with reference to the attached drawings in which:

[0040] Fig. 1 shows HPAE-PAD chromatograms of exemplary oligomolecular tags (OMTs) produced via enzymatic hydrolysis of chicory inulin and size exclusion chromatography. OMT #1 (A; fractions 31-33), OMT #2 (B; fractions 34-36), OMT #3 (C; fractions 37-39) and OMT #4 (D; fractions 40-42). Where F = fructose, G = glucose and the subscripts indicate the number of each residue.

[0041] Fig. 2 shows HPAE-PAD chromatograms of the natural oligosaccharide profiles of laboratory scale apple juice at select processing stages. A: mash prior to enzyme treatment; B: mash after enzyme treatment; C: filtered juice prior to concentration; and D: concentrated juice.

[0042] Fig. 3 shows HPAE-PAD chromatograms of the oligosaccharide profiles of laboratory scale apple juice plus exemplary OMT #2 (fraction 34-36; 40.0 ppm) throughout processing. A: mash prior to enzyme treatment; B: mash after enzyme treatment; C: filtered juice prior to concentration; and D: concentrated juice.

[0043] Fig. 4 shows peak areas of exemplary inulin oligosaccharides before and after storage in apple juice for six months. A: single strength apple juice and B: concentrated apple juice stored at room temperature (22.0 ± 2.0 °C), C: single strength apple juice and D: concentrated apple juice stored at 4.0 ± 1.0 °C. Error bars represent the variation in detector response.

DESCRIPTION OF VARIOUS EMBODIMENTS

I. Definitions

[0044] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present disclosure herein described for which they are suitable as would be understood by a person skilled in the art.

[0045] The term "inulin oligosaccharides" or "inulooligosaccharides" and the like as used herein refer to inulin that has been hydrolyzed into oligosaccharides, *i.e.* hydrolyzed inulin oligosaccharides, and also includes hydrolyzed inulin oligosaccharides that have been modified so they are hydrophobic.

[0046] The term "hydrophilic" as used herein refers to the property of a substance that has attraction to water. If a hydrophilic molecule is mixed with water the two will tend to form one phase. Hydrophilic molecules tend to be polar molecules. The term hydrophilic may be used to refer to a food or other materials described herein, where the food or other materials are, for example, water based or water soluble.

[0047] The term "hydrophobic" as used herein refers to the property of a substance that lacks attraction to water. If a hydrophobic molecule is mixed with water the two will tend to separate and form two individual phases. Hydrophobic molecules tend to be nonpolar molecules that group together to form micelles rather than be exposed to water. The term hydrophobic may be used to refer to a food or other materials described herein, where the food or other materials contain hydrophobic molecules, for example, fats and oils.

[0048] The term "tracing" as used herein also refers to tracking, monitoring, authenticating, distinguishing, and/or identifying a product.

[0049] A "fraction" of inulin oligosaccharides comprises any suitable part of inulin oligosaccharides for the tracing a product. The fraction may be obtained by any suitable fractionation methods, for example, size exclusion chromatography.

[0050] The term "combined fraction" as used herein refers to a composition comprising two or more inulin oligosaccharides of different structures. The inulin oligosaccharides of different structures have different sizes, masses, and/or chemical compositions. A combined fraction is produced and collected from the methods described herein. As such, inulin oligosaccharides are produced by hydrolyzing inulin using an endo-

inulinase. The inulin oligosaccharides are separated by fractionation using size exclusion chromatography procedure, whereby eluent is collected in constant volumes (known as fractions) in different tubes, and the eluent collected from proximal tubes are pooled to provide a "combined fraction".

[0051] The letters "F" and "G" as used herein are in reference to components of inulin oligosaccharide fractions refers to the molecular residues fructose and glucose, respectively. The numerical subscript (n) proceeding the letters "F" and "G" refer to the number of residues of each type in a given molecule. Therefore, for example, F_6 refers to a hexasaccharide containing 6 fructose residues and GF_4 refers to a pentasaccharide containing 1 glucose and 4 fructose residues.

[0052] The term "free D-fructose" as used herein refers to a D-fructose molecule that is not covalently linked to another molecule, such as another carbohydrate molecule.

[0053] The term "and/or" as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that "at least one of" or "one or more" of the listed items is used or present.

[0054] As used in the present disclosure, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise. For example, an embodiment including "a product" should be understood to present certain aspects with one product or two or more additional products.

[0055] In embodiments comprising an "additional" or "second" component, the second component as used herein is different from the other components or first component. A "third" component is different from the other, first, and second components, and further enumerated or "additional" components are similarly different.

[0056] In understanding the scope of the present disclosure, the term "comprising" and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, "including", "having" and their derivatives.

[0057] The term "consisting" and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components,

groups, integers, and/or steps, but exclude the presence of other unstated features, elements, components, groups, integers and/or steps.

[0058] The term “consisting essentially of”, as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps.

[0059] The term "suitable" as used herein means that the selection of the particular compound or conditions would depend on the specific synthetic manipulation to be performed, and the identity of the molecule(s) to be transformed, but the selection would be well within the skill of a person trained in the art.

[0060] The terms "about", “substantially” and “approximately” as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least ±5% of the modified term if this deviation would not negate the meaning of the word it modifies or unless the context suggests otherwise to a person skilled in the art.

[0061] As used herein, the term “effective amount” means an amount of an OMT of the disclosure that is effective to achieve the desired result. For example in the context of tracing a product, an effective amount is an amount that, for example, is sufficient to be detected by a detector described herein, compared to the detection without the OMT.

[0062] The term “detectable” as used herein when referring to detection of carbohydrates, for example, monosaccharides including free D-fructose, or oligosaccharides including F_2, F_3, F_4, F_5, F_6, F_7, F_8, GF_3, GF_4, GF_5, and GF_6, means detection within the limits using methods, instruments and systems known in the art, for example, detections/detectors described herein including Capillary Gas Chromatography, High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), High Performance Anion Exchange Chromatography with Pulsed Electrochemical Detection (HPAE-PED), and liquid chromatography-mass spectrometry (LC-MS).

II. Oligomolecular Tags (OMTs) and Uses

[0063] The present disclosure includes an oligomolecular tag (OMT) for tracing a product, wherein the OMT comprises at least one combined fraction of hydrolyzed inulin, optionally chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin, suitably chicory inulin. In some embodiments, the OMT comprises at least one combined fraction of hydrolyzed chicory inulin. In some embodiments, the OMT comprises at least one combined fraction of hydrolyzed agave inulin. In some embodiments, the OMT comprises at least one combined fraction of hydrolyzed dahlia tubers inulin. In some embodiments, the OMT comprises at least one combined fraction of hydrolyzed Jerusalem artichoke inulin.

[0064] The combined fraction of hydrolyzed inulin contains oligosaccharides that fall within the range of 300 - 1,800 Da. In some embodiments, the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides having the molecular weight between about 300 and about 1,800 Da.

[0065] The combined fraction of hydrolyzed inulin can be characterized by a degree of polymerization (DP), which refers to the number of monomeric units in an oligomer molecule. In some embodiments, the at least one combined fraction of hydrolyzed inulin have a DP of (a) from 5 to 8; (b) from 3 to 7; (c) from 3 to 5; or (d) from 2 to 4.

[0066] The combined fraction of hydrolyzed inulin includes oligosaccharides containing various number of fructose residues, either only by themselves (F_n) or further containing a glucose residues (GF_n). In some embodiments, the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides:

(a) GF_5, GF_6, F_5, F_6, F_7, and F_8;

(b) GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7;

(c) GF_3, GF_4, F_3, F_4, and F_5; or

(d) F_2, F_3, and F_4.

[0067] In some embodiments, the oligosaccharides are GF_5, GF_6, F_5, F_6, F_7, and F_8. In some embodiments, the oligosaccharides are GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7. In some embodiments, the oligosaccharides are GF_3, GF_4, F_3, F_4, and F_5. In some embodiments, the oligosaccharides are F_2, F_3, and F_4.

[0068] In some embodiments, the oligosaccharides comprise hydrophobic oligosaccharides. In some embodiments, the hydrophobic oligosaccharides comprise acetylated oligosaccharides.

[0069] While each of these combined fractions is useful as an OMT, when they are used in varying concentrations in a product, a large selection of unique OMTs are available for tracing a product.

[0070] The OMTs described herein are useful in a variety of forms. In some embodiments, the OMT comprises hydrolyzed inulin in solid, liquid solution or suspension form, optionally powder form. In some embodiments, the OMT comprises hydrolyzed inulin in solid form. In some embodiments, the OMT comprises hydrolyzed inulin in liquid form. In some embodiments, the OMT comprises hydrolyzed inulin in suspension form. In some embodiments, the OMT comprises hydrolyzed inulin in powder form. In some embodiments, the solvent for the solution or suspension is water.

[0071] The OMTs described herein are useful for tracing a variety of products, including agricultural products such as foods, health products such as pharmaceuticals and nutraceuticals, and products related to textile, personal care and industry, all of which can be a hydrophilic or hydrophobic product. In some embodiments, the product is a food product, a pharmaceutical product, a nutraceutical product, a textile product, a personal care product or an industrial product. In some embodiments, the food product is a whole food or a food ingredient. In some embodiments, the food product is a whole food. In some embodiments, the food product is a food ingredient. In some embodiments, the whole food is a beverage. In some embodiments, the food ingredient is flour or a food additive. In some embodiments, the beverage is water, milk, a juice, coffee, tea, or a soft drink. In some embodiments, the water is coconut water. In some embodiments, the juice is apple, pear, grape, cranberry, grapefruit, tomato, blackberry, raspberry, strawberry, orange, winter melon, pomegranate, aloe vera, clam, sugarcane, kiwifruit, lemon, lime, lychee, cantaloupe, honeydew, papaya, pineapple, guava, prune, beet, carrot, celery, cucumber, dandelion-green, parsley, spinach, tejuino, turnip, watercress or hemp juice. In some embodiments, the pharmaceutical product is a biologic or a small molecule. In some embodiments, the nutraceutical product is a dietary supplement or a functional food. In some embodiments, the personal care product is a personal hygiene or a cosmetic product. In some embodiments, the industrial product is paint.

[0072] In some embodiments, the OMTs described herein are useful for tracing a product even when the OMTs are in low concentrations. In some embodiments, the OMT is at a concentration from at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 ppm. In some embodiments, the OMT is at a concentration of at most about 100 ppm. In some embodiments, the OMT is at a concentration of at least about 10 ppm to at most about 100 ppm.

[0073] In some embodiments, the OMTs described herein are stable and useful for tracing a product at a wide range of temperatures. In some embodiments, the OMTs described herein are also stable during ultrahigh temperature (UHT) processing, such as at about 150 °C for up to 15 seconds. In some embodiments, the OMTs described herein are also stable during canning processing, such as at about 120 °C for up to 20 minutes. In some embodiments, the OMTs described herein are also stable at about 90 °C for at least 3 hours. In some embodiments, the OMT is stable at about 150 °C for up to 15 seconds. In some embodiments, the OMT is stable at about 120 °C for up to 20 minutes. In some embodiments, the OMT is stable at about 90 °C for at least 3 hours. In some embodiments, the OMT is stable for at least 6, 9, 12, 15, 18 or 24 months, optionally from about -80, -40, -20, -10, 0, 4.0 °C to about 25, 30, 40, 50, 60, 70, 80, 90 °C. In some embodiments, the OMT is stable for at least 12 months, from about -80 °C to about 90 °C. In some embodiments, the OMT is stable for at least 12 months, from about 4 °C to about 90 °C. In some embodiments, the OMT is stable for at least 12 months, from about -40 °C to about 50 °C. In some embodiments, the OMT is stable for at least 12 months, from about -20 °C to about 40 °C.

III. Methods of Producing OMTs

[0074] In some embodiments, the OMTs described herein are produced by controlled hydrolysis using an enzymatic method. Specifically, OMTs comprising hydrolyzed linulin were produced using endo-inulinases (EC 3.2.1.7) from *Arthrobacter* sp. S37, which hydrolyzes the glycosidic linkages of inulin to produce oligosaccharides and minimal fructose, with less than 0.01% (w/w) free D-fructose in the hydrolyzed inulin. The skilled person can readily recognize alternative endo-inulinases that are suitable for the hydrolysis reaction. For example endo-inulinase from *Aspergillus*, such as *Aspergillus niger*, or from *Pseudomonas*, such *Pseudomonas mucidolens*, may be useful.

[0075] Accordingly, the present disclosure also includes a method for producing an OMT, comprising:

a) hydrolyzing an inulin solution, optionally chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin, suitably chicory inulin, by adding an endo-inulinase to the solution to produce hydrolyzed inulin, wherein the endo-inulinase is from *Arthrobacter*, optionally *Arthrobacter* sp. S37, from *Aspergillus,* optionally *Aspergillus niger*, or from *Pseudomonas*, optionally *Pseudomonas mucidolens*.

[0076] In some embodiments, the method described herein uses an endo-inulinase that produces less than 0.01% (w/w) free D-fructose. In some embodiments, the endo-inulinase produces less than 0.1%, 0.09%, 0.08%, 0.07%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02%, or 0.01% (w/w) free D-fructose. In some embodiments, the endo-inulinase produces no detectable level of free D-fructose. In some embodiments, the endo-inulinase produces less than 0.1% (w/w) free D-fructose. In some embodiments, the endo-inulinase produces less than 0.01% (w/w) free D-fructose. The presence of free D-fructose indicates exo-inulinase activity, the presence of which decreases the levels of fructose-containing inulin oligosaccharides.

[0077] In some embodiments, the method for producing an OMT comprises adding chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin to a solution and hydrolyzing the inulin by adding an endo-inulinase from *Arthrobacter,* optionally *Arthrobacter* sp. S37, from *Aspergillus,* optionally *Aspergillus niger*, or from *Pseudomonas*, optionally *Pseudomonas mucidolens*. In a specific embodiment, the method of producing an OMT comprises adding chicory inulin to a solution and hydrolyzing the inulin by adding an endo-inulinase from *Arthrobacter* sp. S37.

[0078] The skilled person can readily recognize a suitable solvent for hydrolyzing inulin. For example, suitable solvents include water, and buffer solutions containing acetate, citrate and phosphate.

[0079] Inulin hydrolysis described herein is carried out at between about pH 5.00 and about pH 7.50, for at least 24 hours at 50.0 ± 5.0 °C. In some embodiments, the hydrolysis condition is at between about pH 5.00 and about pH 7.50, for at least 24 hours at 50.0 ± 5.0 °C. In some embodiments, the hydrolysis condition is at between about pH

5.00 and about pH 7.50, for about 24 hours at 50.0 ± 5.0 °C. In some embodiments, the hydrolysis condition is at about pH 5.00 for at least 24 hours at 50.0 ± 5.0 °C. In some embodiments, the hydrolysis condition is at about pH 5.00 for about 24 hours at 50.0 ± 5.0 °C.

[0080] Hydrolyzed inulin oligosaccharides can be further processed to produce hydrophobic hydrolyzed inulin oligosaccharides, such as acetylated hydrolyzed inulin oligosaccharides. Acetylation may be carried out prior to or after hydrolyzed inulin oligosaccharides have been fractionated by size exclusion chromatography. Acetylation takes place at hydroxyl groups of the oligosaccharides such that these hydroxyl groups are esterified to acetate. Acetylation reaction occurs in the presence of an acetylating agent, for example, acetyl chloride or acetic anhydride. Acetylation of hydrolyzed inulin oligosaccharides can be controlled and/or maximized by varying temperatures and duration, in the presence or absence of a solvent and/or a catalyst (*e.g.* sodium acetate) that are familiar to the skilled person. For example, at least about 0.1% to at least about 98%, 99%, 99.9%, or about 100% of available hydroxyl groups of the inulin are acetylated. In some embodiments, inulin acetylated to a lesser degree may be used, such as inulin with at least about 0.1% of available hydroxyl groups acetylated. In embodiments, at least about 0.1%, at least about 0.5%, at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.1%, at least about 99.2%, at least about 99.3%, at least about 99.4%, at least about 99.5%, at least about 99.6%, at least about 99.7%, at least about 99.8%, at least about 99.9%, at least about 99.99%, at least about 99.999%, or about 100%, of the available hydroxyl groups may be acetylated for various inulin oligosaccharides useful as OMTs. Acetylated hydrolyzed inulin oligosaccharides can be identified by capillary gas chromatography, for example, with flame ionization detection under appropriate temperature programming conditions known to the skilled person. In some embodiments, acetylated inulin is considered to be inulin acetate when the hydroxyl peak of its infra-red

spectrum disappears and acetyl peaks appear. Inulin oligosaccharides acetate is hydrophobic and insoluble in water even at elevated temperatures, but is soluble in various organic solvents such as acetone, ethyl acetate, chloroform, and dichloromethane, and in for example, hydrophobic foods and bioproducts. The skilled person can readily recognize alternative options that are suitable for acetylating hydrolyzed inulin and methods for removal of solvents, catalysts and alkylating agents. In some embodiments, the method for producing OMTs further comprises acetylating the oligosaccharides, thereby providing hydrophobic oligosaccharides. In some embodiments, the method for producing OMTs further comprises acetylating the oligosaccharides, thereby providing acetylated oligosaccharides. In some embodiments, the inulin oligosaccharides comprise hydrophobic inulin oligosaccharides. In some embodiments, the inulin oligosaccharides comprise acetylated inulin oligosaccharides. In some embodiments, the hydrophobic inulin oligosaccharides comprise acetylated inulin oligosaccharide. A person skilled in the art would appreciate that hydrophobic hydrolyzed inulin oligosaccharides can be produced using other techniques, such as esterification with other carboxylic acids and/or incorporation of silane and/or siloxane groups.

[0081] The skilled person can readily recognize hydrophobic inulin oligosaccharides of the present disclosure for use in tracing hydrophobic product, such as hydrophobic food product, a hydrophobic pharmaceutical product, a hydrophobic nutraceutical product, a hydrophobic textile product, a hydrophobic personal care product or a hydrophobic industrial product described herein.

[0082] In some embodiments, structurally different OMTs are fractionated by size exclusion chromatography. In some embodiments, Bio-Gel P-2 Gel with a fractionation range of 100 – 1,800 Da is used, and water is selected as the mobile phase as it is inexpensive and food safe. In some embodiments, sodium or potassium phosphate is selected as the mobile phase for hydrolyzed inulin. In some embodiments, sodium or potassium phosphate is selected as the mobile phase for hydrophobic hydrolyzed inulin. In some embodiments, sodium or potassium phosphate is selected as the mobile phase for acetylated hydrolyzed inulin. In some embodiments, the hydrolyzed inulin is separated by size exclusion chromatography. The skilled person can readily recognize alternative options that are suitable for the isolation/fractionation hydrolyzed inulin.

[0083] A number of methods can be used to accurately identify hydrolyzed inulin comprising oligosaccharides. For example, the present disclosure provides High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) for the identification of hydrolyzed inulin. The skilled person can readily recognize alternative options that are suitable for identification of hydrolyzed inulin. For example, Capillary Gas Chromatography, High Performance Anion Exchange Chromatography with Pulsed Electrochemical Detection (HPAE-PED) and liquid chromatography-mass spectrometry (LC-MS) are also useful for tracing or identifying hydrolyzed inulin. In some embodiments, the hydrolyzed inulin is identified by Capillary Gas Chromatography, HPAE-PAD, HPAE-PED, or LC-MS. In some embodiments, the hydrolyzed inulin is identified by Capillary Gas Chromatography. In some embodiments, the hydrolyzed inulin is identified by HPAE-PAD. In some embodiments, the hydrolyzed inulin is identified by HPAE-PED. In some embodiments, the hydrolyzed inulin is identified by LC-MS. In some embodiments, the oligosaccharides are identified by Capillary Gas Chromatography, HPAE-PAD, HPAE-PED, or LC-MS. In some embodiments, the oligosaccharides are identified by Capillary Gas Chromatography. In some embodiments, the oligosaccharides are identified by HPAE-PAD. In some embodiments, the oligosaccharides are identified by HPAE-PED. In some embodiments, the oligosaccharides are identified by LC-MS.

[0084] The methods described herein produced hydrolyzed inulin having molecular weights in the range between 300 and 1,800 Da, which were comprised in a combined fraction having a degree of polymerization (DP) of (a) from 5 to 8; (b) from 3 to 7; (c) from 3 to 5; or (d) from 2 to 4. In some embodiments, the hydrolyzed inulin comprises oligosaccharides having the molecular weight between about 300 and about 1,800 Da. In some embodiments, the hydrolyzed inulin is comprised in a combined fraction of hydrolyzed inulin having a degree of polymerization (DP) of (a) from 5 to 8; (b) from 3 to 7; (c) from 3 to 5; or (d) from 2 to 4.

[0085] The methods described herein produced combined fractions containing oligosaccharides having particular glucose and fructose residues. In some embodiments, the combined fraction of hydrolyzed inulin comprises oligosaccharides:

(a) GF_5, GF_6, F_5, F_6, F_7, and F_8;

(b) GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7;

(c) GF_3, GF_4, F_3, F_4, and F_5; or

(d) F_2, F_3, and F_4.

[0086] In some embodiments, the oligosaccharides are GF_5, GF_6, F_5, F_6, F_7, and F_8. In some embodiments, the oligosaccharides are GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7. In some embodiments, the oligosaccharides are GF_3, GF_4, F_3, F_4, and F_5. In some embodiments, the oligosaccharides are F_2, F_3, and F_4.

[0087] In some embodiments, the oligosaccharides comprise hydrophobic oligosaccharides. In some embodiments, oligosaccharides GF_5, GF_6, F_5, F_6, F_7, and F_8 comprise hydrophobic oligosaccharides. In some embodiments, oligosaccharides GF_5, GF_6, F_5, F_6, F_7, and F_8 comprise hydrophobic oligosaccharides. In some embodiments, oligosaccharides GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7 comprise hydrophobic oligosaccharides. In some embodiments, oligosaccharides GF_3, GF_4, F_3, F_4, and F_5 comprise hydrophobic oligosaccharides. In some embodiments, oligosaccharides GF_5, GF_6, F_5, F_6, F_7, and F_8 comprise acetylated oligosaccharides. In some embodiments, oligosaccharides GF_5, GF_6, F_5, F_6, F_7, and F_8 comprise acetylated oligosaccharides. In some embodiments, oligosaccharides GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7 comprise acetylated oligosaccharides. In some embodiments, oligosaccharides GF_3, GF_4, F_3, F_4, and F_5 comprise acetylated oligosaccharides.

IV. Methods and Systems for Tracing a Product

[0088] The OMTs described herein are useful for tracing a product described herein. Introduction of the OMTs into the product can be carried out during the preparation or production of the product or after the product has been produced, and/or before or during packaging. Accordingly, the present disclosure also provides a method of tracing a product comprising introducing an OMT described herein into the product, optionally during or after production of the product, optionally before or during packaging of the product. In some embodiments, an OMT is introduced into a product during production of the product. In some embodiments, an OMT is introduced into a product after production of the product. In some embodiments, an OMT is introduced into a product before

packaging of the product. In some embodiments, an OMT is introduced into a product after packaging of the product. In some embodiments, detection of the OMTs comprises use of a method, instrument or system described herein.

[0089] The OMTs described herein are useful as part of a system for tracing a product described herein. Accordingly, the present disclosure further provides a system of tracing a product, comprising an OMT described herein and a detector. The detector can be any suitable detector known in the art, for example Capillary Gas Chromatograph, HPAE-PAD, HPAE-PED and LC-MS. In some embodiments, the detector is Capillary Gas Chromatograph, HPAE-PAD, HPAE-PED or LC-MS. In some embodiments, the detector is Capillary Gas Chromatograph. In some embodiments, the detector is HPAE-PAD. In some embodiments, the detector is HPAE-PED. In some embodiments, the detector system is LC-MS.

[0090] The following non-limiting examples are illustrative of the present disclosure:

EXAMPLES

MATERIALS AND METHODS

[0091] **Chemicals.** Agar, ammonium sulfate, ampicillin, antifoam A, chloramphenicol, imidazole, inulin (chicory), isopropyl β-D-1-thiogalactopyranoside (IPTG), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide (NaOH) solution (50% w/w) and Tris-HCl were obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Glycerol, LB broth (Miller, granulated), sodium acetate (NaOAc) and sodium chloride (NaCl) were obtained from Fisher Scientific (Ottawa, ON, Canada). Bio-Gel P-2 gel was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Inulin oligosaccharide powder Orafti® P95 was obtained from Beneo (Mannheim, BW, Germany). The water used throughout this disclosure was obtained from a Milli-Q™ water system (Millipore Corp., Milford, MA, USA).

[0092] **Production of Endo-Inulinase.** A plasmid (pRSET C) containing the gene for an *Arthrobacter* sp. S37 endo-inulinase was obtained from Dr. S. Kang and stored at -80 °C (Kang *et al*, 1998. Purification and properties of an endo-inulinase from an Arthrobacter sp. *Biotechnology Letters*, 20: 983-986). The gene for the endo-inulinase had been modified to contain a *lac* operon and a His6 tag. Stock BL21(DE3)pLysS cells

(Novagen, Millipore Canada Ltd., Etobicoke, ON, Canada) were transformed using the frozen plasmid following the manufacturer's instructions. The cells were then transferred to LB plates containing ampicillin (50 µg/mL) and chloramphenicol (35 µg/mL) to select for the transformed clones. Transformed colonies were selected and transferred to 2.0 mL of LB broth containing ampicillin (50 µg/mL) and chloramphenicol (35 µg/mL) and incubated overnight at 37.0 ± 2.0 °C. The overnight culture was then used to inoculate 20.0 mL of LB broth containing ampicillin (50 µg/mL) and chloramphenicol (35 µg/mL) and incubated at 37.0 ± 2.0 °C until an optical density at 600 nm of approximately 0.5 (OD_{600} ~ 0.5) was reached. Enzyme production was induced by the addition of IPTG to a final concentration of 1.0 mM and the incubation temperature was reduced to 25.0 ± 2.0 °C. Samples of the supernatant were taken after 20.0 and 48.0 hours, and were analyzed by SDS-PAGE using a Mini-Protean II electrophoresis cell (Bio-Rad, Hercules, CA, USA) to confirm the production of endo-inulinase by a band at ~75 kDa. In brief, 50 µL of sample was mixed with a 50 µL β-mercaptoethanol and SDS and heated at 100 °C for 5 minutes prior to being loaded onto a 12% polyacrylamide gel with 1% SDS. Gels were run for 1.5 hour at a constant voltage (200 V) and stained with Coomassie blue dye. BLUeye prestained protein ladder (GeneDireX, Inc., Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) was used for comparison.

[0093] Endo-inulinase activity was confirmed by the transfer of 150 µL supernatant to an aqueous solution (pH 7.5) of inulin (5.0% w/w), which was subsequently analyzed after 3.0 h of incubation at room temperature (22 ± 1 °C) by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD; Dionex ICS 5000 HPLC system; Thermo Fisher Scientific, Waltham, MA, USA) for the presence of inulooligosaccharides (*i.e.* hydrolyzed inulin oligosaccharides) using the gradient program described below.

[0094] Scale up of the transformed bacteria was carried out using 3.0 L of LB broth containing ampicillin and chloramphenicol at the aforementioned concentrations spilt equally into two separate 2.0 L buffer bottles. To each of the bottles 300 µL of antifoam A and 15 to 20 mL of overnight culture was added. The resulting culture was incubated at 35.0 ± 2.0 °C and IPTG was added to a final concentration of 1.0 mM to induce enzyme production when the culture reached an OD_{600} of ~0.5. The mixture was incubated at 25.0 ± 2.0 °C overnight, and cells were sedimented by centrifugation (Sorvall Instruments

Centrifuge, DuPont, Wilmington, DE, USA) at 6,000 rpm for 30 minutes at 4.0 ± 2.0 °C. The cell pellet was re-suspended in 20.0 mM Tris-HCl (pH 7.5), and the cells were disrupted using a French press cell disruptor at 15 kPsi and then centrifuged at 12,000 rpm for 20 minutes at 4 ± 2.0 °C. The supernatant was collected and ammonium sulfate was added to achieve a 20% (w/w) saturation level and the mixture was stored static at 4.0 ± 2.0 °C overnight. The solution was centrifuged at 12,000 rpm for 30.0 minutes at 4.0 ± 2.0 °C and the supernatant was collected. Ammonium sulfate was added to achieve 60% (w/w) saturation and the solution was kept static at 4.0 ± 2.0 °C for 3.5 hours. The solution was then centrifuged at 12,000 rpm for 30.0 minutes at 4.0 ± 2.0 °C and the pellet was re-suspended in 4.0 mL of binding buffer (20.0 mM NaH_2PO_4, 500.0 mM NaCl, 5.0 mM imidazole, 10.0% glycerol, pH 7.5) and dialyzed against the binding buffer for 72 hours.

[0095] The solution was then syringe filtered (cellulose acetate, 0.45 µm pore size; 13 mm diameter; Chromatographic Specialties) and purified by fast protein liquid chromatography (FPLC; BioLogic LP System; Bio-Rad Laboratories; Hercules, CA, USA) employing a HiTrap chelating HP column (GE Healthcare Life Sciences, Mississauga, ON, Canada). The stationary phase was initially equilibrated with 20.0 mL of charging buffer (50.0 mM $NiSO_4$, pH 4.0) at a flow rate of 1.0 mL/min for 20.0 minutes, followed by binding buffer at the same flow rate for 20 min. Three milliliters of sample was loaded onto the stationary phase followed by sequential treatment with 25.0 mL of binding buffer, 25.0 mL of washing buffer (20.0 mM NaH_2PO_4, 500.0 mM, 10.0 mM imidazole, 10.0% glycerol, pH 7.5) and 30.0 mL of eluting buffer (20.0 mM NaH_2PO_4, 500.0 mM NaCl, 500.0 mM imidazole, 10.0% glycerol, pH 7.5) at a flow rate of 1.0 mL/min. All mobile phase buffers were filtered (0.45 µm; 47 mm diameter; Kimble, Fisher Scientific) prior to use. Fractions were collected during the eluting buffer stage based upon enzyme absorbance at 280 nm.

[0096] Appropriate fractions were collected and concentrated employing an Amicon stirred cell (Millipore Canada Ltd.) using a 20.0 kDa cut-off filter. Glycerol was added to the concentrated enzyme to achieve a final concentration of 50% (v/v) and the resulting solution was stored at -18.0 ± 2.0 °C.

[0097] **Production of Inulin Oligosaccharides.** A 2.5% (w/w) aqueous solution of chicory inulin was brought to 50.0 ± 5.0 °C in an AquaTherm shaking water bath (New Brunswick Scientific Co. Inc., Edison, NJ, USA) with mild shaking and 200 µL/g inulin of

endo-inulinase (~50 µg protein/g inulin) solution was added. Various time and pH conditions were tested for hydrolysis: from 30 minutes to 48 hours and between pH 5.00 and 7.50. The final hydrolysis condition was 24 hours at ~pH 5.00, the latter is the natural pH of the water-inulin solution. Enzyme activity was terminated by heating at ~95 °C for 10 minutes.

[0098] Inulooligosaccharide Separation. Inulooligosaccharides were fractionated employing a Bio-Gel P-2 gel stationary phase with water as the mobile phase. The stationary phase (~180g) was initially hydrated for 16 h at room temperature in water prior to packing into a glass column (120 x 2.5 cm to achieve a bed height of 100 cm). Ten grams of a 3.0% (w/w) aqueous solution of produced inulooligosaccharides was placed onto the column with water elution at a flow rate of 0.6 mL/min with 10 mL fractions collected for a period of 13 hours. Individual fractions were monitored for the presence of carbohydrates employing HPAE-PAD. Based on these results, combined fractions representing four elution time ranges were collected (OMT #1: fractions 31-33, OMT #2: 34-36, OMT #3: 37-39 and OMT #4: 40-42) and dried using a combination of rotary evaporation (Büchi, Flawil, Switzerland) and freeze drying (Heto Lab Equipment, Allerod, Denmark).

[0099] Inulooligosaccharide Analysis Employing High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD). Inulooligosaccharide analysis was carried out using a Dionex ICS 5000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Dionex AS autosampler, and an ICS 5000 electrochemical cell with a disposable gold electrode. The potentials and durations of the gold electrode were as follows: E1 = 0.10 V, t1 = 0.00 s; E2 = −2.00 V, t2 = 0.41 s; E3 = 0.60 V, t3 = 0.43 s; E4 = −0.10 V, t4 = 0.44 s; E5 = −0.10 V, t5 = 0.50 s and data acquisition was carried out using Dionex Chromeleon 7.0 software. Analyte separation was accomplished using a Dionex CarboPac PA1 column (4 x 250 mm) in series with a CarboPac PA1 guard column (4 x 50 mm) at room temperature. A linear gradient elution program was used for inulooligosaccharide separation where solvent A was 160 mM NaOH, solvent B was 160 mM NaOH/1.0 M NaOAc and solvent C was 1.0 M NaOH. Initial conditions were 100.0% A for 1.5 min; linear gradient to 60.0% B at 60.0 min and hold for 1.0 min; 100.0% C at 61.1 min with a hold until 65.0 min; 100.0% A at

65.1 min with a hold until 75.0 min. The flow rate was 1.0 mL/min. The injection volume was 20.0 µL.

[00100] Production of Laboratory Scale Apple Juice. Red delicious apples were obtained at a local supermarket and stored at 4 °C until use. Laboratory scale apple juice was produced using enzymes/dosages and conditions (time/temperature) used in commercial juice production (Willems & Low, 2016. Oligosaccharide formation during commercial pear juice processing. *Food Chemistry*, 204: 84-93). Apples were washed and the cores removed prior to maceration in a Sunbeam blender (Jaden Customer Solutions, Brampton, ON) with 10.0 mL water to produce a puree. A 100.0 g aliquot of apple puree was transferred to a beaker and 6.6 µL of Pectinex Ultra Mash enzyme was added. The mixture was then allowed to react at room temperature (22 ± 1 °C) with stirring for 90.0 minutes. The enzymes were inactivated by heating in a 90 °C water bath for 10.0 minutes. The juice was extracted by vacuum filtration with VWR Grade 417 filter paper (40 µm pore size; VWR International Incorporated LLC, Mississauga, ON). Juice clarification was achieved through the addition of 3.0 µL of Amylase AG 300 L and 3.8 µL of Pectinex Ultra Clear and the mixture was allowed to react at 50.0 ± 5.0 °C for 90.0 minutes prior to enzyme inactivation. Particles were removed by vacuum filtration through VWR Grade 413 filter paper (5 µm pore size). Juice was concentrated to 70.0 ± 5.0 °Brix using a rotary evaporator. Samples were taken after mash production, after enzyme treatment of the mash, the final juice and concentrate. Laboratory scale apple juice samples were stored at -20 °C until required for further analysis.

[00101] Tagging of Laboratory Scale Apple Juice with Oligomolecular Tags (OMTs). Two of the oligomolecular tags (OMTs) were selected (Fractions 34-36 and 40-42) and intentionally added at the mashing stage (*i.e.*, after blending but prior to enzyme addition) of laboratory scale apple juice production at two different concentrations (10.0 and 40.0 mg OMTs/100 g mash) to determine if inulooligosaccharides could be utilized as an internal traceability system. In addition, the untagged samples at the same stages of laboratory scale apple juice production were used as controls. Samples of the tagged and untagged stages were diluted to 5.5 ± 0.1 °Brix (Auto Abbe refractometer; Leica Inc., Buffalo, NY, USA), syringe filtered and analyzed for their inulooligosaccharide profiles by HPAE-PAD. All samples were analyzed in duplicates.

[00102] **Inulin Oligosaccharide Stability Testing.** Orafti® P95 was used as a representative standard for inulooligosaccharide storage stability testing. Orafti® P95 was added to 11.5 °Brix commercial apple juice at 0.2% (w/v) and at 1.2% (w/v) in a commercial apple juice concentrate (70.3 °Brix). Non-concentrate samples were syringe filtered into 2.0 mL sterilized centrifuge tubes (Fisher Scientific), with one set maintained at room temperature (22.0 ± 2.0 °C) and a second at 4.0 ± 2.0 °C. The same conditions were used for the concentrate samples. Samples were analyzed at the following time periods: 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months and 6 months by HPAE-PAD. Apple juice samples were diluted to 5.5 ± 0.1 °Brix with water prior to analysis.

RESULTS & DISCUSSION

[00103] Oligosaccharides produced from enzymatic inulin hydrolysis were selected as potential internal oligomolecular tags (OMTs) as they, (1) can be produced to yield a wide range of structural diversity; (2) have generally recognized as safe (GRAS) status for their addition to foods (FDA, 2014. GRAS notice 000537: Short-chain fructo-oligosaccharides); (3) are stable to human and food glycosidic enzyme activities; (4) offer long term stability under the acid conditions of foods (see results below); and (5) have high water solubility which is useful in the majority of food products.

[00104] Currently, there are numerous uses for inulin and related oligosaccharides in the food industry, with the majority involving their role as a prebiotic/soluble fibre ingredient. Other industrial uses include, lipid replacement in foods such as table spreads, baked goods, dairy products and frozen desserts (López-Molina *et al*, 2005. Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus L.*). *Phytochemistry,* 66: 1476-1484); and as a low calorie and low glycemic index sweetener (Morris and Morris, 2012. The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: a review. *Food Chemistry,* 133: 237-248). Inulin has also been used as the starting material for ultra-high fructose syrup (UHFS; ~85 to 97% fructose) production via acid or enzyme hydrolysis (Bajpai and Bajpai, 1991. Cultivation and utilization of Jerusalem artichoke for ethanol, single cell protein, and high fructose syrup production. *Enzyme and Microbial Technology,* 13: 359-362; Nakamura *et al*, 1995. Continuous production of fructose syrup from inulin by immobilized inulinase from *Aspergillus niger*

Mutant 817. *Journal of Fermentation and Bioengineering,* 80: 164-169; Kim *et al*, 1997. Production of inulo-oligosaccharides using an endo-inulinase from a *Pseudomonas* sp. *Biotechnology Letters,* 19: 369-371; Chi *et al*, 2011. Biotechnological potential of inulin for bioprocesses. *Bioresource Technology,* 102: 4295-4303).

[00105] **Production of Inulin Oligosaccharides.** Oligosaccharides can be produced using either enzymatic or chemical hydrolysis of inulin. Chemical hydrolysis results in poorer inulin oligosaccharide production due to the harsh environmental conditions employed (*i.e.* HCl [~0.5N]/60 °C/15 min) resulting in the production of high levels of fructose (Ávila-Fernández *et al*, 2011. Production of functional oligosaccharides through limited acid hydrolysis of agave fructans. *Food Chemistry,* 129: 380-386), which makes the material unsuitable as a source for OMTs. Acid hydrolysis of inulin has a number of additional drawbacks for OMTs production including: final product colour (due to caramelization and Maillard reactions); the use of non-environmentally friendly chemicals; the production of unwanted by-products such as furfurals (*e.g.* 5-hydroxymethylfurfural [HMF]); requirement of corrosion resistant materials; and the production of unwanted salts during neutralization (Waleckx *et al*, 2011. Use of inulinases to improve fermentable carbohydrate recovery during tequila production. *Food Chemistry,* 124: 1533-1542).

[00106] Although inulin oligosaccharides can be synthesized from sucrose by the addition of fructose units using either β-fructofuranosidase (EC 3.2.1.26) or β-fructosyltransferase (EC 2.4.1.9) (Barthomeuf & Pourrat, 1995. Production of high content fructo-oligosaccharides by an enzymatic system from *Penicillium rugulosam*. *Biotechnology Letters,* 17: 911-916), these biochemically controlled reactions result in the production of three oligosaccharides [*i.e.* 1-kestose (β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-α-D-glucopyranoside), nystose (β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-α-D-glucopyranoside) and fructosylnystose (β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-α-D-glucopyranoside)], which greatly limits this method as a source of OMT production (Barthomeuf & Pourrat, 1995).

[00107] An alternate enzymatic method that is useful to produce inulin oligosaccharides is via hydrolysis using endo-inulinases (EC 3.2.1.7), which hydrolyzes

the glycosidic linkages of inulin producing oligosaccharides and minimal fructose. The exact composition of the finished carbohydrate syrup depends upon the enzyme source and reaction conditions employed (Chi *et al*, 2009).

[00108] Without wishing to be bound by theory, it has been postulated that the appropriate choice of an endo-inulinase would result in the production of larger concentrations of higher molecular weight inulin oligosaccharides compared to other methods (*i.e.*, sucrose addition). An endo-inulinase from *Arthrobacter* sp. S37 was specifically selected for the production of inulin oligosaccharides as it was shown to have exclusively endo-activity unlike commercial enzymes so as to minimize fructose production and maximize oligosaccharide yield. Chicory inulin was selected as the substrate as it is a common source of inulin used in the food industry and commercially available with a high degree of purity containing minimal oligosaccharide/monosaccharide concentrations prior to hydrolysis. Other inulin sources were also tested by the present inventors and were found to be useful, which include agave inulin, dahlia tubers inulin, and Jerusalem artichoke inulin.

[00109] To investigate their impact on oligosaccharide production, the optimum temperature and pH for this endo-inulinase (*i.e.* 50.0 ± 3.0 °C and pH 7.50 ± 0.10; Kang *et al*, 1998) were initially employed experimentally on chicory inulin hydrolysis. It was found that temperatures below (*i.e.* room temperature [22.0 ± 2.0 °C] to 40.0 ± 3.0 °C) the optimal value significantly reduced inulin hydrolysis/oligosaccharide production, whereas those higher (*i.e.*, >60.0 °C) resulted in enzyme inactivation. Enzymatic activity at pH 7.50 and at the unadjusted pH of the aqueous inulin solution of 5.00 ± 0.10 were also examined. It was found that there was no difference in oligosaccharide production between these two pHs, therefore, the unadjusted pH was used as it eliminated the need for the use of buffers or pH adjustment. Under these experimental conditions (50 °C/pH 5.00), approximately 95% of the starting material was converted to oligosaccharides with degrees of polymerization (DP) of 2-15 after 48 hours of hydrolysis.

[00110] **Separation and Identification of OMTs.** In order to isolate structurally different OMTs the hydrolysis mixture was subjected to size exclusion chromatography employing Bio-Gel P-2 Gel based on its fractionation range (100 – 1,800 Da). Without wishing to be bound by theory, water was selected as the mobile phase as it is inexpensive and food safe. The skilled person can readily recognizes that alternative

options are available for mobile phase, for hydrolyzed inulin or where the hydrolyzed inulin has been further processed into hydrophobic (*e.g.* acetylated) hydrolyzed inulin.

[00111] Under these chromatographic conditions, four distinct OMTs (*i.e.* combined fraction #1 to #4) were obtained, each containing a variety of oligosaccharides (Fig. 1). Inulin oligosaccharide identifications were determined by High Performance Anion Exchange-Pulsed Amperometric Detection (HPAE-PAD) analytical comparison to a previously characterized commercial inulin oligosaccharide mixture (P95) and the structure of inulin, as standards were not commercially available (Ronkart *et al*, 2007). Isolation and identification of inulooligosaccharides resulting from inulin hydrolysis. *Analytica Chimica Acta,* 606: 81-87; Willems & Low, 2012. Major carbohydrate, polyol, and oligosaccharide profiles of agave syrup. Application of this data to authenticity analysis. *Journal of Agricultural and Food Chemistry,* 60: 8745-8754). Based on the base structure of inulin, the oligosaccharides were identified as either being solely comprised of D-fructose linked through β-(1→2) glycosidic bonds or being comprised of D-fructose with β-(1→2) bonds with terminal D-glucose units linked via an α-(1→2) glycosidic bond. Chromatographic separated oligosaccharides were collected into the following four combined fractions: #1, tubes 31-33 (based on a mobile phase flow rate of 0.6 mL/min and a tube volume of 10.0 mL), contained oligosaccharides with degrees of polymerization (DP) ranging from 5 to 8 (Fig. 1A); #2, tubes 34-36, had DPs ranging from 3 to 7 (Fig. 1B); #3, tubes 37-39, had DPs ranging from 3 to 5 (Fig. 1C); and #4, tubes 40-42, had DPs ranging from 2 to 4 (Fig. 1D). To confirm DP assignments, each of the four fractions were analyzed by mass spectrometry and had protonated ions ($[M+H]^+$) corresponding to the correct molecular weights. For example, combined fraction #4 showed protonated ions at m/z 343.2, 505.4 and 667.6 which agree with the presence of di-, tri- and tetrasaccharides, respectively.

[00112] In order to distinguish oligosaccharides which contained both fructose and glucose (GF_n, where G represents glucose and F represents fructose) from those comprised of only fructose (F_n), the fractions were compared to the previously characterized Orafti® P95 oligosaccharide mixture by HPAE-PAD spiking experiments (Ronkart *et al*, 2007; Willems & Low, 2012). Combined fraction #1 was identified to contain GF_5 (*i.e.*, a hexasaccharide containing one glucose residue and five fructose residues), GF_6, F_5, F_6, F_7, and F8, combined fraction #2 contained GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7,

combined fraction #3 contained GF_3, GF_4, F_3, F_4, and F_5, and combined fraction #4 contained F_2, F_3, and F_4 (Fig. 1). Less than 0.01% (w/w) of free D-fructose was detected when hydrolyzing inulin with the endo-inulinase described herein.

[00113] While there were overlaps in oligosaccharide structures between fractions (*e.g.* F_6 and F_7 in combined fractions #1 and #2), there were significant differences between these tags in overall oligosaccharides structures, their ratios, and their concentrations (as shown by differences in peak area), such that each could be and readily differentiated providing four unique combined fractions for food traceability and/or authenticity. In addition, if multiple combined fractions were added to a product, coupled with varying concentrations of each, then a large selection of unique OMTs would be available for use by food processors. Also, these OMTs are useful in pharmaceuticals, nutraceuticals and other industrial products (*e.g.* paints).

[00114] **Addition of OMTs to Laboratory Scale Apple Juice.** To test their effectiveness in a real food system, OMT #2 (*i.e.*, combined fraction #2 consisting of fractions 34-36; Fig. 1B) was used during the production of a laboratory scale apple juice. Apple juice was selected as the model system as it is a commonly consumed and adulterated food product (Brause & Ratterman, 1982. Verification of authenticity of apple juice. *Journal of the Association of Official Analytical Chemists,* 65: 846-849; Low *et al*, 1999. Capillary gas chromatography detection of invert sugar in heated, adulterated, and adulterated and heated apple juice concentrates employing the equilibrium method. *Journal of Agricultural and Food Chemistry,* 47, 4261-4266; Thavarajah & Low, 2006. Adulteration of apple with pear juice: emphasis on major carbohydrates, proline, and arbutin. *Journal of Agricultural and Food Chemistry, 54*, 4861-4867), it naturally contains a number of structurally different oligosaccharides (Low *et al*, 1999; Willems & Low, 2016) and it has a low pH (3.4 to 4.0), each of which could compromise other oligosaccharide identification and structural integrity. Laboratory scale apple juice production with the same apples without the addition of OMT #2 was also conducted for reference (*i.e.*, as a control).

[00115] In these experiments red delicious apples were washed and had their cores removed prior to being mashed and then OMTs #2 was added at a concentration of 40.0 ppm. The resulting mash was treated with pectinase (Pectinex Ultra Mash) at room temperature (22.0 ± 2.0 °C) with stirring for 90.0 minutes. The enzyme treated mash was

then filtered and the juice was subjected to clarification through the addition of enzymes (Amylase AG 300 L and Pectinex Ultra Clear) at 50.0 ± 3.0 °C for 90.0 minutes prior to enzyme inactivation. Remaining particulates were removed through filtration and the juice was concentrated to 70.0 ± 5.0 °Brix using a rotary evaporator (Willems & Low, 2016).

[00116] Oligosaccharide analysis (HPAE-PAD) was conducted on the laboratory scale apple juice containing OMT #2 (40.0 ppm) (Fig. 3A-D) and the blank (Fig. 2A-D) at each stage of processing, specifically, the mash prior to enzyme addition, the mash after enzyme treatment, filtered juice after clarification/prior to concentration and the final juice concentrate. The large off-scale peak at the beginning of the chromatogram (<10.0 minutes) is due to the major carbohydrates/polyol in apple juice, namely fructose, glucose, sucrose and sorbitol (Thavarajah & Low, 2006). As previously reported for pear, the apple mash contained minimal naturally occurring oligosaccharides (retention times >10 minutes; Fig. 2A; Willems & Low, 2016). The added OMTs were readily distinguishable from the naturally occurring apple juice oligosaccharides at this stage (Fig. 3A). After enzyme treatment the formation of oligosaccharides was observed as shown by the appearance of peaks in the chromatograms with retention times >12 minutes (Fig. 2B-D). These oligosaccharides were produced from the hydrolysis of pectin, hemicellulose (*i.e.*, xyloglucan) and starch by the commercial enzymes (Willems & Low, 2016). The large peak at approximately 20 minutes in the enzyme treated samples was identified as D-galacturonic acid, a product of the hydrolysis of pectin. After enzyme treatment the OMTs were still clearly distinguishable from the oligosaccharides formed during juice production (Fig. 3B-D).

[00117] The carbohydrases used during juice production, which include a variety of pectinases, amylases and hemicellulases (Kashyap *et al*, 2001. Applications of pectinases in the commercial sector: a review. *Bioresource Technology, 77,* 215-227), have not been reported to have activity on inulin based oligosaccharides. This was supported by the minimal change in oligosaccharide concentration (as measured by peak height; *i.e.*, a detector response variation of 51.0 nC initially to 46.6 nC in the final concentrate). Without wishing to be bound by theory, any changes in concentration may be due to harsh processing conditions (*i.e.*, high temperature) and potential loss during steps such as filtration. Therefore, for ease of use and to minimize any potential concentration changes, the present disclosure recommends that the OMTs be added later

during production, for example just prior to concentration. This would allow easier addition and more consistent distribution through the addition to a liquid rather than the mash. The same experiment was repeated with a final OMT concentration of 10.0 ppm and the results were similar.

[00118] Addition of OMTs to Commercial Apple Juices. To determine the effectiveness of inulin oligosaccharides in commercial products (*i.e.*, distinguishable from naturally occurring oligosaccharides), OMT #2 (*i.e.* combined fraction #2) were added to a commercial apple juice concentrate to achieve a final concentration of 40.0 ppm. The commercial juice concentrate was analyzed by HPAE-PAD before and after OMT addition. It was found that the OMTs could be readily differentiated from the oligosaccharides found in commercially produced apple juice, further showing the applicability of these oligosaccharides as markers. The same experiment was repeated with a final OMT concentration of 10.0 ppm and the results were similar.

[00119] Shelf Stability of Inulin Oligosaccharides. In order to determine the long-term stability of inulin oligosaccharides under typical storage conditions, Orafti® P95 was added to concentrated (70.3 °Brix) and single strength (11.5 °Brix) commercial apple juice. The single strength juice was syringe sterilized and both the concentrated and single strength products were stored in sterilized containers at room temperature (22.0 ± 2.0 °C) and 4.0 ± 1.0 °C. Orafti® P95 was selected as it contains inulin oligosaccharides with DPs ranging from 2 to 7 and was commercially available. Apple juice was selected for the reasons described above and room temperature and 4.0 °C were selected as they are common storage temperatures for food and related products. Samples were stored for six months and analyzed by HPAE-PAD for changes in the oligosaccharide content. It was found that at both room temperature and at 4.0 °C that there was no significant change in concentration as determined by peak area after six months of storage for both the single strength and concentrated apple juice samples (Fig. 4).

[00120] In conclusion, inulin oligosaccharides were produced, purified and analyzed for use as oligomolecular tags (OMTs) for traceability and authenticity. Inulin based OMTs were readily produced from commercially available chicory inulin using an endo-inulinase. Through this process a variety of unique OMTs were produced which could be mixed in various combinations/ratios to produce an even greater assortment of readily distinguishable OMTs for use in traceability and authenticity. Due to their GRAS status

these OMTs are useful in both whole foods (*e.g.*, apple juice) and food ingredients (*i.e.*, flour, food additives) allowing these products to be traced throughout the production chain. As well, changes in the OMT concentration/structure would be indicative of mistreatment or adulteration of a product adding an additional layer of security, as any manipulation of the food or ingredient would also lead to a change in the OMT. Finally, in addition to foods, OMTs are useful in high value products such as pharmaceuticals, nutraceuticals, personal care products including personal hygiene and cosmetics, and industrial products (*e.g.* paints).

[00121] While the present disclosure has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the disclosure is not limited to the disclosed examples. To the contrary, the present disclosure is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00122] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present disclosure is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

CLAIMS:

1. An oligomolecular tag (OMT) for tracing a product, wherein the OMT comprises at least one combined fraction of hydrolyzed inulin, optionally chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin.

2. The OMT of claim 1, wherein the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides having the molecular weight between about 300 and about 1,800 Da.

3. The OMT of claim 1 or 2, wherein the at least one combined fraction of hydrolyzed inulin having a degree of polymerization (DP) of (a) from 5 to 8; (b) from 3 to 7; (c) from 3 to 5; or (d) from 2 to 4.

4. The OMT of any one of claims 1 to 3, wherein the at least one combined fraction of hydrolyzed inulin comprises oligosaccharides:

(a) GF_5, GF_6, F_5, F_6, F_7, and F_8;

(b) GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7;

(c) GF_3, GF_4, F_3, F_4, and F_5; or

(d) F_2, F_3, and F_4.

5. The OMT of claim 4, wherein the oligosaccharides are GF_5, GF_6, F_5, F_6, F_7, and F_8.

6. The OMT of claim 4, wherein the oligosaccharides are GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7.

7. The OMT of claim 4, wherein the oligosaccharides are GF_3, GF_4, F_3, F_4, and F_5.

8. The OMT of claim 4, wherein the oligosaccharides are F_2, F_3, and F_4.

9. The OMT of any one of claims 1 to 8, wherein the hydrolyzed inulin is in solid, liquid solution or suspension form, optionally powder form.

10. The OMT of any one of claims 1 to 9, wherein the product is a food product, a pharmaceutical product, a nutraceutical product, a textile product, a personal care product or an industrial product.

11. The OMT of claim 10, wherein the product is hydrophobic.

12. The OMT of claim 10, wherein the food product is a whole food or a food ingredient.

13. The OMT of claim 12, wherein the whole food is a beverage.

14. The OMT of claim 12, wherein the food ingredient is flour or a food additive.

15. The OMT of claim 13, wherein the beverage is water, milk, a juice, coffee, tea, or a soft drink.

16. The OMT of claim 15, wherein the water is coconut water.

17. The OMT of claim 15, wherein the juice is apple, pear, grape, cranberry, grapefruit, tomato, blackberry, raspberry, strawberry, orange, winter melon, pomegranate, aloe vera, clam, sugarcane, kiwifruit, lemon, lime, lychee, cantaloupe, honeydew, papaya, pineapple, guava, prune, beet, carrot, celery, cucumber, dandelion-green, parsley, spinach, tejuino, turnip, watercress or hemp juice.

18. The OMT of claim 10, wherein the pharmaceutical product is a biologic or a small molecule.

19. The OMT of claim 10, wherein the nutraceutical product is a dietary supplement or a functional food.

20. The OMT of claim 10, wherein the personal care products is a personal hygiene or cosmetic product.

21. The OMT of claim 10, wherein the industrial product is paint.

22. The OMT of any one of claims 1 to 21, wherein the OMT is at a concentration of at least about 10 ppm and at most about 100 ppm.

23. The OMT of any one of claims 1 to 22, wherein the OMT is stable for at least 12 months, optionally from about -80 °C to about 90 °C.

24. The OMT of any one of claims 1 to 10, wherein the OMT comprises hydrophobic oligosaccharides.

25. The OMT of claim 24, wherein the hydrophobic oligosaccharides comprise acetylated oligosaccharides.

26. A method for producing an oligomolecular tag (OMT), comprising
    a. hydrolyzing an inulin solution, optionally chicory inulin, agave inulin, dahlia tubers inulin, or Jerusalem artichoke inulin, by adding an endo-inulinase to the solution to produce hydrolyzed inulin, wherein the endo-inulinase is from *Arthrobacter*, optionally *Arthrobacter* sp. S37, from *Aspergillus,* optionally *Aspergillus niger*, or from *Pseudomonas*, optionally *Pseudomonas mucidolens*.

27. The method of claim 26, wherein hydrolysis condition is at about pH 5.00 for about 24 hours at 50.0 ± 5.0 °C.

28. The method of claim 26 or 27, wherein the hydrolyzed inulin is separated by size exclusion chromatography.

29. The method of any one of claims 26 to 28, wherein the hydrolyzed inulin is identified by Capillary Gas Chromatography, High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), High Performance Anion Exchange Chromatography with Pulsed Electrochemical Detection (HPAE-PED), or liquid chromatography-mass spectrometry (LC-MS).

30. The method of any one of claims 26 to 29, wherein the hydrolyzed inulin comprises oligosaccharides having the molecular weight between about 300 and about 1,800 Da.

31. The method of any one of claims 26 to 30, wherein the hydrolyzed inulin is comprised in a combined fraction of hydrolyzed inulin having a degree of polymerization (DP) of (a) from 5 to 8; (b) from 3 to 7; (c) from 3 to 5; or (d) from 2 to 4.

32. The method of claim 31, wherein the combined fraction of hydrolyzed inulin comprises oligosaccharides:

    (a) GF_5, GF_6, F_5, F_6, F_7, and F_8;

(b) GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7;

(c) GF_3, GF_4, F_3, F_4, and F_5; or

(d) F_2, F_3, and F_4.

33. The method of claim 31 or 32, wherein the oligosaccharides are GF_5, GF_6, F_5, F_6, F_7, and F_8.

34. The method of claim 31 or 32, wherein the oligosaccharides are GF_4, GF_5, F_3, F_4, F_5, F_6, and F_7.

35. The method of claim 31 or 32, wherein the oligosaccharides are GF_3, GF_4, F_3, F_4, and F_5.

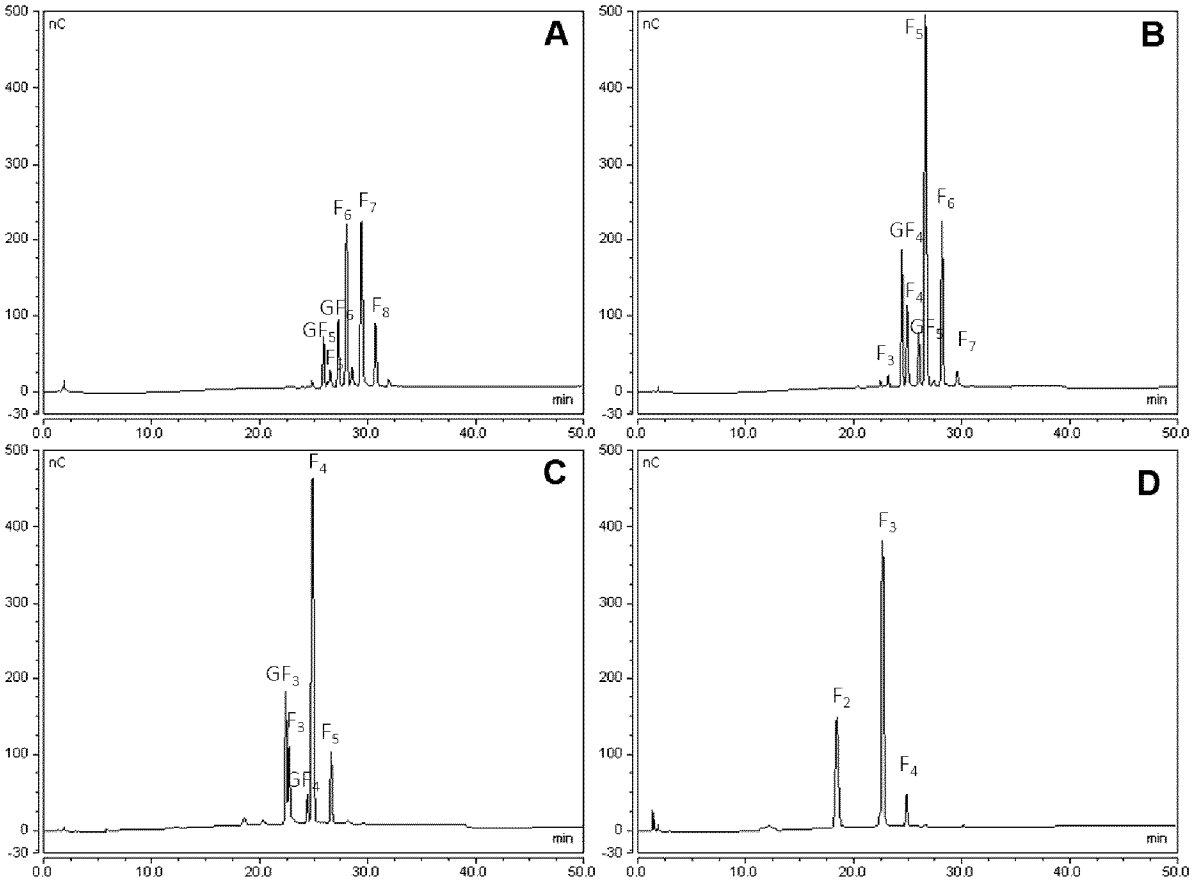
36. The method of claim 31 or 32, wherein the oligosaccharides are F_2, F_3, and F_4.

37. The method of any one of claims 26 to 36, further comprising acetylating the oligosaccharides, thereby providing acetylated oligosaccharides.

38. A method of tracing a product, comprises introducing the OMT of any one of claims 1 to 25 into the product, optionally during or after production of the product, optionally before or during packaging of the product.

39. A system of tracing a product, comprising the OMT of any one of claims 1 to 25 and a detector, wherein the detector is Capillary Gas Chromatograph, High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), High Performance Anion Exchange Chromatography with Pulsed Electrochemical Detection (HPAE-PED), or liquid chromatography-mass spectrometry (LC-MS).

FIG. 1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nC
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F6
F7
GF6
GF5
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F5
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min
F3
F2
F4

FIG. 2

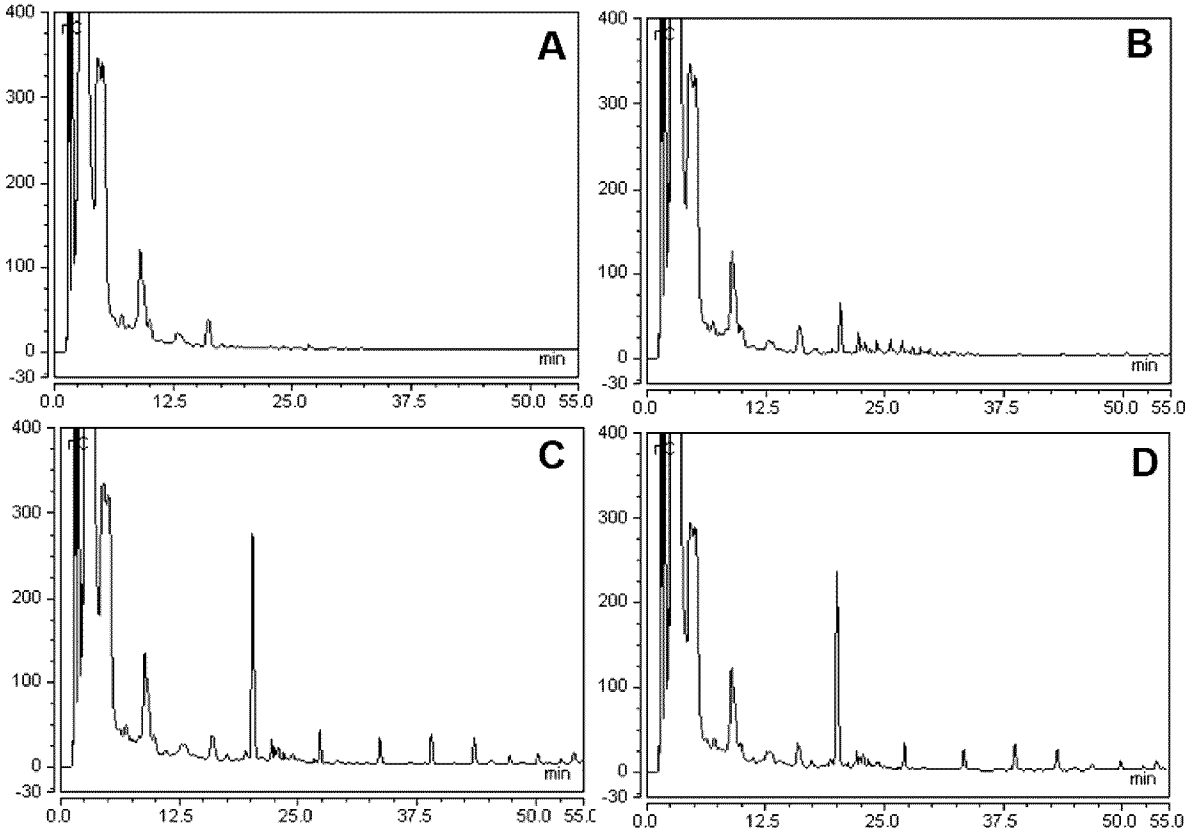


FIG. 3

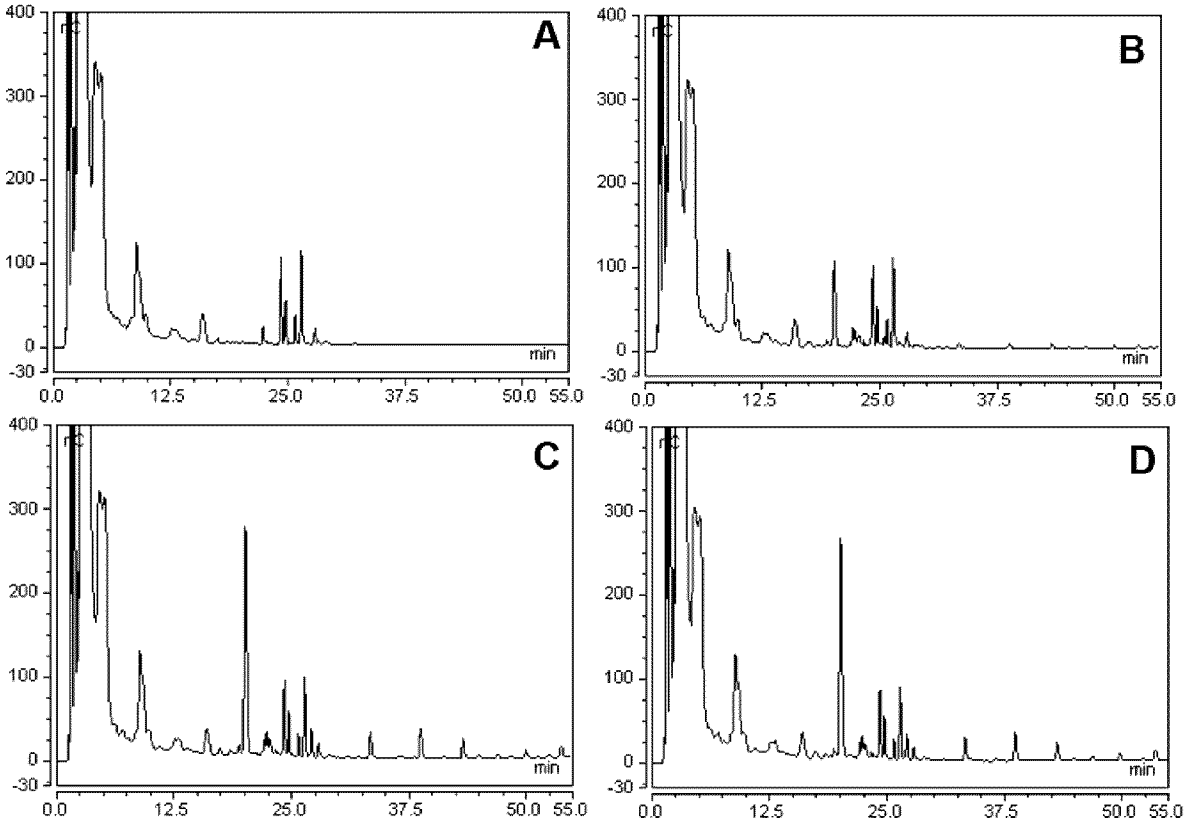
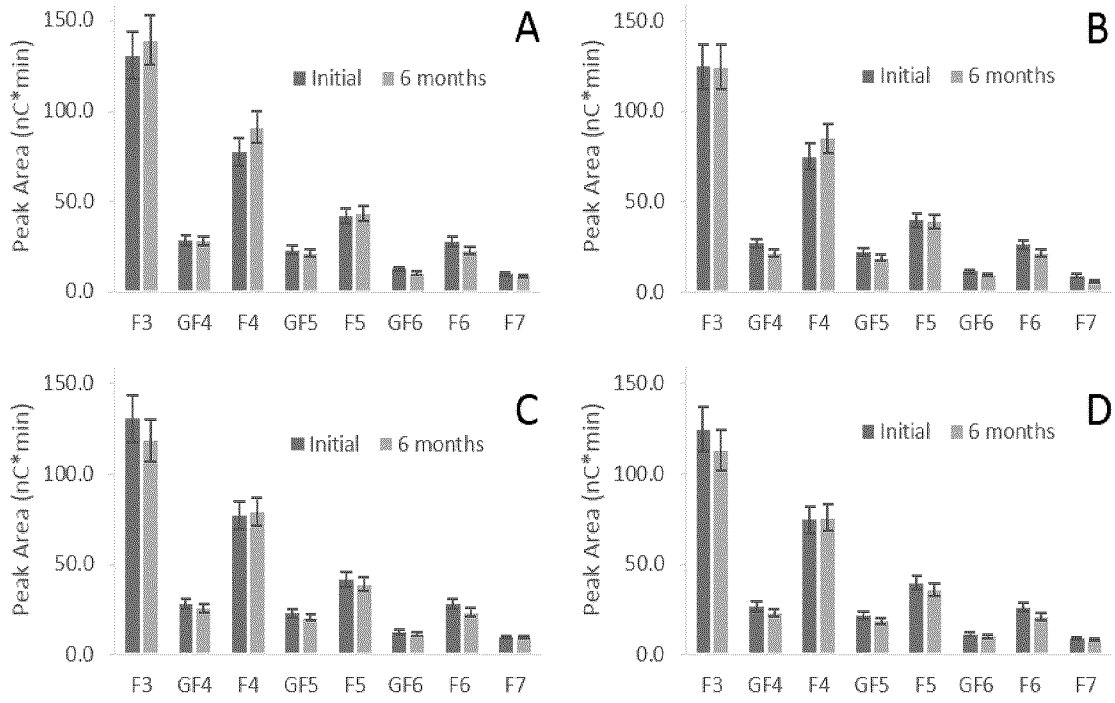


FIG. 4


A
B
C
D
Initial
6 months
Peak Area (nC*min)
150.0
100.0
50.0
0.0
F3
GF4
F4
GF5
F5
GF6
F6
F7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/051058

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***G01N 33/02*** (2006.01) , ***A23L 29/00*** (2016.01) , ***A23L 5/00*** (2016.01) , ***A61K 47/26*** (2006.01) , ***A61K 8/73*** (2006.01) , ***C07H 3/04*** (2006.01), ***C07H 3/06*** (2006.01) , ***C09D 7/63*** (2018.01) , ***C09D 7/65*** (2018.01) , ***C13K 11/00*** (2006.01), ***D06M 15/00*** (2006.01)

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)
IPC: ***G01N 33/02*** (2006.01) , ***A23L 29/00*** (2016.01) , ***A23L 5/00*** (2016.01) , ***A61K 47/26*** (2006.01) , ***A61K 8/73*** (2006.01) , ***C07H 3/04*** (2006.01), ***C07H 3/06*** (2006.01) , ***C09D 7/63*** (2018.01) , ***C09D 7/65*** (2018.01) , ***C13K 11/00*** (2006.01) , ***D06M 15/00*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel Orbit, Canadian Patent database (Intellect), Scopus
Keywords : inulin, oligofructose, fructooligosaccharide, tracer, food, oligomolecular tag, traceability, track*, trace*, tag, endoinulinase, chromatography, alduterations, hydroly*, GF*, additive

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ruiz-Matute et al. (2010) "Detection of adulterations of honey with high fructose syrups from inulin by GC analysis". *J. of Food Composition & Analysis*, 23: 273-276. *whole document*	1-39
X	Singh et al. (2010) "Production of Frutooligosaccharides from Inulin by Endoinulinases and Their Prebiotic Potential". *Food Technol. Biotechnol.*, 48 (4): 435-450. *whole document*	1-39
X	Borromei et al. (2009) "Evaluation of Fructooligosaccharides and Inulins as Potentially Health Benefiting Food Ingredients by HPAEC-PED and MALDI-TOF MS". *Int. J. of Anal. Chem..*, Article ID 530639, 9 pages. *Abstract and pages 1-2*	1-39

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"D"	document cited by the applicant in the international application	"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
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 18 October 2019 (18-10-2019)	Date of mailing of the international search report 18 October 2019 (18-10-2019)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Celine Dumais (819) 635-4825

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Informa (2010) "Internal Tagging In-depth Research", Research report by Informa Market Research Co. Ltd. submitted to the University of Guelph in June 2010. Retrieved from the Internet: https://www.informaresearch.com/sites/default/files/reports_pdf/U_of_G_Report.pdf. *page 40*	1-39
X	US 2011/0020510 (Frooninckx et al.) 27 January 2011 (27-01-2011) *Abstract and paragraphs 0016, 0031, 0037, 0041-0042, 0054, 0058, 0059 and 0071*	1-39
X	US 2010/0272652 (Levinson, Douglas A.) 28 October 2010 (28-10-2010) *Abstract and paragraphs 0021, 0028 and 0041*	1-39
A	Borromei et al. (2009) "Characterization and quantitation of short-chain frutooligosaccharides and inulooligosaccharides in fermented milks by high-performance anion-exchange chromatography with pulsed amperometric detection". *J. Sep. Sci.*, 32:3635-3642. *whole document*	1-39
A	Willems et al. (2014) "Authenticity Analysis of Pear Juice Employing Chromatographic Fingerprinting". *J. Agric. Food Chem.*, 62:11737-11747. *whole document*	1-39

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2019/051058

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2011020510A1	27 January 2011 (27-01-2011)	US2011020510A1	27 January 2011 (27-01-2011)
		US10150819B2	11 December 2018 (11-12-2018)
		AU2009240274A1	29 October 2009 (29-10-2009)
		AU2009240274B2	20 September 2012 (20-09-2012)
		CA2719064A1	29 October 2009 (29-10-2009)
		CA2719064C	18 February 2014 (18-02-2014)
		CN102007148A	06 April 2011 (06-04-2011)
		CN102007148B	14 November 2012 (14-11-2012)
		EP2283044A1	16 February 2011 (16-02-2011)
		EP2283044B1	06 June 2018 (06-06-2018)
		EP3418307A1	26 December 2018 (26-12-2018)
		ES2685263T3	08 October 2018 (08-10-2018)
		US2015079245A1	19 March 2015 (19-03-2015)
		US9801401B2	31 October 2017 (31-10-2017)
		WO2009129985A1	29 October 2009 (29-10-2009)
US2010272652A1	28 October 2010 (28-10-2010)	US2010272652A1	28 October 2010 (28-10-2010)
		EP2411055A2	01 February 2012 (01-02-2012)
		WO2010110916A2	30 September 2010 (30-09-2010)
		WO2010110916A3	02 December 2010 (02-12-2010)